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Urban mining of Municipal Solid Waste Incineration (MSWI) residues with emphasis on bioleaching technologies: A critical review

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Abstract

Metals are essential in our daily lives and have a finite supply, being simultaneously contaminants of concern. The current carbon emissions and environmental impact of mining are untenable. We need to reclaim metals sustainably from secondary resources, like waste. Biotechnology can be applied in metal recovery from waste streams like fly ashes and bottom ashes of municipal solid waste incineration (MSWI). They represent substantial substance flows, with roughly 46 million tons of MSWI ashes produced annually globally, equivalent in elemental richness to low-grade ores for metal recovery. Next-generation methods for resource recovery, as in particular bioleaching, gives the opportunity to recover critical materials and metals, appropriately purified for noble applications, in waste treatment chains inspired by circular economy thinking. In this critical review, we can identify three main lines of discussion: 1) MSWI material characterization and related environmental issues; 2) currently available processes for recycling and metal recovery; and 3) microbially-assisted processes for potential recycling and metal recovery. Research trends are chiefly oriented to the potential exploitation of bioprocesses in the industry. Biotechnology for resource recovery shows increasing effectiveness especially downstream the production chains, i.e. in the waste management sector. Therefore, this critical discussion will help assessing the industrial potential of biotechnology for urban mining of municipal, post-combustion waste.

31 **Key Words:** Circular economy; Waste-to-Energy (WtE) plants; Incineration wastes; Critical raw materials;

32 Secondary raw materials; Resource recovery

33

35 1. Introduction

36 Municipal Solid Waste Incineration (MSWI) is a predominant management practice in many
37 countries, and it has been increasingly adopted in countries like China (Fan et al., 2021). According to the
38 World Bank, 11% of the global MSW is incinerated, corresponding to an estimated 220 million tonnes (Kaza
39 et al., 2018). In the European Union EU-27, in 2019, 60 million tonnes of municipal solid waste were
40 incinerated (Eurostat, 2019). Despite reducing the waste volume and recovering energy, MSWI also produces
41 two main kinds of residues, called bottom (BA) and fly ashes (FA), that must be sustainably managed. MSWI
42 residues' features (chemical and mineralogical composition, grain size heterogeneity, etc.) and their disposal
43 strategy influence their after-use in applications, for example, reuse its mineral fraction in the construction
44 industry as secondary raw material. MSWI residues can be returned to secondary raw materials markets after
45 appropriate treatment to enhance production cycles in urban mining actions, aiming to remove, recover and
46 recycle the mineral resource that may be contained in anthropogenic materials with high economic potential
47 (e.g. critical raw materials) or environmentally positive balance (e.g., producing acceptable secondary raw
48 material with low environmental impacts). Copious research proposed innovative technologies with
49 simultaneous improvements of environmental and financial drawbacks associated with MSWI residues, both
50 BA and FA. BA and, to a lesser extent, FA can be recycled to produce concrete, soil improvers and fillers, glass
51 and ceramics, or used to produce absorbents, stabilizing agents, and zeolites (Quina et al. 2018; Lam et al.
52 2010). So far, urban mining attempts from MSWI residues are promising for application in integrated waste
53 management to boost incomes and minimize environmental impacts, as demonstrated by Life cycle
54 assessment (LCA) (Fellner et al., 2015). Combined separation, extraction, and recovery processes based on
55 physical-mechanical methods, acid and alkaline leaching, biorecovery and electroplating, or
56 bioelectrochemical systems seem particularly efficient for recovering metals from bottom ash and fly ash
57 (Gomes et al., 2020).

58 New options to improve MSWI residues management are needed, especially those capable of the
59 twofold benefit of metal recovery and quality enhancement of the post-treatment residue. Insights from
60 chemical and mineralogical data on MSWI residues can inform recovery of secondary raw materials and
61 marketable metals. For example, among metals of strategic interest and potentially mineable from MSWI
62 residues, silver (Ag), antimony (Sb), cerium (Ce), lanthanum (La), niobium (Nb), nickel (Ni), vanadium (V) are
63 enriched in the fine fractions, while gadolinium (Gd), chromium (Cr), scandium (Sc), tungsten (W), and yttrium
64 (Y) partition in the coarse fractions (Mantovani et al., 2021).

65 MSWI residues are a potential low-grade urban mine of ore metals thanks to the significant flows of
66 substance bearing metals downstream of the municipal waste incineration process (Funari et al., 2015). For
67 MSWI-BA, Funari and co-workers estimated a total flow of more than 350 t/a magnesium (Mg), 8.5 t/a Cr,

68 4.3 t/a cobalt (Co), and nearly 3 t/a Sb. The overall annual flow of the light rare earth elements (LREE: La, Pr,
69 Ce, Nd, Gd, Sm, Eu) and Sc and Y reach 2 t/a; while only the flow of heavy REE (HREE: Lu, Tb, Ho, Dy, Tm, Er,
70 Yb) is about 0.1 t/a. The Substance Flow Analysis (SFA) also shows considerable amounts of gallium (Ga) and
71 Nb (0.3 t/a) and the precious metals gold (Au) and silver (Ag) (0.01 t/a and 0.12 t/a, respectively). SFA analysis
72 on MSWI-FA showed relatively high flows of Mg (79 t/a), Sb (2.4 t/a), Cr (1 t/a), Ce (0.05 t/a), Co (0.04 t/a),
73 and also volatile elements such as Ag, Zn, and Sn have a considerable output retained in the solid FA. With
74 further estimates coming from these figures, a total of 4500 tons Cu, 130 tons REEs, and 0.5 tons gold, are
75 potentially recoverable from all MSWI-BA flowing on a national level. At the same time, the MSWI-FA output
76 is a promising source of Zn, Sn, Sb, and Pb. Besides the relevance of metal recovery, successful urban mining
77 strategies favour i) the reduction of the environmental impact, providing less dangerous leachates, ii) more
78 control over nanoparticle pollution, and iii) high quality of post-treatment residues. In parallel, investigation
79 of MSWI residues and related environmental media (e.g., topsoil nearby incinerators) would favour the
80 development of finely tuned methods for urban mining with a close eye on sustainability. Looking ahead, the
81 quality of MSWI feedstock materials and final solid residues, especially considering the 10-to-20-year life
82 cycle of MSWI technology, needs continuous improvements from synergistic actions of both private and
83 public stakeholders and the local communities.

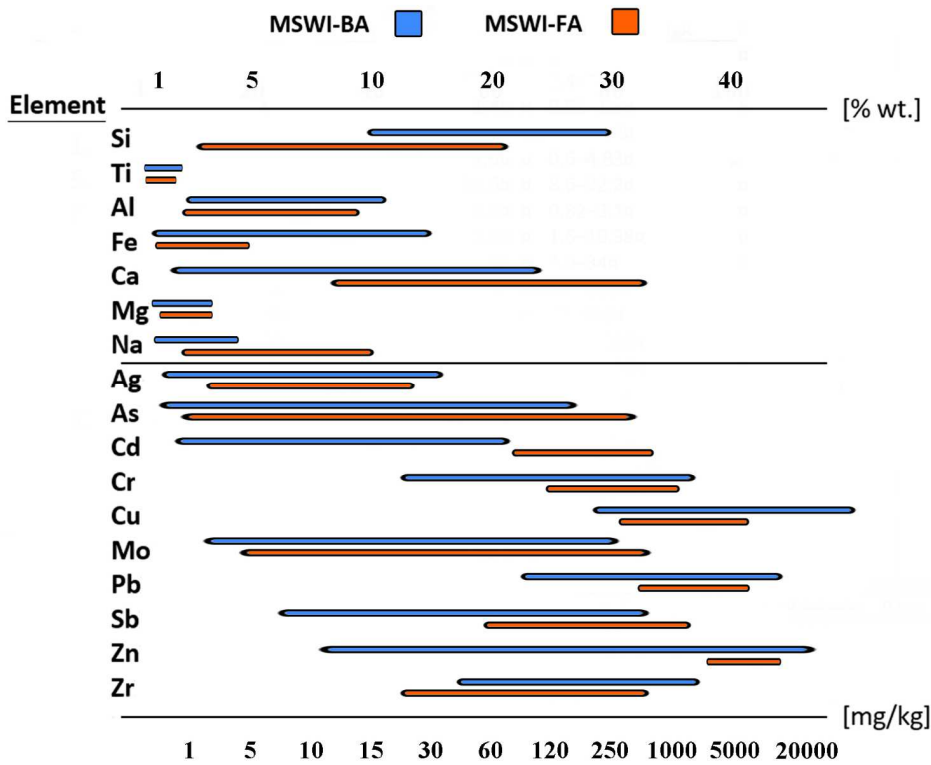
84 This paper aims to critically reflect on the application of biotechnologies for urban mining of waste
85 streams in the context of the circular economy. Our objectives are to focus on: 1) MSWI material
86 characterization and related environmental issues; 2) currently available processes for recycling and metal
87 recovery; and 3) microbially-assisted processes for potential recycling and metal recovery.

88 2. Mineral resources and secondary raw materials from MSWI residues

89 2.1. Chemistry and Mineralogy of MSWI residues

90 MSWI residues can be thought of as a mineral matrix mixed with a small fraction of partly combusted
91 organic matter and secondary organic by-products (approx. 4% by weight) resulting from temperature
92 changes through the processing line of MSWI technology leading to the establishment of different
93 thermodynamic equilibria (Guimaraes, et al. 2006). Eusden et al. (1999) described a detailed petrogenesis of
94 the MSWI solid materials sent to incinerators. The major elements in MSWI residues are Ca, Si, Al, Fe, Mg,
95 Na, K and Cl in the form of silicates, aluminosilicates, carbonates (e.g., calcite, trona), most of their oxides
96 (e.g., calcium oxide, hematite, sodium oxide, titanium dioxide and potassium oxide) and alkaline salts (e.g.,
97 halite, sylvite; preferably present in MSWI-FA). Usually, the most abundant components are Ca and Si oxides.
98 Cu, Cr, Pb, Cd, Zn, Hg, Sb, and Ni metals are also found in these ashes as minor and trace elements potentially
99 risky for the environment. Studies of element fractionation found that elements with high melting
100 temperature tend to remain in the MSWI-BA, while the volatile ones tend to break down in the MSWI-FA

101 (Funari et al., 2015). The heterogeneity of the urban waste input feed directly influences the mineralogical
 102 and chemical composition and the physical-mechanical properties of the incinerator ashes. Different
 103 spectrometers are used to determine major, minor, and trace elements in MSWI residues together with other
 104 analytical techniques depending on the analyte sought and, in general, from the purpose of the
 105 characterization. Figure 1 shows the compositional range reported in the literature for measured
 106 analytes. In MSWI-FA, the heavy metals content is generally higher than in BA due to the metal vaporization
 107 during the combustion and adsorption on a higher specific surface area. Harmful compounds such as
 108 chlorides and metal oxide nanoparticles from MSWI-FA are controlled by wet scrubbers in the Air Pollution
 109 Control (APC) system, which primarily removes acid gases such as HCl and HF (Sabbas et al., 2003).



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110
 111 Figure 14. Chemical composition ranges of MSWI-BA and MSWI-FA for selected major, minor and trace elements (Izquierdo et al.,
 112 2002; Sabbas et al., 2003; Bayuseno et al., 2010; Funari et al., 2015; Astrup et al., 2016; Xiaomin et al., 2017; Huber et al., 2019;
 113 Wong et al., 2021; Maldonado-Alameda et al., 2021; Mantovani et al., 2021; Clavier 2021) in wt. % and mg/kg, respectively.

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114 Numerous works elaborated the mineralogical composition of MSWI residues, e.g., by scanning
 115 electron microscope defining morphology, single-point chemical composition and the interaction between
 116 the different phases present inside the grains (Bayuseno and Schmahl, 2010; Bogush et al., 2015; Funari et

117 al., 2018; De Boom and Degrez, 2012). The thin sections show structural variability and complexity under the
118 microscope. Moreover, it is possible to verify the presence of glassy and crystalline material together with
119 metallic and empty parts (e.g., Mantovani et al., 2021). The presence of wollastonite (CaSiO_3), with a dendritic
120 crystallization in the glass matrix indicates a fast crystallization is frequent. There are also evident zoning and
121 evidence of core recrystallization; sometimes, recrystallizations of fresh structures are observed within a
122 metal matrix (Bogush et al., 2015). Iron, ubiquitous and present as a major element ($> 0.1\%$ by weight),
123 undergoes a complex petrogenesis and can form a series of oxides and hydroxides, but also remain as nuggets
124 of metallic Fe, or Fe-phosphides (e.g., schreibersite, associated with reducing conditions) and -sulfides
125 (pyrite, pyrrhotite, greigite among many) (Funari et al., 2018; 2020), making hard to determine minor iron-
126 bearing phases. Mineralogical analysis on magnetic separates showed the presence of small ($<5 \mu\text{m}$)
127 spherules containing Fe in the form of agglomerates of particles or loose particles that could be attributable
128 to technogenic spheres (*sensu* Magiera et al., 2011) readily dispersible during handling, being generally
129 MSWI-FA dustier than MSWI-BA. Despite the great uncertainty on the stoichiometry and quantification of
130 the crystalline phases, the X-ray diffractograms readily detect various carbonates such as calcite, soluble salts
131 such as halite, silicates such as quartz, solid solutions gehlenite-akermanite, pyroxenes and feldspars, sulfates
132 and phosphates and oxides of iron. However, the origin of certain mineralogical phases, i.e., if the minerals
133 observed in MSWI-BA or MSWI-FA are derived from the incoming waste or freshly formed remains puzzling.
134 This is due to different processing technologies, chemical composition of the incoming waste and combustion
135 temperatures reached, which, in turn, can depend on local policies and have seasonality effects. The
136 commonly identified minerals from MSWI-BA and MSWI-FA by XRD are in [Table 1Table 1](#).

137 The data reported in the literature also suggested the residues' particle size as a proxy of element
138 enrichment or, in other words, a tool for mineral beneficiation. Carbonates and sulfatic phases typically occur
139 in the finer fractions ($<0.065 \text{ mm}$). Analytical determinations show a higher concentration of S, Cl and metals
140 such as Zn, Pb, Cr, and Sr in fine particle sizes ($<1 \text{ mm}$) (Speiser et al., 2000; Chimenos et al., 2003). In the
141 finer fraction, there is a higher content of metals within mineralogical phases less resistant to weathering
142 (carbonates and sulfates), that is, more available to environmental leaching. Analyzing the particle size's
143 cumulative distribution, about 60% of total weight of MSWI-BA is composed of particles with a size between
144 1 and 8 mm (belonging to the category coarse sand or gravel), while 20% has a particle size bigger than 10
145 mm and the remaining 20% is made up of grains smaller than 1 mm. MSWI-FA is more homogeneous than
146 MSWI-BA in its particle size, which results averagely silty. Both ashes show a minor but significant ultrafine
147 fraction ($<1 \mu\text{m}$). Overall, although not always agreeing on the identification of phases, the mineralogical
148 data in the scientific literature confirms that the MSWI residues contain minerals of potential economic
149 interest. However, the chemical composition can vary significantly according to the particle size, the quality
150 of the incoming waste, the combustion process, and the type of residue.

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151 Table 1 -Most common mineralogical phases detected by XRD from MSWI residues (Wan et al., 2006; Liu et al., 2009; Bayuseno and
 152 Schmah, 2010; De Boom and Degrez, 2012; Bogush et al., 2015; Funari et al., 2018; Mantovani et al., 2021). O: rare; X: common;
 153 XX: very frequent; n.d.: not detected.

Mineral phase	Chemical formula	MSWI-FA	MSWI-BA
<i>Silicates, aluminates, and aluminosilicates</i>			
Quartz	SiO ₂	X	XX
Cristobalite	SiO ₂	n.d.	X
Corundum	Al ₂ O ₃	X	XX
Alkali Feldspars	(K,Na)(Al,Si) ₃ O ₈	n.d.	XX
Plagioclase feldspars	NaAlSi ₃ O ₈ -CaAl ₂ Si ₂ O ₈	X	X
	(K, Ca, Na)(Al,Si) ₄ O ₈	O	X
Gehlenite	Ca ₂ Al ₂ SiO ₇	X	XX
Anorthite	CaAl ₂ Si ₂ O ₈		
Akermanite	Ca ₂ MgSi ₂ O ₇	X	XX
	Ca ₂ (Mg,Fe)Si ₂ O ₇	O	X
Calcium Pyroxene	Ca(Mg,Fe)Si ₂ O ₆	n.d.	X
	Ca(Mg, Al)(Si,Al) ₂ O ₆	O	X
	(Na,Ca)(Fe,Mn)(Si,Al) ₂ O ₆	n.d.	X
Wollastonite	CaSiO ₃	X	X
	Ca ₂ SiO ₄	X	X
Portlandite	Ca(OH) ₂	X	X
Gibbsite	Al(OH) ₃	O	XX
Ettringite	Ca ₆ Al ₂ (SO ₄) ₃ (OH) ₁₂ 26H ₂ O	X	X
<i>Carbonates</i>			
Calcite	CaCO ₃	XX	X
Other	(Pb,Cd,Zn)CO ₃	X	O
<i>Fe-bearing phases</i>			
Magnetite	Fe ₃ O ₄	O	XX
Hematite	Fe ₂ O ₃	X	O
Wüstite	FeO	X	X
Goethite	FeO(OH)	n.d.	X
	Fe(OH) ₃	X	X
	FeCO ₃	O	O
	Fe(Cr,Ti) ₂ O ₄	O	X
	FeSO ₄ 7H ₂ O	n.d.	X
<i>S-based phases</i>			
Anhydrite	CaSO ₄	XX	X
Gypsum	CaSO ₄ 2H ₂ O	X	XX
	Ca ₆ Al ₂ O ₆ (SO ₃) ₃ 32H ₂ O	n.d.	X
	PbSO ₄	X	n.d.
<i>Other oxides</i>			
Lime	CaO	XX	X
	TiO ₂	X	X
	PbO	X	O
	ZnO	X	X
	Na ₂ O	XX	X
	CuO	X	XX

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	CaMoO ₄	O	X
	NaAsO ₂	O	X
<i>Cl-based phases</i>			
Friedel's salt	Ca ₂ Al(OH) ₆ Cl 2H ₂ O	<i>n.d.</i>	X
Hydrocalumite	Ca ₂ Al(OH) ₆ Cl _{1-x} (OH) _x 3H ₂ O	<i>n.d.</i>	X
	Ca ₅ (PO ₄) ₃ Cl	O	X
	Ca ₂ SiO ₃ Cl ₂	O	X
	CaCl ₂	X	<i>n.d.</i>
	KCaCl ₃	X	<i>n.d.</i>
	PbCl ₂	X	<i>n.d.</i>
	ZnCl ₂	X	<i>n.d.</i>
	NaCl	XX	X
	KCl	X	<i>n.d.</i>
<i>Other halides</i>			
fluorides	CaF ₂	X	<i>n.d.</i>
bromides	not specified	O	O
iodides	not specified	<i>n.d.</i>	<i>n.d.</i>
<i>Other compounds</i>			
organometallic compounds	organoarsenic compound	<i>n.d.</i>	O
<i>Native elements</i>			
zinc	Zn(0)	X	X
aluminium	Al(0)	X	X
copper	Cu(0)	O	X
gold	Au (0)	<i>n.d.</i>	X
other elements	Ti(0), Pb(0), Ag (0), Hg(0)	X	O

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154 2.2. Hydrometallurgy for MSWI residues Urban Mining

155 Hydrometallurgical solutions in waste management typically involves the dissolution of the metals
 156 present in the mineralogical matrix in acids or bases. During the leaching procedures, minerals dissolve under
 157 varying thermodynamic conditions. Metals can be separated in the dissolution step when not soluble in the
 158 solvent used, producing a solid precipitate as a part of the process chain. Hydrometallurgical separation can
 159 rely on solvent extraction and solid ion exchangers and membranes, ionic liquids, and on adsorption capacity
 160 of other materials (e.g., carbon). Following the separation of the metals, the single metal can be purified, for
 161 instance, by sequential precipitation or electrowinning.

162 Water, mineral acids (i.e., sulfuric acid, aqua regia), bases (i.e., sodium hydroxide, ammonia), organic
 163 acids (such as maleic acid), salt solutions, and combinations of these are common leaching reagents. The
 164 process optimization can be achieved by playing with pressure, temperature, reaction time, but also by
 165 adding oxidizing (e.g., H₂O₂, Cl₂, HClO, NaClO) or reducing (e.g., Fe²⁺, SO₂) agents. The most used leaching
 166 methods include reactor leaching, heap leaching, vat leaching, dump leaching (heap without crushing), in-
 167 situ leaching (extractant pumped directly in the ore deposit) and autoclave leaching (high pressure and

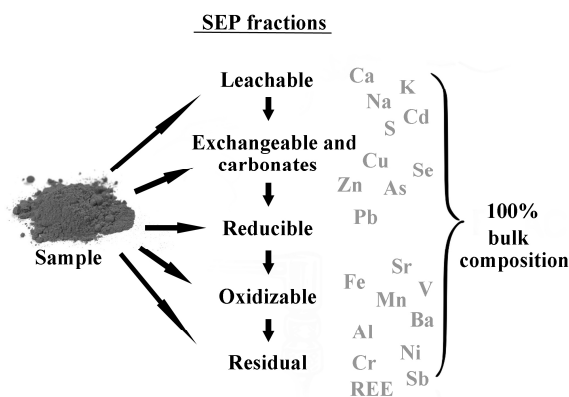
168 temperature). Galvanic, microwave, and ultrasound-assisted leaching are other methods investigated to
169 enhance the efficiency of traditional leaching.

170 A technique mainly used to treat MSWI-BA before the process of metal recovery takes place or the
171 residue is landfilled, is ageing (or natural weathering). It reduces the water content of the material up to an
172 optimal humidity (10–15 wt. %) for metal recovery, improves environmental leaching properties and/or
173 stabilizes the reactive matrix. Ageing occurs naturally during storage, which normally lasts from 4 to 12 weeks
174 and sporadically up to one year. During ageing, the precipitation of carbonates, degradation of organic
175 matter, and pH changes can occur (Nørgaard et al., 2019) as well as total or partial immobilization of Cu, Pb,
176 Zn and chloride can be achieved. Conversely, oxyanion-forming elements (e.g., Cr, Mo, Sb and sulfate) may
177 become more prone to mobilization (e.g., Arickx et al., 2006; Costa et al., 2007), likely impacting metal
178 recovery.

179 Hydrometallurgical applications' primary purpose for managing MSWI residues is decontamination
180 from harmful metals. In the earliest studies on pH-dependent leaching using HNO₃ (Eighmy et al., 1995), Ca,
181 Cl, K, Na, and Zn dissolved easily, while other elements (Cr, Pb, Zn, Cu, and Al) exhibited amphoteric behavior
182 with enhanced solubilization at low pH over a leaching period of 3h. Nagib and Inoue (2000) reported the
183 recovery of different metals from MSWI-FA using acetic acid, sulfuric acid, sodium hydroxide, and
184 hydrochloric acid, with a fixed L/S ratio of 7 ml/g. They found that most of Zn is dissolved quickly using sulfuric
185 acid (10 wt. % H₂SO₄) leaching, while temperature was mainly affecting Fe and Mg solubility. Therefore, 30°C
186 temperature and 5 min time were determined to be suitable for Zn acid leaching to suppress the solubility
187 of Fe and Mg, which is significantly enhanced at 60°C (Nagib and Inoue, 2000). Hydrochloric acid (10 wt. %
188 HCl) leaching dissolves 63% Zn and 40% Pb in 5 minutes, together with impurities such as Fe, Mg and Ca.
189 Acetic acid (10 wt. % CH₃COOH) leaching was effective, and most Pb and Zn were dissolved in 60 minutes
190 (Nagib and Inoue, 2000). Acid leaching is efficient because it can dissolve nearly all Pb and Zn, but further
191 separation and purification steps may be required since other potential impurities (e.g., Fe, Mg, Al, Ca) are
192 acid soluble. Further implementation was applied to the recovery method of Al and Fe, combining physical
193 and mechanical processes (e.g., Nayak and Panda, 2010). The use of thermal treatment combined with acidic
194 leaching allows overcoming some limitations (with recovery efficiencies up to 86 % Al and 94 % Fe using
195 sulfuric acid) and produced sintered pellets suitable as an inert and lightweight aggregate (Matjie et al., 2005;
196 Li et al., 2007, 2009). However, the high costs for energy to reach the calcination temperatures (800-1200°C)
197 and the time demand (up to 24h) make these processes uneconomic. The leaching behavior of antimony (Sb)
198 is particularly chased because of its elevated concentrations in MSWI residues and environmental relevance
199 tied to its speciation also during natural ageing. Cornelis et al. (2012) investigated the leaching of antimonate
200 (Sb⁵⁺) and antimonite (Sb³⁺) in MSWI-BA as a function of degree of carbonation and pH. Results showed that
201 acidification and carbonation increased Sb⁵⁺ leaching and decreased Sb³⁺ leaching, and pointed out that Sb

202 solubility depends on pH and calcium cations availability (romeite minerals are found to play an important
 203 role in the antimonate leaching) (Cornelis et al., 2012). Alkaline leaching, on the other hand, is hampered by
 204 the limited solubility of valuable metals (e.g., Zn), but can have the advantage of leaving a lesser amount of
 205 impurities in the solid residue. Bipp et al. (1998) were among the first to suggest alkaline leaching for heavy
 206 metals extraction. They tested gluconic acid and molasses hydrolysate leaching with sugar acid addition
 207 (1.8%) in the typical pH range of MSWI residues, achieving good recovery performances for Zn, Pb, Cu, Cr,
 208 and Cd with the molasses hydrolysate under weak alkaline condition. In general, alkaline leaching carried out
 209 in pH conditions near the MSWI residue's starting pH showed limited performances (Lee and Pandey, 2012).

210 Significant impact on leaching of MSWI residues comes from selective extractions used in typical
 211 geochemical investigation to understand metal behaviours under different environmental conditions.
 212 Although it is not possible to reach complete selectivity in each step, a sequential extraction procedure is
 213 applied to MSWI residues (Figure 2) and corroborated by experimental and theoretical models (Kirby
 214 and Rimstidt, 1993; Van Herck and Vandecasteele 2001; Chou et al., 2009; Funatsuki et al., 2012). The
 215 sequential extraction procedures highlight that metals like Zn, Cu, Pb, and Cd are soluble at low pH (>3.5),
 216 but oxidizing conditions are necessary to leach additional Zn. While Ca, K, Na, chlorides, and sulfates exhibit
 217 high solubility in water (step 1), Al and, to a lesser degree, Fe remain in the residual fraction. Most trace
 218 elements including REE tend to endure in the residue. The desired pH of the extractions and the sample
 219 matrix influence the chemical species found in the fractions.



220
 221 **Figure 23.** Generalization and summary of the fractions and analytes interested by Sequential Extraction Procedure (SEP) after Van
 222 Herck et al., 2000; Wan et al., 2006; Huang et al., 2007; Zhao et al., 2008; Chou et al., 2009; Chang et al., 2009.

223 Several authors investigated metal extraction using various aminopolycarboxylic acids such as DTPA,
 224 EDTA, NTA (Hong et al. 2000). Hong et al., 2000 underlined that the efficiency is not pH-dependent and
 225 solvent demanding (reagents concentration ranging between 0.5 wt. % and 1.0 wt. %); the extraction
 226 performed well for Pb (80 % recovery) in moderate alkaline condition with EDTA and DTPA, but the

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227 application of such chelating agents is uneconomic at the full scale for their high selling costs. Finally,
228 experiments using batch extraction under similar conditions have shown that applying electric current can
229 improve the solubility of some metals. Pedersen et al. (2005) evaluated different assisting agents for
230 electro-dialytic removals: the best aid in the removal of Cd was an NH₃ solution, perhaps because it helped
231 build stable tetraamine complexes, while the best aid in the removal of Pb was Na-citrate. The optimum for
232 removing a group of metals (up to 86% Cd, 81% Cu, 62% Zn, 44% Cr and 20% Pb) used 0.25 M ammonium
233 citrate/1.25 % NH₃ solution (Pedersen et al., 2005).

234 2.3. Current Options for Resource/Material Recovery from MSWI 235 residues

236 Since the early 1990s through the research programs known as NITEP (National Incinerator Testing
237 and Evaluation Program) and WASTE (Waste Analysis, Sampling, Testing, and Evaluation) which were
238 pioneered by Canada and the USA, MSWI-BA and MSWI-FA have been the focus of years of research efforts
239 (Chandler et al. 1997). Several processing techniques for MSWI residues have been proposed to recover
240 metals of economic interest and secondary raw materials, minimize harmful metals releases, and improve
241 the final residue environmental status. Commonly, MSWI residues are treated initially with separation
242 techniques, sometimes tailed by thermal treatments or stabilization or solidification processes (Kuboňová et
243 al. 2013; Sabbas et al. 2003). Separation technologies consisting of physical-mechanical separation have been
244 the most popular options because of their relative technical and economic feasibility compared to advanced
245 treatment processes. Physical and mechanical treatments of MSWI residues aim primarily at:

- 246 i. Recovering concentrated stream fractions (e.g., ferrous- and non-ferrous metals)
- 247 ii. Improving the final residue quality for its reuse or inert landfilling
- 248 iii. Achieving mineral beneficiation before hydrometallurgical processes (as a pre-treatment)

249 A plethora of metals, notably aluminum, iron, copper and other base metals, can be obtained at
250 different purity levels by simple physical/mechanical separation. Before the MSWI-BA are piled up, a drum
251 magnetic separator often recovers the biggest magnetic bars/alloys that can be sold to metal refiners.
252 Various systems can further divide non-ferrous and ferrous fractions of MSWI-BA with rather high efficiency
253 during the processing of these stockpiles. The non-ferrous part is rich in Au, Ag, Cu, Al, Pb, Zn and Sn and is
254 commonly preferred for their recovery (Muchova et al., 2009; Biganzoli and Grosso, 2013). To optimize the
255 recovery of Cu, Ag, Pb, Sn, and Zn from a heavy fraction and an Al-rich product from the light fraction,
256 separation techniques such as magnetic density separation, kinetic gravity separation, and Eddy current
257 separation are frequently employed. A final thermal treatment step to stabilize inorganic compounds and
258 destroy organic contaminants could be suitable. However, due to high costs, such as in the case of vitrification
259 by re-melting (1200-1400°C), they are hardly applied, although they can suite in post-processing of mineral
260 concentrates or stabilization in dedicated plants.

261 The recovery efficiency likely increases after size reduction steps and washing with water is suitable
262 for removing unwanted compounds such as easily soluble salts and sulfates. In fact, natural or accelerated
263 ageing and water washing are the most adopted treatment for MSWI residues.

264 2.3.1. Bottom ashes

265 MSWI-BA depending on the type of discharge system can usually be treated by wet or dry processing.
266 While the wet discharge is most adopted, leading to the production of typical quench products of MSWI-BA,
267 dry discharge systems are rare and technically demanding despite demonstrating some advantages like a
268 minor number of mineralogical phases formed and the low levels of corrosions and inter-mineral reaction
269 edges, thus higher recovery potential (Chandler et al., 1997; Eusden et al., 1999; Šyc et al., 2020). In the late
270 90's dry discharge technology raised limited interest likely because the recovery of secondary resources from
271 waste was believed to be a less critical issue.

272 Ageing, washing, and limited crushing are the key process for re-using MSWI-BA in the construction
273 industry. To further promote residue stabilization and reduce leaching, the addition of Al(+3) and Fe(+3) salts,
274 cement or other bonding agents during ageing is also used. The MSWI-BA treatment trains rely on physical-
275 mechanical treatments including density separation, sieving, sensor-based sorting, Eddy current separation,
276 and even hand-picking. The recovery of ferrous (FeF) and non-ferrous (n-FeF) metal fractions by Eddy current
277 separators is widespread (Smith et al., 2019), favoring the marketability of added-value streams as well as
278 BA acceptance at smelters or refiners. Dry technologies are more efficient than wet processes regarding
279 water consumption and, to some extent, reduced transport costs (due to reduced weight and volume).
280 However, the main drawback of dry processing is abundant dust formation (Šyc et al., 2020).

281 The first installation of MSWI-BA treatment plant came in 1995. Only two sieved fractions, fine
282 (<4mm) and coarse (4–45 mm), were designed to undergo stepwise magnetic and Eddy current separators
283 achieving average outputs of 36 wt. % FeF and 1.9 wt. % n-FeF (Chandler et al., 1997; Sabbas et al., 2003).
284 However, the total Fe content in FeF was only 20–30 wt. % due to agglomeration with other minerals. Similar
285 treatment trains built after the 2000s suited medium- to low-capacity MSWI plants and showed recovery
286 efficiency of around 80 wt. % FeF and 9–48 wt. % n-FeF with enhanced Fe concentrations and aluminium
287 products recovery (Grosso et al., 2011; Šyc et al., 2020). In countries like Switzerland and The Netherlands,
288 implementing the best available practices is mandatory by legislation (e.g., The Netherlands' Green Deal).

289 Regarding commercially available treatment methods, MSWI-BA are usually sieved using bar seizers,
290 trommel, vibrating screens, and flip flow screens (the latter only for wet treatments). In advanced plants,
291 tens of fractions can be sorted for enhanced metal recovery. However, sieving can be expensive with a water
292 content < 10 % because appropriate dust control during the handling of the material must be assured, and
293 the crushing changes the size distribution likely precluding residual fraction utilization where well-sorted
294 materials are required (Hyks and Hjelmar, 2018). Density separation is another effective method for the

295 recovery of different components, such as copper, gold, and brass showing a significant density contrast
296 compared to MSWI-BA matrix (2700 kg/m³). Density separation does not apply for Al recovery because its
297 density resembles that of bulk MSWI-BA, so it is preferably recovered using magnetic methods. Belt and drum
298 are the two main devices commercially available. Multi-step magnetic separation is typically used for sieved
299 fractions in advanced treatment plants before the Eddy current separation stage. Ballistic separation (patent
300 WO 2009/123452 A1) is a cutting-edge technology used in advanced dry recovery processes that
301 mechanically separates the fine particles (< 2 mm) associated with the moisture content. This device can
302 couple with conventional dry separation processes, improving performance. Sensors-based separation
303 technologies in MSWI residues processing are quite innovative and mainly used for separating glass and
304 metal particles (Bunge, 2018). Among these, magnetic induction separation based on electromagnetic
305 sensors is capable of identifying types of metals and alloys in the fraction coarser than 4 mm. Other types of
306 sensors include X-ray fluorescence to detect different metals, optical sensors for distinguishing shapes,
307 colored or transparent materials. Still, they are rarely applied to MSWI residues processing.

308 The most used processing options are dry technologies tailored for wet discharged MSWI-BA (e.g.,
309 Holm and Simon, 2017), even though dry discharge is experiencing a renaissance tied to its ability to avoid or
310 minimise the negative effects of the formation of reaction by-products after quenching, mainly credited by
311 Ca(OH)₂, CaCO₃, Friedel's salt and hydrocalumite (Inkaew et al., 2016). The KEZO MSWI plant in Switzerland
312 is one example of dry treatment of dry discharged MSWI-BA that yields around 10 % FeF, 4.5 % n-FeF, and
313 1.1 % glass, generating a total revenue of 95 CHF/t of dry MSWI-BA with a total consumption of about 16
314 kWh/t of treated waste (Böni and Morf, 2018). However, efficient recovery of the heavy n-FeF can increase
315 the revenues due to its precious metals content. Notably, the fraction with particles < 0.3 mm is sold without
316 treatment at a likely depreciated value despite a potentially significant content of marketable metals.

317 On the other hand, fervor is on the development of wet technologies for the treatment of MSWI-BA
318 that, however, implies a massive use of water as a primary limiting factor. Ageing is typically not included in
319 the treatment to avoid a detrimental effect from the formation of mineral coatings. The first wet technology
320 pilot plant for metal recovery came in 2005 in Amsterdam, The Netherlands. The treatment plant allows to
321 recover inert granulates for building materials and marketable metal fractions of different levels of purity,
322 equipped with several wet processing stages such as wet gravity separator, the wet eddy current separator,
323 and the wet magnetic separator (Muchova et al., 2009). Although a recovery efficiency up to 83 % FeF and
324 73 % n-FeF, the plant never went to full scale, mainly due to the high water-demand and costs for water
325 treatment. Another example is the Brantner&Co. plant in operation since 2013, located on an Austrian landfill
326 site (Stockinger, 2018). With a treatment capacity of about 40,000 Mg/year of MSWI-BA, it counts on two-
327 step magnetic separation, including overbelt magnets, separating iron scraps, and fine (>50mm) and large
328 (<50 mm) fractions. A wet jig further separates material streams by density: a fraction of carbon-based

329 materials and floating plastics, the heavy (density < 4,000 kg/m³) n-FeF containing copper, brass, stainless
330 steel, and precious metals, and the light n-FeF mainly composed of Al-bearing materials. One wet technology,
331 installed in 2016 in Alkmaar, The Netherlands, and first developed by the Boskalis Company in response to
332 the Netherlands' Green Deal, has a treatment capacity of about 240,000 Mg/year of MSWI-BA. This
333 technology separates different fractions using dry sieving instead of a wet drum sieve, and then each fraction
334 is washed to remove soluble salts and metals. A bar sizer separates fine (>40mm) and large (<40mm)
335 particles, followed by magnetic separation for large particles, which, in turn, removes iron scrap and stainless
336 steel as a first value-added material. The fine particles fraction undergoes a wet drum sieve and a vibrating
337 screen. However, the main drawback is the production of large amounts of sludge with a high concentration
338 of heavy metals according to the mass balance of the Alkmaar plant (Born, 2018).

339 Metal recovery can occur on-site, preferably at big MSWI plants where the flow of residues can justify
340 the investment leveraging on transportation costs. Another option is to establish centralized or mobile
341 treatment plants serving several MSWI plants, but they usually demonstrate lower efficiency than on-site
342 plants (Šyc et al., 2020). Nowadays, seven MSWI plants implemented with dry extraction system for MSWI-
343 BA are operational in Europe (5 plants in Switzerland and 2 plants in Italy). The main drawbacks of this
344 technology are tied to the need for further treatments to allow afteruse of MSWI-BA in the construction
345 industry and control or better recover the finest fractions that must be safely managed (Böni and Morf, 2018).
346 Especially the numerous stages of crushing lead to abundant dust formation and unfavorable grain-size
347 distribution curve for residue's reuse in the production of building materials. The high investment required
348 for upgraded treatment plants stems from the demanding crushing stages, the presence of multi-step
349 magnetic separation, and sensor-based sorting systems.

350 Each treatment plant is unique, although the processing methods can be the same. The recovery rate
351 increases with the number of recovery devices: more than ten eddy-current separators can be used in series,
352 still influencing the capital costs. According to the 2018 technical report of European Integrated Pollution
353 Prevention and Control Bureau, the electricity consumption of MSWI treatment plants averages 3 kWh/t of
354 treated waste, sometimes reaching up to 15 kWh/t (EIPPCB, 2018).

355 2.3.2. Fly ashes

356 Disposal of MSWI-FA through backfilling also after packaging in "big bags" made of a resistant
357 material is viable underground in natural cavities such as salt mines. The most used option is landfilling after
358 an appropriate treatment such as stabilization or solidification using other types of wastes (e.g., co-landfilling
359 with red mud) or binders (Quina et al., 2018; 2008). The stabilization processes suffer, however, some
360 limitations such as increased mass and volumes that may result in unsustainable space demand. Landfilling
361 after thermal stabilization (vitrification, sintering, thermal treatment with mixed wastes) is widely used as it
362 can reduce leaching of inorganic pollutants and destroy toxic organic components. However, LCA analysis

363 demonstrated that thermal treatments of MSWI-FA are uneconomic due to the high energy demand to
364 achieve suitable treatment temperatures (Fruegaard et al., 2010). The poor magnetic susceptibility of MSWI-
365 FA compared to MSWI-BA, due to lower concentration of Fe and magnetic minerals (Funari et al., 2020),
366 prevents the use and scalability of magnetic separation methods for material upgrading and FeF and n-FeF
367 recovery that is rarely attempted.

368 Decontamination/detoxification is the first pathway towards recovery and recycling of MSWI-FA as
369 secondary raw material for other applications avoiding landfilling (e.g., reuse for preparation of geopolymers;
370 Sun et al., 2013). Different methods can be performed, such as carbonation (Costa et al., 2007; Wang et al.,
371 2010), washing, leaching or bioleaching (Benassi et al., 2016; Funari et al., 2017), electrodialysis (Parés Viader
372 et al., 2017), and mechanical methods, e.g., ball milling (Chen et al., 2016). MSWI-FA for the production of
373 secondary raw materials is well suited for ceramic materials, epoxy composites, glass-ceramics, zeolite-like
374 materials, low-cost stabilizers and buffering agents, lightweight aggregates and secondary building materials
375 for geotechnical applications, adsorbents including high capacity materials for energy storage (Quina et al.,
376 2018 and reference therein). Other practical applications include biogas production, CO₂ sequestration
377 (Baciocchi et al., 2010), filler for embankment and landfill top cover (Brännvall and Kumpiene, 2016). The
378 primary aim of MSWI-FA washing is the removal of easily soluble salts to improve
379 decontamination/detoxification treatments. The Solvay Process developed during the 1860s is extensively
380 used to recycle sodium chloride from MSWI-FA produced by wet or semi-dry APC system (Chandler et al.,
381 1997), especially from FA collected at the sodic bag filters. Recently, Stena Recycling A/S developed the
382 HALOSEP® process to remove and recover chlorine salts (mostly CaCl₂) and a concentrate metal cake from
383 MSWI-FA. The key application for these salt products is road de-icing in compliance with the criteria CEN TC
384 337 WG1. The metal filter cake shows an average concentration of around 38-40 wt. % Zn, so it is particularly
385 suited for Zn recovery at the smelter.

386 Several efforts have been made in recent years for metal recovery from MSWI-FA with commercial
387 potential, such as Zn (Fellner et al., 2015), P (Kalmykova et al., 2013), Cu, and other precious and rare metals
388 (Morf et al., 2013; Allegrini et al., 2014; Funari et al., 2016). The removal or stabilization of hazardous
389 substances using traditional robust means such as water washing or co-landfilling is preferred over methods
390 aiming at metal recovery so that, for example, bioleaching and electrocoagulation or eventual landfill mining
391 strategies are still far from industrial rollout. However, some successful examples exist at the demonstration
392 scale such as co-mixing with rice husks (Benassi et al. 2016) to recover an environmentally compatible
393 secondary raw material. The FLUWA process dedicated to recovering Cu and volatile toxic metals such as Zn,
394 Pb, Cd, and organic substances started in 1997 in Switzerland (Bühler and Schlumberger, 2010). It further
395 allows metal separation and recovery through multistep acidic and neutral scrubbing and oxidation,
396 providing a residual MSW-FA less prone to environmental leaching. Organic substances remaining in the filter

397 cakes again represent a key issue, requiring further incineration cycle in the MSWI plant for complete thermal
398 destruction. The new FLUWA + FLUREC process allows up to 60–80% Zn, 80–95% Cd and 50–85% Pb and Cu
399 removal (Quina et al., 2018). The FLUREC implemented in 2012 at MSWI plant Zuchwil, Switzerland, can
400 recover up to 300 Mg/year Zn; however, purification of Zn-rich cake and filtrates is a prerequisite.

401 The use of MSWI-FA for the production of cement is sought because this can limit the enormous
402 environmental impact of the cement industry tied to massive anthropogenic CO₂ (from calcination) and other
403 gaseous emissions (NO_x, organic compounds, and toxic volatile elements), the consumption of energy and
404 natural resources (Lederer et al., 2017). The main options for MSWI-FA reutilization in the cement industry
405 include the production of blended cement and the co-processing in the cement kiln to produce the clinker
406 (e.g., Bertolini et al., 2004). Co-processing of MSWI-FA containing high amounts of Ca-bearing phases is viable
407 to substitute a part of the raw material input (Chandler et al., 1997) up to about 40-50% (Saikia et al., 2015).
408 Earlier studies suggested that the leaching rates of potentially toxic elements are very low in the short term.
409 However, Lederer et al. (2017) surmised that volatile metals such as Cd and Pb are reincorporated into the
410 cement during the regular production process. Considering that the chemical composition of MSWI-FA is not
411 stable over time, particular care should be given to the final cement quality and the emissions at the
412 smokestacks.

413 3. Biotechnology for MSWI ash management

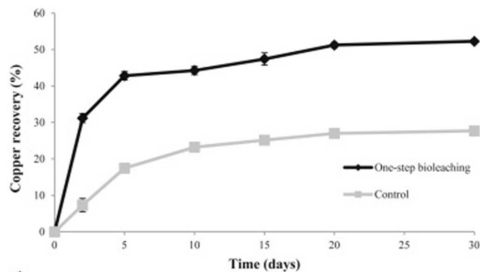
414 3.1. Brief Overview of Biohydrometallurgy

415 Biohydrometallurgy is a branch of metallurgy devoted to hydrometallurgical extraction mediated by
416 microorganisms. Its use in the recovery of metal from primary ores and in the treatment of mine tailings is
417 factual while gaining more and more popularity in treating secondary resources.

418 Extremophile microorganisms adapted to thrive under extreme environmental conditions (e.g.
419 salinity, pH, temperature). In particular, acidophilic bacteria are able to grow, for example, in acid mine
420 drainage (AMD) at low pH (< 2), high concentrations of sulfate and metals, particularly iron, giving it a deep
421 red color. Microbiological studies conducted on Rio Tinto water streams demonstrated the occurrence of
422 precise ecological niches of microbes. It is also not rare to identify new species of microorganisms. Their
423 metabolism evolved towards the use of available nutrients (e.g., metals) contained in solid minerals (e.g.,
424 pyrites) for their energy supply, enabling life in extreme environments. Minerals supposedly oxidize without
425 bacteria or biological interactions, but microorganisms make the process much faster. It was demonstrated
426 in the '70s that the oxidation of ferrous iron operated by *Acidithiobacillus ferrooxidans* was about a million
427 times faster than abiotic chemical oxidation (Lacey and Lawson, 1970). Strains of extremely thermophilic
428 archaea (*Acidianus sulfidivorans*) are found to withstand pH between 0.35 and 3.0, temperatures of 45-83°C
429 in the presence of sulfur minerals such as pyrite, chalcopyrite and arsenopyrite (Brierley and Brierley, 2013).

430 Recently, improved kinetics of biomineralization and biodissolution has also been studied for carbon capture
431 and storage e.g., by observing microbial carbonic anhydrase catalyses (Bhagat et al., 2018). Thermophilic
432 microorganisms can survive at higher temperatures than mesophiles and guarantee faster kinetics and higher
433 yields. However, the use of special catalysts can make the mesophiles exceptionally performing to bioleach
434 complex minerals. For example, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* strains
435 supplemented with ferrous iron are valuable for treating chalcopyrite or molybdenite (Brierley and Brierley,
436 2013). Empirical studies demonstrated that *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* reduce
437 sulfur accumulation and improve process efficiency, e.g., by enabling bioleaching for sphalerite and
438 arsenopyrite (e.g., Suzuki, 2001; Vera et al., 2013). Bioleaching and biooxidation processes promoted
439 sustainability in the recovery of base metals (Zn, Cu, Ni, Mo) and precious metals (Au, Ag) trapped inside
440 sulfur minerals.

441 The knowledge of usable microorganisms has significantly increased over the past decades, with
442 higher extraction rate of metals even from complex mineralogical assemblages like waste materials, as
443 demonstrated by the use of extremophiles (e.g., Ramanathan and Ting, 2016) , which is illustrated in Figure
444 3 for the kinetics and extent of recovery of metals via single-stage bioleaching. However, some criticalities
445 were promptly highlighted, such as the need to monitor bacterial growth and the difficulty in guaranteeing
446 the correct and stable functioning of the treatment plants over time. The development of corrosive
447 conditions inside the reactors evoked investments in special building materials, reactors and propellers
448 designs. It is necessary to continue contaminating knowledge in bioleaching by encouraging “among
449 scientists and engineers to enhance development of this very important technology for an industrial sector
450 whose successful future is increasingly dependent on technological advances”, as postulated by Brierley and
451 Brierley (2013).



* Copper recovery in one-step bioleaching(%) denotes the recovery obtained with the bacteria without subtracting the recovery obtained with the uninoculated media (control).

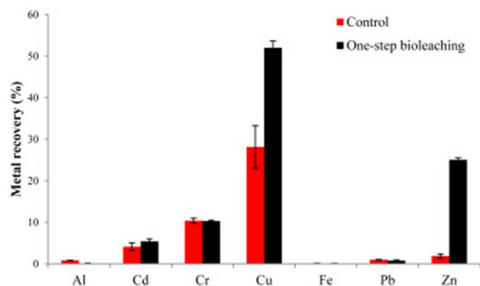


Figure 3. Kinetics of Cu bioleaching (top) and recovery of metals (bottom) from municipal solid waste incineration fly ash by *Alkalibacterium* sp. TRTYP6 ($n = 3$). Reproduced with permission from Elsevier (5493660184237) (Ramanathan and Ting, 2016).

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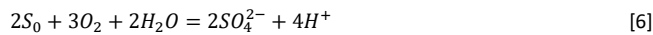
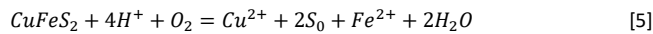
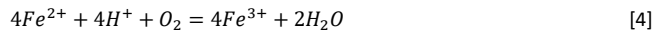
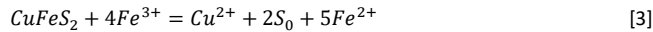
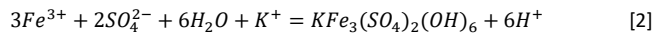
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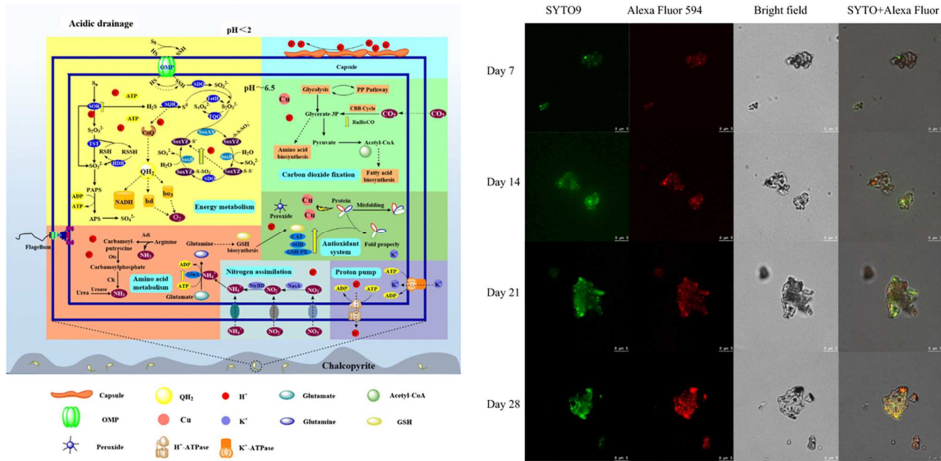
3.2. Bacterial leaching in sulfidic environments and their industrial applications

The first attempt to understand the mechanism behind bacterial leaching was studying the metal sulfides bioleaching reactions. This effort was accomplished with a multi-disciplinary approach, including mineralogy, chemical bound theory, and biochemistry. In sulfidic environments it is possible to find many microorganisms sulfur and iron oxidizers such as, *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Acidianus/Sulfolobus* spp., *Metallogenium* spp., that can operate direct and indirect leaching *sensu* Sand et al. (1995). Although debated (Sand et al., 2001; Vera et al., 2013; Yin et al., 2019), some co-participated reactions can be drawn:



469 Direct or contact bioleaching generally assumes a metal sulfide-attached cell oxidizes the mineral by
470 an enzyme system with oxygen to sulfate and metal cations. To dissolve a metal sulfide [Eq. 1] the indirect
471 or non-contact mechanisms grounds on the oxidizing capacity of Fe^{3+} ions. During this chemical reaction, Fe^{2+}
472 ions and elemental sulfur (S_0) are poly-sourced [Eq. 3, 5], promoting a cyclic reaction where Fe^{3+} and sulfide
473 moiety is reduced and oxidized progressively [Eq. 4, 6] thanks to an ancillary engine of S-oxidizers. It is worth
474 mentioning Extracellular Polymeric Substances (EPS) allow contact and mineral decomposition that
475 preferably start in crystal defects (Fletcher and Savage, 2013; Gehrke et al., 1998), where the Fe^{2+} ions are
476 more accessible (Dziurla et al., 1998). The need for iron is as important as the S-cycle: when the Fe^{3+} interacts
477 with the electronic structure and (leach) the surface of the mineral, and sulfur de-bonds from sulfide crystal
478 lattice, the thiosulfate releases Fe^{2+} ion and protonation forms H_2S , which reacts with the oxidative
479 compounds (e.g., Fe^{3+} , O_2). This starts a radical chain reaction that produces S_0 as an end-product, which is
480 used by bacteria (e.g., *Acidithiobacillus thiooxidans*) to produce sulfuric acid.

481 The regulatory strategies of *A. thiooxidans* during bioleaching of low-grade chalcopyrite were studied
482 in-depth by Yin et al. (2019), illustrated in Figure 4, through physiological observations matched with
483 transcriptomic approach. The authors observed that during the CuFeS_2 bioleaching process the bacterium
484 endeavor's three mechanisms to keep the pH homeostasis: i) externalizes H^+ by ATPase activity; ii) the amino
485 acid metabolism becomes more active lowering cytoplasmic acidification by proton consumption via the
486 tricarboxylic acid (TCA) cycle (i.e., Krebs cycle); iii) prevents proton invasion increasing the amount of
487 unsaturated fatty, particularly cyclopropane, and so far the density of the cell membrane. At the
488 transcriptomic level the genes involved in sulfur metabolism were significantly up-regulated while those
489 associated with the flagellar assembly and carbon metabolism were down-regulated, suggesting a strategy
490 of alternative energy production from the first and reduction of energy consumption with the second.
491 Noteworthy, confocal laser scanning microscopy (CLSM) analysis indicated that EPS and biofilm formation
492 might also improve strain resistance to the stress condition (Figure 4). Niu et al. (2016) studied a real-scale
493 bioleaching system of the Dexing Copper Mine (Jiangxi, China) to provide insights into the bacterial
494 community structure and mechanisms involved at three different processing stages. According to
495 phylogenetic analysis based on 16S rRNA metabarcoding, all three groups shared 259 OTUs (Operation
496 Taxonomic Unit), but demonstrated a significant microbial shift in the process line. Gene arrays revealed a
497 difference in functional gene structures of the microbial communities and metabolic pathways potentially
498 related to bioleaching. Genes involved in carbon fixation, polyphosphate degradation, sulfur oxidation, and
499 denitrification were abundant in a sample from the heap; while genes related to carbon degradation,
500 polyphosphate synthesis, sulfite reductase, and nitrification in the spent medium leachate (Niu et al., 2016).



501
 502 Figure 4. Adaptation mechanism model of *A. thiooxidans* in bioleaching of low-grade chalcopyrite (left); visualization of EPS and cell
 503 attachment on chalcopyrite surface when bioleaching at 7, 14, 21, and 28 days by CLSM (right). Reproduced with permission from
 504 Springer Nature (5493660654399) (Yin et al., 2019).

505 There is a long list of microorganisms used for sulfur-bearing ores bioleaching, but their adaptive
 506 mechanism to harsh environments remains disputed. Such acquired knowhow is of fundamental importance
 507 for creating strains with greater stress tolerance, crucial for commercial use in industrial bioleaching (Jerez,
 508 2008). A review of bacterial strains possessing unique characteristics critical for commercial-scale bio-
 509 processing is reported elsewhere (Brierley and Brierley, 2013). The first significant biohydrometallurgical
 510 operation took place at the Rio Tinto copper mine in Spain from 1950 to 1980, where bioleaching was
 511 primarily done in heaps and dumps on-site. Several industrial plants for metal recovery (especially Cu and
 512 Au) have been started in America, South Africa, Uganda, and Australia. The percentages of minerals extracted
 513 were very high: up to 95% Au was extracted from crude high-graded ores and Cu yields were up to 65% from
 514 chalcopyrite and up to 98% from some sulfosalts (enargite). Around 85% Mo bioleaching is achievable from
 515 molybdenite (MoS_2) using *A. ferrooxidans* and *L. ferrooxidans*, in a six-month timeframe (Bosecker 1997;
 516 Brombacher et al., 1998). After numerous developments, the BioCOP™ technology owned by BHP Billiton
 517 was commercialized at the Chuquicamata Mine in Chile, showing a production rate of 20000 Mg/year Cu
 518 using thermophilic microorganisms to leach sulfide mineral concentrates at temperatures up to 80 °C (Batty
 519 and Rorke, 2006). The BioCOP™ technology yields a high-value copper metal product after conventional
 520 solvent extraction and electrowinning.

521 In commercial applications, biohydrometallurgical methods' advantages can offset net smelter
 522 royalties for metals production. For example, mineral beneficiation through bioleaching can decrease
 523 refineries and smelters penalty charge associated with high levels of impurity (Gericke et al., 2009).
 524 Moreover, secondary bioleaching or spent medium leaching can be used for on-site acid bioproduction to

525 replace mineral acid purchases (e.g., Funari et al. 2017) although sulfuric acid costs can be volatile (Moore,
526 2008). Biohydrometallurgical methods can fit existing infrastructure, such as wet technologies or
527 electrorefining, avoiding new investments by the companies. Biomining processes are mostly carried out by
528 stirred-tank and heap reactors, or both combined, especially when a spent medium leaching process is
529 attempted. In general, they can be divided into irrigation-type and stirred tank-type, as the two main
530 categories. Irrigation-type processing is primarily deployed *in situ* (e.g., heap, dump, and slope bioleaching
531 techniques), being slope bioleaching more affordable compared to other techniques, while heap bioreactors
532 represent the primary option because they are cheaper and easier to operate than stirred-tank reactors
533 (Gahan et al., 2013; Rawlings, 2004). The latter, however, are less time-consuming and offer more control
534 and predictable performances. A typical heap bioleaching system operates over a 400–600-day period,
535 starting with the preconditioning phase of 1–6 weeks. In Chile, the Bala Ley plant for low-grade Cu minerals
536 ore processing equips a dump bioleaching system, where cycles of preconditioning, irrigation, maturation,
537 and washing can last years (Rawlings, 2002). Several (hybrid) irrigation-type methods were used to treat low-
538 grade uranium ore at the Denison Mine in Ontario, Canada. The primary problems associated with impending
539 leachate loss in the environment were addressed by these procedures (Bosecker, 1997; Rawlings, 2002; 2004;
540 Rawlings et al., 2003). In South Africa and Australia, pilot-scale plants have demonstrated technical feasibility
541 for Ni recovery, with Queensland Nickel as a relevant stakeholder (Gahan et al., 2013). Similarly, the
542 Talvivaara mine in Finland tested heap bioleaching, with operations likely terminated in 2018. A famous
543 example of biooxidation plant in stirred tank reactors is at the Fairview Mine in South Africa, which used the
544 BIOX process for pretreatment of gold-bearing sulfide ores (Kaksonen et al., 2014). Bioleaching as a
545 pretreatment in a multi-stage process increased recovery efficiency, especially for extraction of precious
546 metals and Co. Gu et al. (2018) provide a new list of pretreatment methods based on bioleaching. BacTech
547 Mining Company, Canada, can treat refractory Au concentrates with the further aim of recovering Co, Ni, and
548 Ag, and remediating As-tailings (Rawlings et al., 2003; Gahan et al., 2013). Biooxidation plants equipped with
549 stirred tank reactors to recover Co from enriched mining waste and tailings can be found at Sansu, Ghana,
550 Liazhou, Shandong province, China, the Kasese Cobalt Kilembe Mine, Uganda, and Youanmi Gold Mine,
551 Australia. The latter (Youanmi project) exploits some thermophilic bacteria possessing an optimum
552 temperature between 45 °C and 55 °C (e.g., *Sulfobacillus thermosulfidooxidans*). Numerous pilot and
553 demonstration-scale processes of stirred tank bioleaching prove the potential for recovering other metals
554 from sulfides, including Ni, Co, Zn, and rare metals. Patented processes of Zn bioleaching involving solvent
555 extraction and electrowinning are also available (Steemson et al., 1994).

556 3.3. Biohydrometallurgical processes for the circular economy

557 In order to promote a circular economy, biotechnology for metal production should be eco-friendly
558 and cost-effective and adapt to the waste management sector. The continuously increasing high demand for

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559 critical raw materials and rare metals for technological development has led not only Europe, but also other
560 industrialized countries, to look at diversified sources of supply such as mining waste, mine tailings, and
561 alternate anthropogenic stocks and flows that frequently exhibit a hidden metal value (Baccini and Brunner,
562 2012). Biomining is suitable for treating such materials because they are flexible for optimization and can
563 prove beneficial to decontamination, coupling metal recovery with environmental remediation. This has led
564 to recent advances in fine-tuned methods to treat anthropogenic wastes. Despite the modest and variable
565 ore metal concentrations in anthropogenic flows like MSWI residues, bioleaching methods can allege lower
566 capital costs than other robust technologies in waste management (Funari et al., 2017).

567 Bioleaching for metal extraction from anthropogenic materials such as electronic scraps, various
568 types of slag and flying ashes, secondary solid wastes, and sludge is largely investigated as an economical and
569 eco-friendly process (Gahan et al., 2013; Meawad et al., 2010; Lee and Pandey, 2012; Srichandan et al., 2019).
570 Bioleaching of metals through the use of thermophilic and acidophilic bacteria has been primarily
571 investigated to recover metals from electronic scrap, especially printed circuit boards (Ilyas et al., 2007).
572 Among the strategies adopted to achieve higher speeds of metal leaching from electronic waste, it was found
573 that a mixed consortium can show the maximum efficiency of leaching, and a pre-washing might be useful
574 to remove easily soluble metals (e.g., Cl, Na) or light fractions (e.g., plastics) toxic for bacteria. Satisfactory
575 leaching yields are achievable with *S. thermosulfidoxidans*, an example of moderate thermophilic bacteria,
576 but the presence of Pb and Sn precipitation complicated separation and purification (Ilyas et al., 2007).
577 Recently, Becci et al. (2020) confirmed that pre-crushing to obtain a granulometry > 0.5 mm is a good strategy
578 to enhance bioleaching of printed circuit boards using iron oxidizers. However, the formation of passivation
579 layers (e.g., jarosite) remains a limiting factor that reduces kinetics dramatically. In their experiments,
580 bioleaching processes using monoclonal cultures of *A. ferrooxidans* and *L. ferrooxidans* were compared,
581 emphasising oxidation of iron species. The latter microorganisms are very sensitive to metals toxicity and
582 perform a slow conversion of Fe^{2+} in Fe^{3+} , resulting in relatively low recovery of around 40% Cu and 20% Zn,
583 while bioleaching with *A. ferrooxidans* yielded around 95% Cu and 70% Zn and showed high conversion of
584 Fe^{3+} . Further comparing different scenarios in terms of carbon footprint (Figure 5), they found the optimum
585 condition with further bioreactor size reduction can achieve four times reduction of the CO_2 -eq per kilogram
586 of treated material compared to the best bioleaching processes reported in the literature (Becci et al., 2020).

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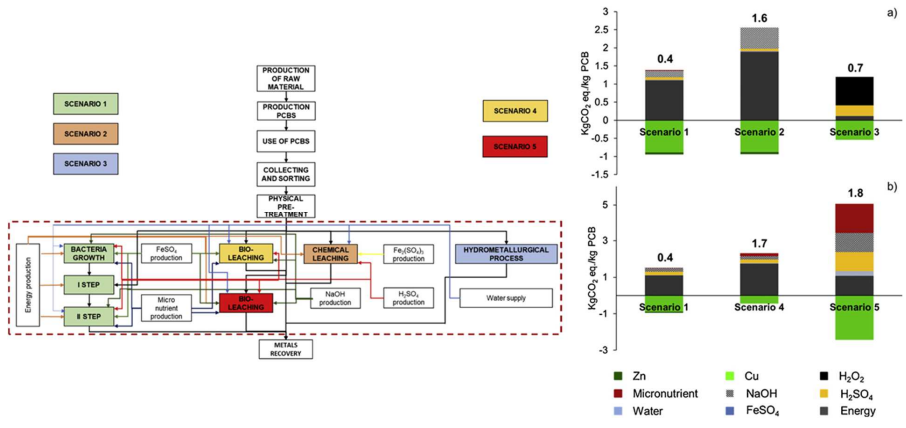


Figure 5. System boundaries considered for the carbon footprint assessment (left); carbon footprint of the five considered approaches (right). (Function unit: 1 kg of shredded PCBs). Reproduced with permission from Elsevier (5493661285952) (Becci et al., 2020).

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591 The ability of microorganisms to interact with Rare Earth Elements (REE) is suitable for metallurgical
592 separation and environmental technology. The microbial capacity to interact with REE and the REE adsorption
593 sites were investigated in depth using synchrotron-based techniques on genetically engineered strains
594 clarifying the REE adsorption mechanisms. Recognized patterns can be generalized: surface adsorption,
595 adsorption on extracellular biopolymers, cellular absorption, and adsorption on extracellular biominerals
596 (Moriwaki and Yamamoto, 2013). The binding sites of the bacterial cell wall suggested to interact the most
597 with REEs and determine the strain selectivity are phosphate and carboxyl groups. In the recovery of metals
598 such as Ni, Co, and Mo from spent catalysts low yield were reported for acidophilic bacteria, however, it
599 could be improved using *Escherichia coli* due to its capacity to produce reducing conditions even in acidic
600 environments (Vyas and Ting, 2020). Vyas and Ting (2020) reported that higher Mo extraction (from 72 to 96
601 %) was observed in the spent medium leaching when *E. coli* biomass was kept in contact with the pregnant
602 solutions in a two-step bioleaching procedure. The result suggests a possible biosorption or bioaccumulation
603 mechanism operated by *E. coli* using spent medium indicating a significant involvement of active metabolites
604 such as amino acids. Also, recent studies demonstrate that autochthonous bacteria can be present in wastes
605 and that they could be isolated and tailored for bioprocessing (Ramanathan and Ting, 2016) and
606 bioremediation (Pierivandi et al., 2020).

3.3.1. Bioleaching of MSWI residues

607 Bioleaching of MSWI residues mainly involves ferrous iron and sulfur-oxidizing bacteria (e.g., *A.*
608 *ferrooxidans*, *A. thiooxidans*), and several species of fungi (e.g., *Aspergillus niger*), which can grow on and
609 around the waste material. Despite numerous bench-scale experiments, no commercial processes for MSWI
610 residues bioleaching are available so far. Biohydrometallurgical processing of MSWI residues suffers
611

612 considerable limitations when a high pulp density is required to justify short to medium term investments.
613 The high content of toxic metals, organic materials, and a highly alkaline starting pH can impede microbial
614 growth and process efficiency. Various leaching organisms show a high tolerance to toxic metals (i.e., 50 g/l
615 Ni, 55 g/l Cu, 112 g/l Zn), but also the mineralogical composition is of primary importance: a high calcium
616 carbonate content, such as for several types of MSWI-FA, would be unfavorable because high alkalinity and
617 the precipitation of gypsum can occur, affecting the overall costs.

618 Bioleaching can extract valuable metals, especially Cu, Zn, Pb, As, Sb, Ni, Mo, Au, Ag and Co, from
619 MSWI residues with less energy and environmental impact than pyro- or hydro-metallurgical methods.
620 Ishigaki et al. (2005) studied the bioleaching of Cr, Cu, Zn, Cd, and As from MSWI-FA by sulfur-oxidizing and
621 iron-oxidizing bacteria, as pure cultures and a mixture of both. The mixed culture showed the best
622 performance (67% Cu, 78% Zn and 100% for Cr and Cd at 1 % w/v pulp density). Characterization of metal
623 bioleaching revealed that the acidic and oxidizing conditions remained stable throughout the experiment.
624 The redox mechanisms coupled with the sulfate leaching brought an increase of ferrous iron enhancing the
625 Cr, Cu, and As leaching. However, they found that at a higher pulp density (3 % w/v) chromium remained
626 virtually undissolved (4 % Cr yield). The presence of degradable and non-degradable organic compounds in
627 MSWI residues exerted no significant changes in the leachability of metals other than Zn (Ishigaki et al., 2005).
628 An earlier study on microbial leaching (Mercier et al., 1999) elucidated that the following elements can be
629 removed in decreasing order of extraction rate: Cd, Zn, Pb, Cu, using a pure culture of *A. ferrooxidans*. In the
630 same work, four different leaching tests were conducted for environmental compliance of the final residues,
631 and the authors concluded that the leachate from Toxicity Characteristic Leaching Procedure (TCLP) was
632 within the acceptance criteria only if the final residue's pH was increased to five after the biological
633 treatment. Still, Cd releases could be an issue concerning regulatory limits. The investigation by Krebs (2001)
634 reported an example of co-treatments of MSWI-FA using a mixture of strains (*Thiobacillus* genus) in a
635 suspension of water and nutrients (1 % w/v S_0 powder) and 4 % w/v sewage sludge. The cultivation was
636 compared to pure *A. thiooxidans* or sewage sludge alone, over 1-3 months. The inoculation with the
637 combination of sewage sludge and bacteria showed a fast decrease in pH and increased microbial growth. In
638 the final pH of 1, the efficiency of metal leaching was very similar, with pulp density ranging from 0.5 % to 1
639 %. More than 80 % Cd, Cu, and Zn, around 60 % Al, up to 30 % Ni and Fe, less than 10% Cr and Pb were
640 mobilized (Krebs, 2001). Autochthonous bacteria can be used for bioleaching as reported in a study on MSWI-
641 FA produced in Singapore incineration plant (Ramanathan and Ting, 2016). Thirty-eight different microbes
642 were isolated and characterized to find the most suitable autochthonous microbe with inherent fly ash
643 tolerance and ability to thrive in alkaline pH (thus avoiding any pre-acidification of the ash). Besides
644 *Firmicutes* (90 % relative abundance), three other phyla were identified: *Bacteroidetes*, *Actinobacteria*, and
645 *α-Proteobacteria*. Among six isolates displaying Cu recovery of about 20% or more, *Alkalibacterium* sp. was

646 tolerant to pH and fly ash, making it a suitable candidate for MSWI-FA bioleaching. Indeed, a one-step
647 bioleaching with *Alkalibacterium sp.* on MSWI-FA showed 52% Cu and 25% Zn recovery. The high tolerance
648 of *Alkalibacterium sp.* to metals and substantial bioleaching ability can prompt scaled-up bioleaching with
649 alkaline bacteria that, at present, do not reach acidophile bioleaching in terms of Cu and Zn removal rates. A
650 clear advantage of alkaline bioleaching is the higher pulp density (more than 20% w/v), signifying less water-
651 demand. Hong et al. (2000) tested saponin, a biosurfactant produced by microorganisms and plants, for
652 metal removal from MSWI-FA. They compared the efficiency of saponins with that of other solvent extraction
653 (HCl and EDTA) in the pH range 4-9. The saponins leaching was more effective than control acid treatments
654 for Cr, Cu, and Pb with yields up to 45 %, 60 % and 100 %, respectively, whereas the Fe, Si, Al, and Zn
655 extraction was not significant. Gonzalez et al. (2017) used cementing bacteria to stabilise MSWI-FA. After
656 bacteria cementation assays and the assessment of the adsorption of metals in the cemented fly ash, they
657 concluded that *Sporosarcina pasteurii* and *Myxococcus xanthus* are suitable for multiple metal stabilization
658 (As, Cd, Cr, Cu, Ni, Pb, Sn, and Zn) with some differences concerning trace elements mobility, depending on
659 the starting concentrations in the samples (Gonzalez et al., 2017).

660 MSWI-BA bioleaching has received less attention in the scientific literature because of its less
661 hazardous nature than MSWI-FA, which allows for direct reuse in the construction sector with minimum
662 pretreatment. Aouad et al. (2008) studied *Pseudomonas aeruginosa* and MSWI-BA interactions foreseeing
663 real exposition of MSWI residues to halotolerant bacteria at landfill site. Bioleaching experiments using a
664 pure culture of *P. aeruginosa* was carried out for 133 days at 25 °C using a modified Soxhlet's device and a
665 culture medium in a closed, unstirred system and resulted in an increase in pH, a greater immobilization of
666 Pb, Ni and Zn, and weaker alteration rate of treated MSWI-BA compared to the abiotic control. The authors
667 explained that the biofilms acted as a protective barrier, thus preventing dissolution by promoting
668 biomineralization (Aouad et al., 2008). Many halotolerant bacteria can be found at MSWI disposal site since
669 tolerating the salinity of MSWI residues, but little information is available about the interaction between
670 bacteria and landfill waste (Sun et al., 2016). *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* as the dominant
671 phyla, with dominant genera as *Halanaerobium*, *Lactococcus*, *Methylohalobius*, *Ignatzschineria*,
672 *Syntrophomonas*, *Fastidiosipila*, and *Spirochaeta* are characteristic in municipal waste landfills (Wang et al.,
673 2017)

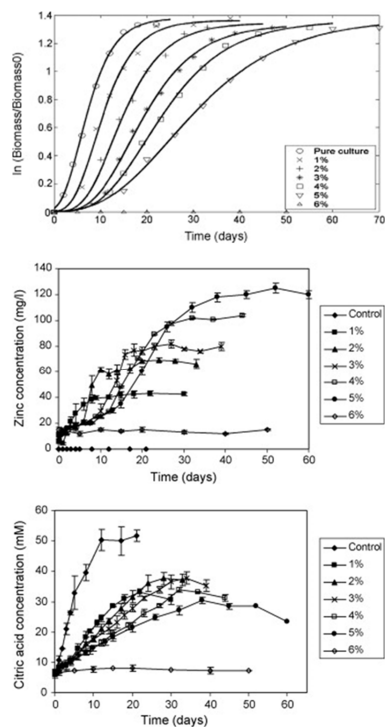
674 The acid bioleaching behavior of both MSWI FA and MSWI-BA was investigated at the bench scale
675 and compared to abiotic leaching (H₂SO₄, 10% pulp density, 30 °C, 150 rpm) looking at a wide range of metals
676 (Funari et al., 2017). A mixed acidophilic culture composed of iron and sulfur-oxidizing bacteria tested on a
677 one-step bioleaching process yielded >85% Cu, Al, Mn, Mg, and Zn and significant removals of Co, Cr, Pb, Sb,
678 and REE. Unvalued elements like Ca, Si, and Ti showed low mobility with the tendency to remain in the solid
679 phase, while the solubility of other trace elements might be selectively enhanced by the cyclic supply of Fe⁺³

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680 produced by iron-oxidizing bacteria. Moving ahead, Mäkinen et al. (2019) tested the possibility of heap
681 bioleaching for the recovery of Zn and Cu from MSWI-BA at the bench scale via column experiments. Leaching
682 yields varied 18–53% Zn and 6–44% Cu, and they noted that appropriate aeration is the main critical factor
683 needing further adjustments in future testing. Potentially high Fe and Al, easily dissolved in sulfuric acid
684 solutions, and different heap behavior due to the heterogeneity of the material can also impede bioleaching
685 utilization in MSWI residues treatment. However, a balance between bacterial adaptive mechanisms and
686 nutrient supply can generate savings compared to processes relying on abiotic procedures. Electrochemical
687 technologies are also promising in the optimization of acidophile bioleaching for MSWI residues (Gomes et
688 al., 2020), possibly to offset the CO₂ generation of full-scale applications.

689 Fungi have considerable industrial importance in biomining. Several studies demonstrate the
690 applicability of a bioleaching process to MSWI residues using fungal metabolic substances and reactions.
691 However, data on a limited spectrum of genera are available (i.e., *Aspergillus* and *Penicillium*). The
692 bioleaching ability of *Aspergillus* has been primarily ascribed to metal dissolution by organic acid excretion
693 (e.g., citric acid). Bosshard et al. (1996) compared biological leaching of MSWI-FA by *Aspergillus niger* in batch
694 cultures 5% pulp density to chemical leaching, and they found that bioleaching was only slightly lower than
695 chemical leaching with commercial citric acid. They also noted that, in the presence of MSWI-FA, *A. niger*
696 produced gluconate, whereas, in its absence, citrate. Xu and Ting (2009) investigated the bioleaching kinetics
697 of *A. niger* with MSWI-FA at various pulp densities (1-6%) in a batch system; Figure 6 illustrates the key
698 results. A modified Gompertz model was used to evaluate growth and acid production by the fungus, while
699 a Monod inhibition model served to assess growth kinetics in the presence of toxic and inhibitory
700 components of the MSWI-FA. The metals present in the MSWI-FA at high concentrations acted as inhibitors,
701 decreasing the *A. niger* bioleaching yield. A gradual decrease of the fungal growth rate was observed with
702 the increase of the pulp density, likely in relation to the primary inhibitory mechanisms that include inhibition
703 of critical functional groups of enzymes, conformational changes of cell's polymers, and alteration of the
704 integrity of the cell membrane. Nonetheless, the acid excretion by the fungus played a direct role in metal
705 solubility (Al, Fe, and Zn) since the concentration of organic acid increases with biomass and time during
706 fungal leaching of MSWI-FA (Xu and Ting, 2009). The optimal MSWI-FA concentration for fungal leaching is
707 up to 10 % (w/v) in the medium (Bosshard et al., 1996). Yang et al. (2009a) reported bioleaching experiments
708 of MSWI-FA by using single-metal adapted, multi-metal adapted, un-adapted *A. niger*. The effect of pH and
709 concentration of the extracted metals on the fungus growth was evaluated by comparing the diameter of the
710 fungal colonies. The authors found that multi-metal adapted AS3.879 can tolerate the greatest pulp densities
711 and the Al-adapted strain AS3.879 is the best candidate for MSWI-FA decontamination according to the TCLP
712 test on final residues (Yang et al., 2009a). The biosorption of metals in their ionic form operated by *A. niger*
713 was further elucidated contacting MSWI-FA leachate made from gluconic acid leaching and the fungus for

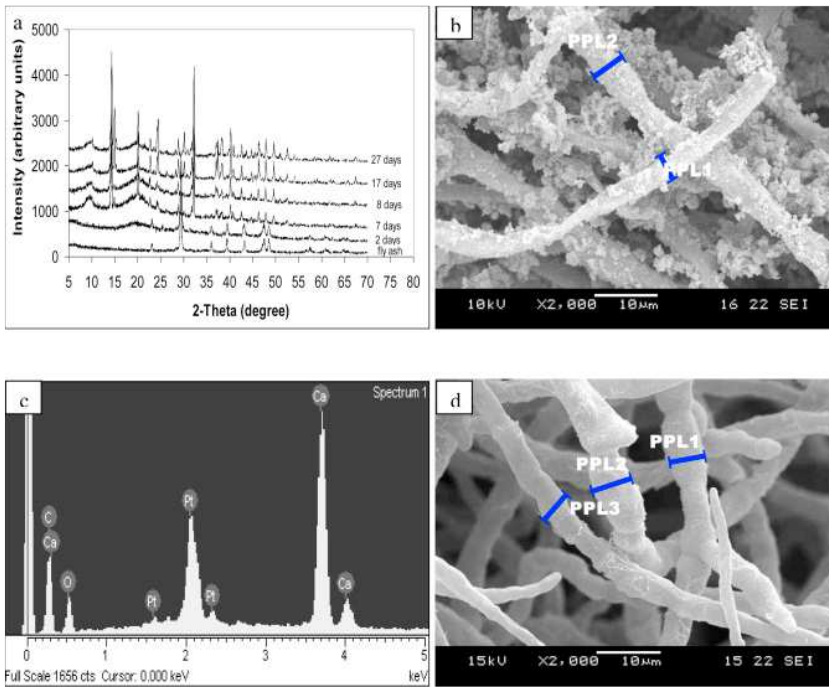
714 120 minutes at 6.5 pH: Al, Fe and Zn fitted a pseudo-first-order kinetic and Pb a pseudo-second kinetic;
 715 regarding the isotherm models, Pb, Zn and Fe fitted the Langmuir model, while Al Freundlich's (Yang et al.,
 716 2009). Moreover, microscope observations revealed that fungal morphology was significantly affected during
 717 both one-step and two-step bioleaching, with precipitation of calcium oxalate hydrate crystals at the surface
 718 of hyphae (Xu et al., 2014), as illustrated in Figure 7, leading to noteworthy implications for after-use. Metal
 719 richness in solution or contact surface can be toxic to microorganisms, but finely tuned pre-treatment and
 720 adaptation strategies would overcome this limitation in industrial bioprocessing. For example, water washing
 721 pre-treatment of MSWI-FA was simultaneously evaluated in both one-step and two-step bioleaching
 722 procedures using *A. niger* (Wang et al., 2009). The results (under optimum pulp density of 1% w/v) showed
 723 that the fungi lag phase (i.e., the timeframe of steady pH level and after which the pH starts to drop quickly)
 724 in the absence of pre-treatment lasts about 260 hours, while less than 150 hours if water washing is deployed
 725 (yielding 96% Cd, 91% Mn, 73% Pb, 68% Zn, 35% Cr, 30% Fe at the end of the experiment; Wang et al., 2009).
 726 Water washing pre-treatment improves the production of organic acids thanks to partial removal of other
 727 components, leading to a reduction of the experiment duration and overall costs.



728

729 Figure 6. Growth of *A. niger* in the presence of fly ash: solid lines are Modified Gompertz model, and no growth was seen at 6%
 730 pulp density (top). Effect of fly ash pulp density (1–6%, w/v) on: the bioleaching of zinc (middle); the production of citric acid
 731 (bottom). Reproduced with permission from Elsevier (5493670696531) (Xu and Ting, 2009).

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732
 733 Figure 7. (a) Undefined XRD analyses of the fungal pellet in two-step bioleaching confirming the formation of calcium oxalate
 734 crystals and dissolution of fly ash particles from Day 7; (b) micrograph of the fungal pellet in two-step bioleaching on Day 7 showing
 735 small particles on the hyphae and increased diameter of hyphae (PPL1 = 4.45 mm and PPL2 = 5.97mm); (c) EDX analyses of the
 736 fungal pellet in two-step bioleaching at Day 7 confirming the presence of calcium oxalate crystals on the fungal surface;
 737 (d) surface of the fungal pellet in two-step bioleaching at Day 8 showing abnormally short, swollen and high-branched hyphae (PPL1 = 5.59
 738 mm, PPL2 = 6.32 mm and PPL3 = 5.32 mm). Reproduced with permission from Elsevier (5493670100485) (Xu et al., 2014).

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739 4. Perspectives and Future Developments

740 5. 4.1 Challenges and Opportunities

741 Bioleaching applied to metal recovery and other biomining operations (e.g., biooxidation for mineral
 742 beneficiation, bioremediation of mining waste) can be considered more environmentally friendly than
 743 traditional methods. The application of bioleaching, which often refers to mimicking natural processes, is one
 744 of the most prominent methods capable of balancing environmental and economic costs in the waste
 745 management sector.

746 MSWI residues are mineral assemblages resulting from an elaborated petrogenesis and, thus, after
 747 appropriate metallurgical treatments, can fit many potential after-uses. MSWI urban ores show metal

748 concentrations equivalent to low-grade primary ores (Funari et al., 2015). Therefore, urban mining can be
749 affordable. Critical raw materials and strategic elements for green and high-tech applications can be
750 recovered from anthropogenic resources like MSWI residues which are an ever-present flow of loose material
751 and not natural ores to be drilled/crushed to the detriment of the environment. Many countries with limited
752 mineral reserves could find urban mines a compelling resource supply and income option.

753 Biotechnologies, with their relatively low running costs and capital, have a central role in the supply
754 of raw materials and eco-friendly alternatives. It is ideal for remediation and metal recovery from legacy sites
755 and other mining wastes, even and especially in developing countries (Acevedo, 2002). Some strength points
756 of MSWI residues bioleaching have been elucidated after about twenty years of laboratory testing at different
757 scales (Rawlings, 2002; Hennebel et al., 2015; Srichandan et al., 2019; Gomes et al., 2020), such as, less energy
758 and solvent consumption, high boosting potential, easiness to suite existing infrastructures, etc. However,
759 bioleaching of MSWI BA and FA results slower than chemical leaching, and the state-of-the-art highlights the
760 need for improvements concerning dissolution kinetics. For industrial rollout, this limiting factor makes the
761 sole bioprocessing unaffordable and less appealing for MSWI plants than aggressive acid extractions or
762 energy-demanding physical methods.

763 Safety measures must be continually adapted to the desired technology, and fundamental
764 information from basic research is required for process development. Using strains from lab collections and
765 indigenous uncultured strains or mixtures from the natural environments may overcome some limitations,
766 such as the long reaction times to obtain satisfactory yields. As a fact, it became clearer that mixed cultures
767 instead of monoclonal strain showed synergistic effects, favoring biomass growth against heavy metals
768 inhibition and maintaining a reasonable trade-off between microbial community succession and their energy
769 type metabolisms. The use of nutrients, e.g., iron and sulfur for acidophilic bacteria or organic sources for
770 fungi and cyanobacteria, and the mineral acids/bases to maintain a predetermined pH setpoint can increase
771 overall processing costs (Srichandan et al., 2019). Moreover, each microorganism must be adapted to the
772 waste material to be treated as its resistance might strongly depend on the heterogeneity the matrix and
773 standard pH. As in the case of alkaline wastes, such as MSWI ashes, where acidophile cultures can be
774 unaffordable in bioleaching, additional data on alkaline bioleaching or fungal bioleaching is required.

775 Reducing carbon footprint could receive attention and leverage the development of bioleaching to
776 mitigate climate change. For example, carbon sequestration and accelerated carbonation of alkaline wastes
777 mediated by microorganisms are promising (Mayes et al., 2018; Gomes et al., 2020). Further opportunities
778 are represented by i) fine-tuned bioleaching (enhanced selectivity for specifically chased metals as Li, Co, Cu,
779 REE); ii) microbial recovery cell (consisting in a combination of galvanic leaching and bioleaching). In relation
780 to the last case, an electrodynamic in-situ bioleaching can be tested expecting great results. Several authors
781 argued that some magnetic separates from MSWI residues via electrodynamic fragmentation (Bluhm et al.,

2000; Seifert et al., 2013), can suite as ideal substrate material (e.g., Panda, 2020) which, at some point, could effortlessly combine to electro-dialytic bioleaching. In Europe, metal recovery exploiting ferrous fraction separation has been valued at 60-100 € per ton (of MSWI-BA), while the economic value of the non-ferrous fraction is significantly higher (Šyc et al., 2020). Interestingly, the ferrous metal fraction >3/4 mm is still virtually unemployed, although it could contain a significant amount of precious metals (Muchova et al., 2009; Holm and Simon, 2017). Since they contain many impurities, these separated by-products are generally sold to a third party at low cost. Again, fine-tuned bioprocessing can enter the treatment chains if sufficient trials are available to achieve better market values. New contractors for MSWI residues bioprocessing indeed produces new job and business models which, in turn, aside from economic feasibility, depends on:

- i. geographic location
- ii. desired final quality of recovery
- iii. throughput (i.e., large vs small MSWI plants)
- iv. type of MSWI residue (e.g., MSWI-BA vs MSWI-FA, quenched MSWI-BA vs dry MSWI-BA)
- v. type of treatment plant (e.g., on-site, at the landfill, mobile processing plant)
- vi. space requirements
- vii. proposed technology (e.g., one step vs multistep bioleaching)
- viii. management options for end-products (landfilling vs inert re-use)

4.2 Future research

The reproducibility of MSWI ashes bioprocessing is uncertain due to the lack of pilot-scale treatment results and considering the significant diversity and obsolescence (lifespan of 20-30 years) of the technologies used in MSWI management and municipal waste feed heterogeneity. During prototyping phase the assessment of biological hazards via standardized tests must be completely developed and adapted to the proposed technologies. BA and FA contain hazardous substances, such as mobile harmful elements Pb, As, Mo, Cd, Zn, and Sb, and also organic contaminants such as halides, hormones, prion, ionic liquids and rare volatile metals like osmium, and other ultrafine particles (Funari et al., 2016; 2020; Turner and Filella, 2017; Richardson and Kimura, 2017). Target and non-target chemical analyses, toxicological studies, and endpoint metrics (such as antibiotic resistance, genotoxicity, superparamagnetism, etc.) are necessary to protect the environment and the human health.

Despite the general skepticism in the application of biological processes, feasibility studies ascertain the urgent need to establish process efficiency in appropriate scale reactors or heaps to optimize the process, reactor design, and cost-benefit analysis towards cleaner waste management and minimization of loss of resources in the production chains. Optimizing the dissolution kinetics to speed up the reaction can be improved by optimizing pH, pulp density, temperature, pre-treatments, reaction time, and the careful choice

816 of bioleaching bacteria and their nutrients. Certainly, a profound knowledge of the leaching mechanism and
817 behavior of microorganisms is vital for identifying new promising species or consortia. Pulp density is another
818 issue that makes the process uneconomic unless clear water recirculation solutions are developed.
819 Evaluation of the process economics may be properly examined in the long run after identifying the best
820 metal to recover based on the commercial bioleaching applications in primary ore mining. Although
821 bioleaching yields are a step below compared to abiotic leaching, engineered inocula can be tailored to the
822 target materials and express their functionalities to sustain or prevent metal leaching from the treated waste.

823 Statements

824 -Ethical Approval

825

826 Not applicable

827

828 -Consent to Participate

829

830 Not applicable

831

832 -Consent to Publish

833

834 Not applicable

835

836 -Authors Contributions

837

838 *Conceptualization: Valerio Funari; Methodology: Valerio Funari, Helena I. Gomes, Rafael Santos; Writing -*
839 *original draft preparation: Valerio Funari; Writing - review and editing: Valerio Funari, Helena I. Gomes,*
840 *Simone Toller, Laura Vitale; Supervision: Valerio Funari, Helena I. Gomes, Rafael Santos.*

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847 -Competing Interests

848

849 *The authors have no relevant financial or non-financial interests to disclose.*

850

851 -Availability of data and materials

852

853 *The data that support the findings of this study are available from the corresponding author, V.F., upon*
854 *reasonable request.*

855

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