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Review

Highlighting the Role of Archaea in Urban Mine Waste Exploitation and Valorisation

Annalisa Abdel Azim [1,*], Ruggero Bellini [1], Arianna Vizzarro [1,2], Ilaria Bassani [1], Candido Fabrizio Pirri [1,3] and Barbara Menin [1,4]

[1] Centre for Sustainable Future Technologies, Fondazione Istituto Italiano di Tecnologia, Via Livorno 60, 10144 Turin, Italy
[2] Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Turin, Italy
[3] Department of Applied Science and Technology, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Turin, Italy
[4] Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Via Alfonso Corti 12, 20133 Milan, Italy
* Correspondence: annalisa.abdelazim@iit.it

Abstract: E-materials become e-waste once they have been discarded without the intent of reuse. Due to its rich content of metals, among which many are Critical Raw Materials (CRMs), e-waste can be considered an urban mine to exploit and valorise. Common metal refining is performed by energy-intensive processes frequently based on the use of fossil fuel. Bio-metallurgy is a promising alternative for e-waste valorisation based on biological routes of specialised microorganisms able to leach solid-containing metals. Because of the physiology of these microorganisms, microbial leaching can be economically feasible, besides being an environmentally sustainable process. Like Bacteria and Fungi, Archaea are also capable of metal leaching activity, though their potential is underestimated. Among them, the extremophiles are the most studied and applied in the field of metal recovery, while mesophilic species are less common but still of high interest. Here we provide the state of industrial application of bio-metallurgy and report on the state of the art of Archaea exploitation in metal recovery from e-waste. Moreover, we give a special highlight to methanogenic archaea, which are able to convert CO_2 into methane in order to highlight the potential for the valorisation of CO_2-rich industrial streams generated by key processes (i.e., anaerobic digestion, concrete, and steel production) in CH_4 for gas grid distribution, while making metals content in e-waste available again as raw material.

Keywords: e-waste; by-products; metals; critical raw material; bioleaching; archaea

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1. Introduction

The growing demand for devices that are indirectly used in products, such as aluminium, steel, metals, concrete, cement, chemicals, wood, paper, plastics, lubricants, fertilisers, and other construction materials, is the main global driver of industry GHG emissions [1]. In the production sector, improvements in the efficiency of material extraction, processing and manufacturing alongside improved material substitution and service efficiency, smartest designs, extended product and maintenance lifetimes, and increased reuse are some of the measures which can be adopted to promote high energy efficiency.

Global economic development has raised the consumption of electric and electronic equipment (e-materials), which includes a wide range of products with circuity or electrical components with a power or battery supply. Besides everyday household and business use (basic kitchen devices, toys, tools for music, and ICT items, such as mobile phones, and laptops), e-materials are also used in transport, health, security systems, and renewable

energy technologies, such as photovoltaics, and sensors or devices in the "smart home" concept.

E-materials become e-waste once discarded without the intent of reuse, but due to their different composition, e-waste can be unfairly harmful to the environment and human health if not managed in an environmentally sound way.

On the basis of the European Commission (2012/19/EU) [2,3], e-wastes have been categorised into 10 groups (i.e., the UNU-KEYS classification) according to comparable average weights, material components, end-of-life qualities and lifetime distributions. The 10-EU classification is listed below:

(1) Large household machines (i.e., washing machines, refrigerators, dryers, air conditioners, dishwashers, etc.);
(2) Small household machines (i.e., vacuum cleaners, microwaves, ventilation equipment, toasters, electric kettles, electric shavers, calculators, radio sets, video cameras, scales);
(3) Information technology and communication (ICT) equipment (i.e., PCs, laptops, mobile phones, fax machines, printers, telephones, and photocopiers);
(4) Consumer electronics (i.e., TV, VCR/DVD/CD players, hi-Fi sets, radios, train sets, coin slot machines);
(5) Lighting Fluorescent lamps, high-intensity discharge lamps, and LED lamps;
(6) Electrical and electronic tools (i.e., drills, electric saws, sewing machines, lawnmowers, large stationary tools);
(7) Toys (i.e., electrical and electronic toys);
(8) Leisure and sports equipment;
(9) Medical devices (surveillance and control equipment, medical instruments and equipment);
(10) Automatic dispensers.

From those, 54 sub-categories with approximately 920 end-of-life device products are included. As a primary component common to almost all e-wastes, we should mention printed circuit board (PCB) holding 28–30% of metals (copper > aluminium > nickel > iron > tin > lead > precious metals) and 70–72% of non-metallic elements such as plastics, ceramics, and polymers [4,5].

In 2019, 53.6 Mt of e-waste was globally generated, with an average of 7.3 kg per capita. The global generation of e-waste has grown by 9.2 Mt since 2014 and is projected to reach up to 74.7 Mt by 2030. The growing amount of e-waste is mainly fuelled by higher consumption rates of e-materials, short life cycles, and few repair options [6]. The amount of e-waste generated per capita in Europe is comparable to the Americas and Oceania, although the EU collection and recycling rate is over four times higher. Nevertheless, the fate of 44.3 Mt of e-waste in 2019 is uncertain, and its location and impact on the environment are distributed differently among regions. In EU countries, about 0.6 Mt of e-waste goes to waste bins [7]. Moreover, a considerable amount of e-waste is still exported illegally with the excuse of being recovered or pretending to be leftover metal. Those flows are usually undocumented and thus not traceable, although developing countries are often the final recipients. The Transboundary E-waste Flows Monitor 2022 estimated that 5.1 Mt of e-waste passed through the borders in a controlled and uncontrolled manner in 2019 [8]. Of these, only the shipment of 1.8 Mt is controlled, while the remaining part is moved without supervision, usually in a legal shipment. This "backyard recycling" poses significant risks to the environment and to human health due to the toxic additives and hazardous substances content of e-waste. Moreover, this uncontrolled trade creates the premise for illicit management.

In 2019 were released, besides 50 t of mercury and 98 Mt of CO_2-equivalents from untreated refrigerants, corresponding roughly to 0.3% of global energy-related annual emissions based on the International Energy Agency. The European Commission has recently presented a new circular economy action plan that has among its priorities the reduction of electronic and electrical waste. EC proposal addresses the principles of circular economy, which range over product design, waste reduction, right to repair, reuse and recycling of end-of-life products, materials, and components. Following all these solutions

can be very challenging because it requires a deep structural change involving massive technological investments that represent a prohibitive cost for the manufacturing sector and SMEs [9,10]. However, a change must occur in order to improve the environment and health system and guarantee the human rights of the countries which are involved in the so-called "conflict minerals" [11]. In addition, proper recycling of e-waste and the application of circular economy principles could mitigate the demand for raw materials and lead to significant economic benefits for society.

E-waste can be considered a huge urban mine to be exploited as it contains up to 69 elements from the periodic table, among which about 50 elements are recognised as critical raw materials (CRMs) [12–14]. Critical (e.g., cobalt, palladium, indium, germanium, bismuth, antimony), precious (e.g., gold, silver, copper, platinum, palladium, ruthenium, rhodium, iridium, and osmium), and other non-critical (aluminium, iron and cadmium) metals in e-waste represent a secondary resource of raw materials. Many of those metals are toxic (such as barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), lead (Pb), lithium (Li), lanthanum (La), mercury (Hg), manganese (Mn), molybdenum (Mo) and persistent organic pollutants (POPs) such as brominated flame retardants (BFRs) [15,16]. As Table S1 reports, the application of each element in industrial manufacturing can be wide and diversified. Moreover, the use of some metals in renewable energy technology is gradually increasing, as in the case of gallium (Ga), indium (In) and tellurium (Te) for photovoltaic (PV) panels solar cells. Because of this, they are classified as critical. In this regard, considering the increasing demand for and the average life of PV modules (i.e., 20–30 years), it has been estimated that more than 78 million tons of them will reach the end of life by 2050 [17]. Thus, proper disposal of dismissed PV panels should be a priority in the rush to a carbon-neutral world. Other CRMs, such as platinum (Pt), palladium (Pd), and rhodium (Rh), which are also part of the platinum group materials (PGM), are largely used in catalysts manufacturing for automotive. Rare earth elements (REEs) such as praseodymium (Pr), lanthanum (La), and neodymium (Nd) have recently found application in battery manufacturing. Lanthanum is also used in alloys with nickel for H_2 storage. Currently, the EU's primary material supply totally depends on China, South Africa, and Latin America, which detain most of the metal reservoirs (Table S1). from this scenario, it is clear how important is the recovery from spent devices of the elements discussed therein.

However, e-waste is not the only source of raw materials though it is the most obvious one; also, by-products of industrial manufacturing generate a significant stream of waste rich in critical metals, called "new scrap or process scrap".

Vanadium (Vd), which is applied in the manufacturing of steel and battery, can be found in the slag produced by the steel processing and titanomagnetite smelting, as well as antimony (Sb) can be recovered from the wastewater from mining and smelting procedures. In, Ga and Ge are obtained by the zinc refining process. Nb, mostly present in stainless steel, can be sourced as a by-product of tin extraction. Te, which will enter the CRM list in 2022, can be recovered from the mud generated during copper refining via electrolysis. Pd, a precious element among the CRMs, can be obtained as a by-product of nickel, copper, and zinc refining beyond extracting it from natural ores.

Overall, the environmental implications of "industrial" metals depend on the metal type; most are known on commonly used metals such as copper and iron, whilst limited information is available for minor metals (e.g., niobium, indium, bismuth, germanium, rhenium, hafnium) although their application is gradually increasing [18]. In this regard, the Compound Annual Growth Rate (CAGR) of world production, either for raw or refined materials, which is reported in Table S1, is a useful indicator attesting to the raw material demand. For instance, Ga, Li and Te have a CAGR value of 16%, 17% and 15%, respectively, which is representative of a growing market in the sector of battery and PV cell manufacturing. The economic value of raw materials in the global e-waste, including both documented and not documented flows, generated in 2019 is equal to approximately 57 billion €. Iron, copper, and gold contribute mostly to this value. However, only 4 Mt of raw materials, corresponding to roughly 10 billion € gained and 15 Mt of

CO_2 saved, can be recovered from properly recycled e-waste (9.3 Mt). To have an idea about the huge potential of e-wastes as a source of raw material, you can visit the ProSUM project dashboard (ProSUM project, EU Horizon 2020, grant agreement N° 641999) [7], which shows all available data on products placed on the market, stocks (in use and hidden), composition and waste streams for e-wastes, vehicles and batteries for Europe, Switzerland and Norway. The end-of-life—recycling input rate (EOL-RIR) is a circular economy indicator that provides information about the contribution to the production system from recycling end-of-life scrap but not processed scrap. The EOL-RIR values reported in Table S1 are available for a few elements and never reach 50%, meaning that the input of scrap metals is not fully exploited. The most recycled element is tungsten (W), followed by two precious metals, i.e., rhodium (Rh) and palladium (Pd), while other metals, such as Ge and Mn, have a low EOL-RIR.

In contrast, we reported the import reliance (IR) indicator for primary and refined materials, also part of circular economy parameters for CRM assessment, which reveals EU dependence on other world countries for raw and processed materials provision (Table S1). The IR values for REEs are equal to 100% because rare elements are mostly available in mineral deposits and in a very low amount worldwide. Thus, Europe totally depends on the import. Also, the supply of processed material based on Li, Mg, Nb, Sc, and Y elements totally relies on exporting countries. Although copper (Cu), iron (Fe), including steel, and molybdenum (Mo) are not critical, their demand (CAGR value) has increased over the last decades.

Given the undeniable value of e-waste and its implication for human and environmental health and safety, the development of a renewable recycling system offers a valuable solution to reduce the environmental and health risks and the economic losses associated with e-waste abandonment. In this context, biological metal recovery is emerging as an alternative to traditional energy-consuming metallurgy processes. With this review, we aim to provide the state of the art on microbial- and especially archaeal- application at both research and industrial level in the recovery of metals from e-waste. Particular attention will be given to methanogenic archaea, in the perspective of coupling CO_2 conversion to biomethane with the use and recovery of metals.

2. Recycling E-Waste via Biological Route

The metallurgy process for metal refinement frequently requires energy-intensive melting steps based on the use of fossil fuel. Pyrometallurgy, hydrometallurgy and biohydrometallurgy are three variants of metallurgy that differ in the mechanism of extraction. While pyrometallurgy uses heat power, hydrometallurgy applies redox chemical reactions in an aqueous or organic liquid solution. Biohydrometallurgy works similarly to hydrometallurgical processes, except that the reagents are directly supplied by the microorganisms because of the by-products of their metabolic reactions. Research is rapidly evolving to manage the challenges in recycling e-waste for metal recovery through both chemical and biological routes. As part of the chemical method, hydrometallurgy is a well-established process for metal leaching from both primary and secondary resources [19,20]. Conversely, biohydrometallurgy is still under investigation in several aspects, such as the physiology of less explored microorganisms, bioprocess operation and scalability [21–23].

The biological route is carried out by specialised leaching microorganisms which implement strategies for recovering metals against their scarcity in the environment.

The microbial systems acquire metals necessary for metabolism and counteract the adverse effects of toxic metals to protect the cell by using a whole repertoire of mechanisms and to acclimatise themselves to hostile environmental conditions [24]. Microbial growth, metabolism and differentiation are intimately linked to the biogeochemical cycle of metals. The metals can be classified into three categories based on their different physiological roles: (a) vital and non-toxic, such as Ca and Mg; (b) vital but toxic at elevated levels, such as Fe, Mn, Zn, Cu, Co, Ni, and Mo; and (c) toxic, such as Hg and Cd [25,26]. Metals such as aluminium (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi),

cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V), and uranium (U) have no established biological functions and are considered as non-essential metals [24]. Nevertheless, there is evidence of microbial removal of some of these non-essential metals [21–23,27].

Natural sources of metal-leaching microorganisms are mine sites or acid mine drainage samples, though other sources exist (e.g., brines and sediments, sludge from anaerobic digestion plants) [28]. Due to the physiology of these microorganisms, bioleaching can be economically feasible (e.g., in situ processing is viable thanks to the simplicity of technology design and operating conditions; the possibility of reusing existing facilities; and reasonable capital and operating costs) and eco-friendly approach with higher efficacy, safety and ease of control [29]. Furthermore, the waste streams, including toxic gas emissions and wastewater, are more restricted and controlled in the context of microbial leaching. However, reaction kinetics can be pointed out as the main limitation of biohydrometallurgy processes, being strictly dependent on the characteristics of the biocatalyst.

Factors affecting the bioleaching process include pH, temperature, oxygen, and carbon dioxide supply, as well as nutrients in the medium. pH is selective only for certain metal compounds (e.g., carbonates, common oxides, acid-soluble sulphides), and of course, it is also a filter for microbial growth and activity [30–32].

Bioleaching essentially occurs via autotrophic and heterotrophic mechanisms, with the former being either direct or indirect, whilst the latter being only indirect [27,31], as depicted in Figure 1.

Direct bioleaching implies the contact between microorganisms and metallic materials. This is valid for both single- and double-step processes, where the solid containing metals are mixed with microorganisms from the beginning or during the exponential microbial growth, respectively. Though direct and indirect bioleaching are both effective methods, the first one may run into the inhibition of microbial activity due to the toxicity of leached metals [31]. However, direct contact promotes the adhesion between microorganisms and the solid surface, mediated by the formation of biofilms and the uptake or complexation of metals through the excretion of bio-lixiviants and chemicals that perturb the environmental state, respectively, ending with the release of metal ions into solution. Recently, insights into the mechanism of interspecies communication within the archaeal biofilm and its regulation have enabled a deeper understanding of the bioleaching processes [33]. Autotrophic bioleaching uses carbon dioxide as a carbon source and iron Fe (II) or sulphur S (0) as the main energy sources to carry out their oxidation [34]. The general chemical reactions occurring in bioleaching are extensively reported in several studies [29,35,36] and more recently by Magoda K. and Mekuto L. (2022) [37]. Sulfur-oxidising bacteria (e.g., *Acidithiobacillus thiooxidans*,), iron- and sulphur-oxidising bacteria (*Acidithiobacillus ferrooxidans*) and iron-oxidising bacteria (*Leptospirillum ferrooxidans*) are the most employed autotrophs in bioleaching processes because of their resistance to heavy metal toxicity and their simple nutritional requirements [38]. The consequent acidification due to the production of sulfuric acid and ferric ions causes metal dissolution, although for acid-insoluble sulphides or some metal oxides. Metals can be recovered either through biological oxidation, when the metal is soluble at a high oxidation state, or biological reduction, occurring when the metal is more soluble in a low oxidation state (e. g., Ni, V, and Mn) [39]. In contrast, heterotrophic bioleaching occurs via an indirect mechanism based on organic acids (e.g., citric or oxalic acid), ligands (e.g., chelators and siderophores) and exopolysaccharides (EPS) biosurfactants [33]. Among archaea, *Haloferax mediterranei* has been chosen as a model organism for EPS production, achieving a technological level of readiness (TRL) of 2 [40]. Heterotrophic bioleaching can be performed via acidolysis (*Acidothiobacillus ferroxidans* and *Leptospirillum ferroxidans*) or complexolysis *(Chromobacterium violaceum, Pseudomonas aeruginosa, Pseudomonas flourescens, and Bascillus megaterium*). In acidolysis, organic acids produce complexes with metal ions to enhance their mobilisation through a protonation mechanism, while complexolysis occurs when a chelating agent combines with metal on the

surface or via biosurfactants acting as complexing agents. A third possible mechanism for heterotrophic bioleaching is the Fe (III) or (Mn (IV) dissimilatory reduction under anaerobic conditions in complex media, where the oxidation of organic matter is coupled to the metal reduction *Shewenella putrfaciens* is a reference microorganism for this process [41].

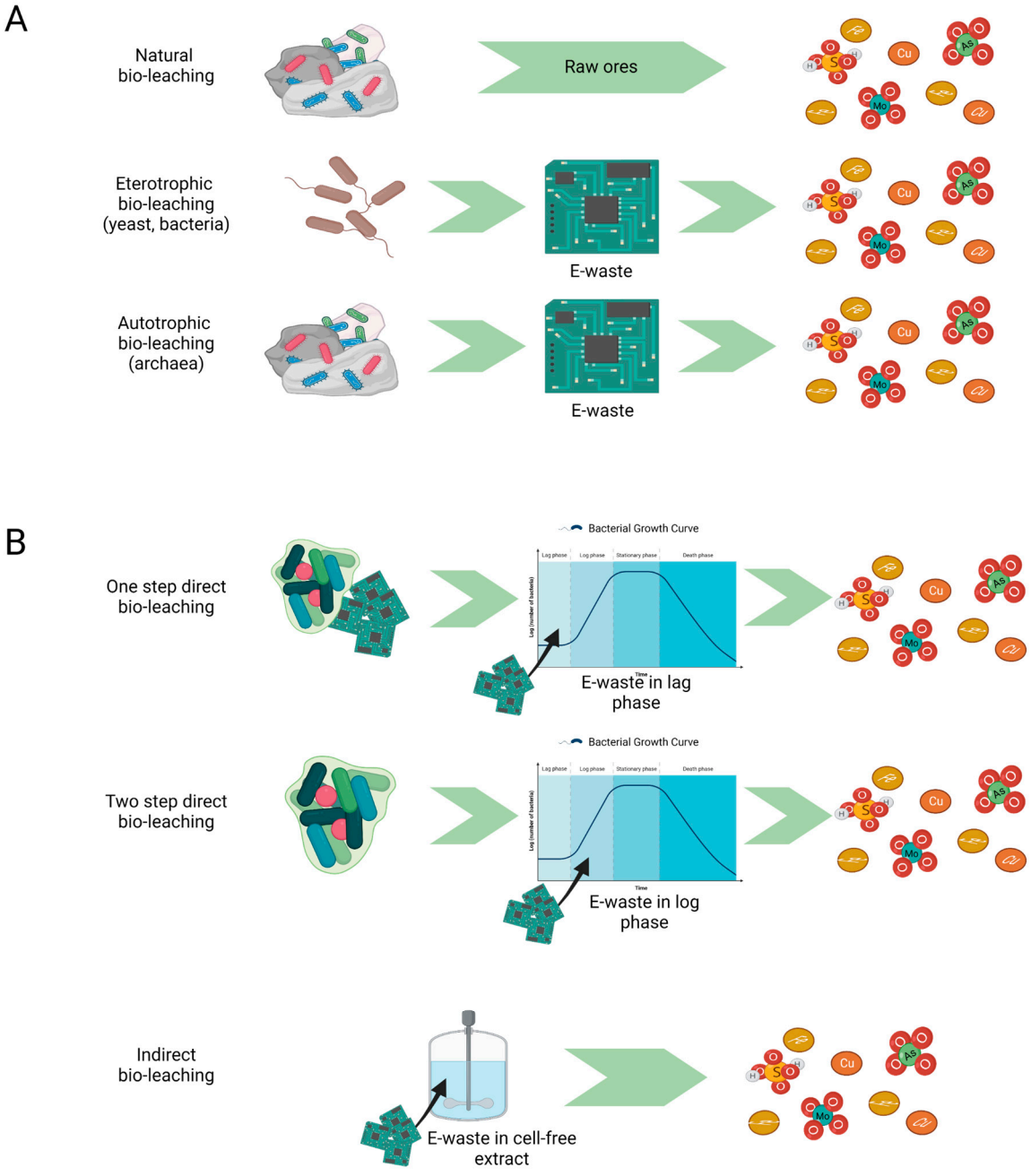


Figure 1. Bioleaching: mechanisms and strategies. (**A**) Heterotrophic and autotrophic bioleaching occurring in natural minerals deposits and in e-waste recovery processes resulting in free metal ions and metal oxides; (**B**) Direct (one-step and two-step) and indirect approach to bioleaching process. In direct bioleaching, e-waste is introduced during the initial microbial growth phase (one-step) or exponential growth phase (two-step). In indirect bioleaching, only microbial metabolites interact with e-waste.

Bioleaching has been successfully applied in indirect and direct approaches for the recovering of valuable metals (i.e., Ni, Si, Cu, Ga, Mg, Te, Zn) from the dismissed light

emitting diode (LED) [27], end-of-life PV solar cells [42], printed circuit board (PCB) [4] and other e-waste [19,43–45].

2.1. Interactions between Archaea and Metals

Microorganisms belonging to the Archaea domain are widely recognised as cosmopolitan organisms capable of adapting to either natural or anthropogenic environments characterised by extreme life conditions (i.e., natural gas and oil reservoirs, acid mines, hydrothermal vents) [46–50]. The relation between metals and thermophilic archaea stems from their natural ability to live at high temperatures and high-metals concentrations [25], thanks to unique cell wall structures, thermostable enzymes, and metabolic features [51].

Archaea also requires metals because of their role in enzymatic structures and co-factors, as final electron acceptors, or for sustaining their growth with metal enzymes and for pathways requiring metal ions as co-factor constituting the archaeal metallosome [48].

Genomic and proteomic studies allowed for the identification of similarities between archaeal, bacterial and eukaryotic metallosome, with the most common domains coding for Fe-, Co-, and Zn- binding proteins and only a low percentage coding for binding domains (<0.3%) for Ni-, Cu-, and Mo- [52]. Nonetheless, a higher percentage of Fe- binding domains were identified in archaeal genomes (≈7.1%) when compared to bacterial (≈3.9%) and eukaryotic (1.1%) ones. Although less represented, metal binding domains related to Ni-, Co- and Cu- utilisation/transport were also identified in archaeal genomes [52,53]. Table 1 resumes the roles that the previously mentioned metals have in Archaea.

Table 1. Physiological role of essential metals in some selected species of Archaea.

Metal	Microorganism	Function in Archaea	References
Fe-	*Halobacterium* spp., *Methanosarcina* spp., *Methanobacterium* spp., *Sulfolobus* spp., *Thermoplasma* spp., *Ferroplasma* spp., *Pyrobaculum* spp.	Fe (II)oxidation, Fe (III) reduction, Fe_4S_4-ferredoxin, Fe_4S_4 cluster for S-adenosylmethionine cleavage, Ni-Fe hydrogenase	[48,52,54–56]
Zn-	*n.s.*	"Small proteins" class genes (Zn finger motifs and Really Interesting gene (RING))	[52]
Co-	*Methanosarcina* spp., *Sulfolobus solfataricus*, *Thermoplasma acidophilum*	Found in co-enzyme B12 structure, Ni/Co uptake system	[57,58]
Ni-	*Sulfolobus* spp., *Halobacter* spp., *Methanococcus* spp.	Enzymatic co-factor for different enzymes:Ni-Fe hydrogenase, CO de-hydrogenase, methyl-CoM reductase, urease	[56–58]
Cu-	*Halobacterium* spp., *Methanosarcina* spp., *Methanobacterium* spp.	Copper-binding proteins, N_2O reductase	[54]
Mo-	*Sulfolobales* spp., *Halobacteriales* spp., *Methanosarcinales* spp., *Methanococcales* spp., *Methanomicrobiales* spp.	Molybdenum co-factor (Moco) involved in W utilization	[59]

With archaeal life requiring metals that are classified as CRMs (i.e., Co) and toxic materials (i.e., Ni, Co, Mo) and being capable of storing them in cellular structures (i.e., enzymes, co-factors, proteins) applications of archaeal cultures for metal detoxification and recovery surely sounds appealing. Furthermore, different studies have highlighted how archaeal-driven biotechnological applications (i.e., biomethanation, anaerobic digestion)

need a stable supply of trace metals, including Fe, Co, Ni, Cu, and Mo, in order to maintain process stability [60–64].

A possible solution for economically viable metal recovery could be represented by its integration within already operating processes. In this logic, the modulation and fine-tuning of waste metals, supplied as nutrients, could sustain the systems requirements while increasing the sustainability of the process through the integration and valorisation of different waste streams.

Although most knowledge about the interaction of archaea with metals focuses on the role of Fe, Co, Ni, Cu, and Mo, many sources have reported interactions with non-essential metals, also listed as CRMs, precious and toxic elements (i.e., As, Cd, Pt, V, and U) [21–23,27]. A deeper insight regarding such archaea-metal interaction will be provided in further sections.

2.2. Bioleaching among Archaea

Despite the peculiar ubiquities and the great biotechnological potential of Archaea, Bacteria and Eukarya have always dominated the scene in terms of research studies, industrial application, and public perception. As detailed in the review authored by [40] on the current status of archaeal cell factories in bioproduction, few companies are investing in Archaea, while commercialisation is limited to extremophilic genera, which are suitable for applications in industrial processes under harsh conditions [65]. As regards the biomining and bioleaching industry, the engagement of Archaea is very limited, while research studies are mainly restricted to thermoacidophiles genera, often cultured with bacterial species whose leaching ability has already been validated [25,66]. However, archaea are also members of non-extreme environments [67]. Thus, the scenarios of possible industrial applications and their operating conditions are multivariate. A panoramic of CRMs typically contained in e-wastes and targeted by the Archaea herein discussed is presented in Figure 2.

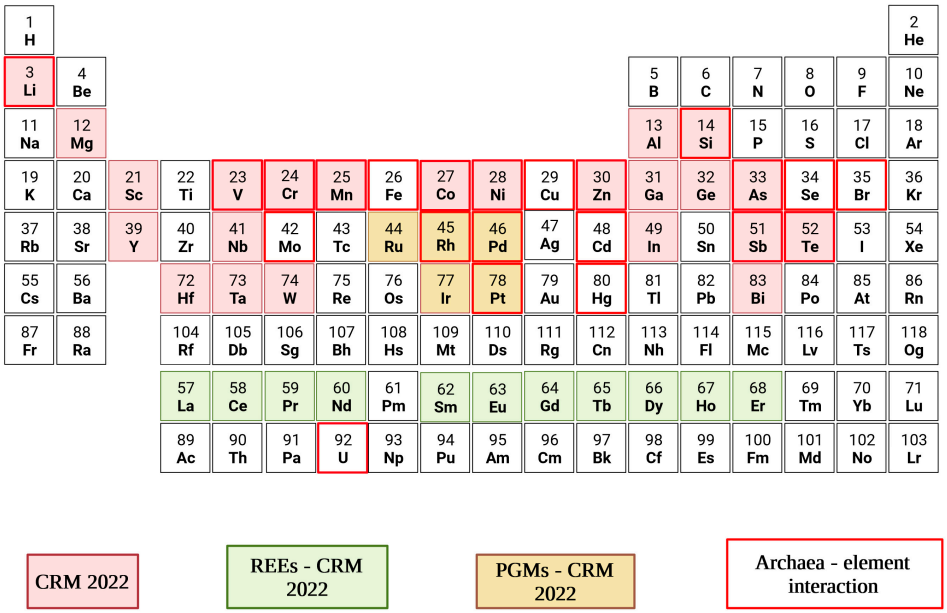


Figure 2. Periodic table of elements categorised as Critical Raw Materials in 2022, comprising PGMs and REEs as listed in Table S1 [13,14]. The red squares represent archaea-element interactions described in the present work.

2.2.1. Extremophile Archaeon

Several species of thermoacidophiles Archaea, such as *Acidianus sp*, *Ferroplasma acidophilum*, *Metallosphaera sp*, *Metallosphaera sedula*, have been identified in stirred-tank mineral bioleaching and bio-oxidation operations [68]. Other species isolated from a hydrother-

mal pool and mineral sulphide ores, respectively *Acidiplasma aeolicum* and *Acidiplasma cupricumulans* [69], can oxidise and reduce iron. The Sulfolobacaee family comprises species representative of acid hot spiring able to oxidise elemental sulphur (i.e., *Sulfolobus acidocaldarius DSM 639, Sulfolobus solfataricus P2, Sulfolobus tokodaii JCM 10545, Stygiolobus azoricus DSM 6296)* [70,71]. Among *Thermoplasmatales*, we can mention *Picrophilus torridus* and *Thermogymnomonas acidicola JCM 13583,* which inhabit dry solfataric fields [72,73], *Thermoplasma acidophilum DSM 1728* and *Thermoplasma volcanium GSS1* [74], respectively identified from coal refuse piles and hot acid springs. Novel species isolated from acid mine drainage (AMD), such as *Cuniculiplasma divulgatum JCM 30642*, *Candidatus Micrarchaeum acidiphilum ARMAN-2 NIA Candidatus Parvarchaeum acidiphilum ARMAN-4 NIA Candidatus Parvarchaeum acidiphilum ARMAN-5* are likely capable of oxidising metal sulphides [30].

Heavy metals, such as As, Hg, and Cd, can be remediated by several extremophilic archaeon species [39]. *Sulfolobus acidocaldarius strain BC* was isolated in an acidic, sulfuric thermal spring in the Yellowstone National Park and recognised to be capable of oxidising arsenite As (IV) to arsenate As (V) [75]. Other Archaea strains, such as *Aeropyrum pernix K1, Pyrobaculum calidifontis JCM 11548, and Sulfolobus tokodaii 7* and the genus *Halorubrum* contain arsenite oxidase genes [76–78]. *Sulfolobus solfataricus* species, *Halococcus*, *Halobacterium*, and, to a lesser extent, *Haloferax* genera were capable of mercury Hg (II) volatilisation into Hg (0), thanks to the presence of mercury reductase genes [79–81]. *Halobacterium noricense* stands out for its ability to adsorb cadmium [82], while *Haloferax strain BBK2* uptakes cadmium intracellularly [83]. Among metalloids included in the CRMs list, Si is of primary importance given its large application in PV solar cells. The hyperthermophilic sulphur-metabolising *Pyrococcus abyssi* was fossilised during and after exposure to a silica-saturated solution (about 500 ppm of SiO_2) in a simulated hydrothermal environment [84], demonstrating the ability to bind silica at the S-layer sites and integrate it in replacement of the cell wall.

Based on the above-mentioned studies, thermophilic and hyperthermophilic archaea can be applied in many types of high-temperature metal-contaminated waste streams, while haloarchaea can be used in the treatment of hypersaline environments and wastewaters for heavy metal removal.

2.2.2. Methanogenic Archaea

Methanogens, which already play an important role in the global methane (CH_4) cycle by producing CH_4 via methanogenesis, can also recover many critical, platinum group metal (PGM) and non-critical metals from the —end-life products, mining residues and waste streams.

Consortia

It has been stated in many studies that anaerobic granular sludge is efficient in PGM metals recovery (Figure 2). The work by Espadas et al. reports Pd (II) removal via reduction to Pd (0) nanoparticles or biosorption into the biomass of a methanogenic granular sludge from a full-scale up-flow anaerobic sludge blanket (UASB) reactor processing brewery wastewater [85]. While the reduction route was supported using external H_2, formate, and ethanol, the biosorption dominated when acetate, lactate and pyruvate were added as external electron donors. A more recent study determined the ability to reduce Pt (II) and Pt (IV) to Pt (0) with the same anaerobic sludge applied by Pat-Espadas et al. 2018 [85]. Rh (III), which is one of the rarest elements in the geosphere (about 0.001 ppm), was also reduced to Rh (0) nanoparticles, with ethanol as an external donor of electrons [86]. Ramos-Ruiz et al., 2016 highlighted the potential of anaerobic granular sludge from a methanogenic reactor as a biocatalyst for the reduction of both Te (IV) and Te (VI) to produce Te (0) nanoparticles [87]. *Methanobacterium* genus was detected as predominant among the archaeal community populating a fluidised bed reactor applied to lithium-ion batteries (LIBs) leaching via biogenic hydrogen sulphide [88].

Pure Culture in H_2/CO_2

Compared to consortia, pure culture applications present some advantages in terms of process reproducibility and simplicity. However, while pure cultures usually require more strict conditions (e.g., nutrient demand and key process parameters), consortia are typically more robust [60]. The mesophilic hydrogenotrophic strain *Methanobacterium bryatii* BKYH has been isolated from a copper mining area in the Upper Peninsula of Michigan and is able to chelate Cu by secreting a specific protein in response to copper exposure [89]. Increased Cu concentration in the medium results in the production of an extracellular Cu response- (CRX) protein encoded by the *crx* gene and promoted by an archaeal Cu-responsive promoter, which could be synthesised as part of a more generic stress response [90].

Zhang et al., 2014 published the first study reporting methanogenic archaeal strains as capable of reducing vanadium (V) [91], classified as CRM in 2022 (Table S1; Figure 2). *Methanosarcina mazei* and *Methanothermobacter thermautotrophicus* could reduce up to 10 mM and 5 mM of vanadate V(V) to vanadyl V(IV), respectively, inducing solid extracellular precipitation as the bioreduction occurred at the cell membrane level. However, at some point, methanogenesis stops with vanadyl generation, possibly due to the redirection of electrons from methanogenesis to vanadate reduction.

According to Singh et al., 2016 *Methanothermobacter thermautotrophicus* is capable of reducing Co (III) present at a maximum concentration of 4 mM [92] and can reduce up to 1 mM of Cr (VI) to Cr (III) without an inhibitory effect on methanogenesis and cell growth through an intracellular and extracellular reduction mechanism [93]. Holmes et al. reported the potential role of acetoclastic methanogens belonging to the genus *Methanosarcina* in reducing U(VI) present in contaminated groundwater [94]. After a period of acetate amendment in the field, the predominance of *Methanosarcina* corresponded to methane accumulation and U(VI) reduction. Like *Pyrococcus abyssi,* the hyperthermophilic, hydrogenotrophic methanogen *Methanocaldococcus jannaschii* could bind Si on its cell wall and accomplish the silicification mechanism, but only when metal cations, particularly Fe (III), were present [95]. However, a more detailed comprehension of this mechanism is pivotal in order to understand its exploitability in the recovery of Si-rich wastes.

2.2.3. Adverse Interactions between Methanogens and Critical Metals

Despite both methanogenic consortia and pure cultures have been proven capable of reducing different metals, studies have also reported potential issues related to the metal-methanogen interactions as:

- Inhibition and toxicity: 50% inhibiting concentrations (IC50) for Cd, selenite, tellurite, and tellurate have been investigated on methanogenic consortia, with acetoclastic methanogens displaying higher IC50 ($IC50_{Cd}$ 8.6 mg/L, $IC50_{selenite}$ 24.1 mg/L, $IC50_{tellurite}$ 8.6 mg/L, $IC50_{tellurate}$ 10.2 mg/L) than those of hydrogenotrophic methanogens ($IC50_{Cd}$ 2.9 mg/L, $IC50_{selenite}$ 18 mg/L, $IC50_{tellurite}$ 8.6 mg/L, $IC50_{tellurate}$ 10.2 mg/L) [96]. Similarly, IC50 for Pd(II), Pt(II) and Pt(IV) for methanogens were reported at 2.7, 2.4 and 3.7 mg/L, respectively [85], whilst full inhibition of methanogenesis occurred at Pt(II) concentration higher than 5 mg/L [97];
- Competition between metal reduction and methanogenesis: Methanogenesis, both in anaerobic digestion and in the biomethanation process, requires electron donors to reduce CO_2. When considering the bioleaching process, the addition of an external electron donor is often needed due to the competition for electrons that are redirected from methanogenesis to metal reduction [91]. In an anaerobic granular sludge, endogenous substrates can provide sufficient electron equivalents for the leaching of metals of interest, although reduction rates may increase with excess electrons, as reported by [87]. Among external electron donors to be added to methanogenic anaerobic sludge, ethanol is considered a safe and economical option. Fermentation of ethanol by the acetogenic community populating the granular sludge generates H_2 that is then redirected to metal reduction. However,it should be mentioned that some studies

have described the abiotic reduction of some metals through direct chemical reduction by H_2 and formate [85,97,98].

2.2.4. Anaerobic Methanotrophic Archaea (ANME)

Microorganisms play an important role in the global CH_4 cycle that is controlled by the balance between anaerobic production via methanogenesis and CH_4 removal via methanotrophic oxidation. Methane oxidation is anaerobic (AOM) in more than 90% of cases [99]. AOM can be coupled to other electron acceptors besides the well-known sulphates SO_4^{2-}, such as nitrates NO^{3-} (N-DAMO), or metals such as manganese Mn (IV), iron Fe (III), As (V), Cr (VI), Se (VI), Sb (V), V (V), and Br (V) [100] through the establishment of a syntrophic partnership as electron sinks. However, it is not fully elucidated whether the reduction of metallic electron acceptors is independent or supported by electron transfer to syntrophic partners, interspecies electron transfer, nanowires or conductive pili [101].

The study by Zhang et al., 2020 investigated the bio-reduction of vanadate V(V) present in groundwater using an anaerobic sludge inoculum taken from a wastewater treatment resource [102]. Biological mediated vanadate removal, corresponding to 95.8 ± 3.1% of 1 mM, occurred after seven days of incubation using CH_4 as the sole electron donor. Microbial community analysis revealed a more significative change in the archaeal population than in the inoculum, with a massive presence of the *Methanobacterium* genus, which is known to oxidise methane via reverse methanogenesis, and the hydrogenotrophic methanogens belonging to *Methanomassiliicoccus.* Thus, vanadate reduction occurred through the anaerobic oxidation of methane and synergistic interactions with methane-oxidising bacteria such as *Methylomonas*. Evidence of the reduction of antimonate Sb (V) to Sb (III) using CH_4 is reported by the work of Lai et al., 2018 [100] in a membrane biofilm batch reactor inoculated with a pre-enriched culture with methane and Sb (V), Figure 2. Increasing concentrations of Sb (V), 0.41, 0.82, 1.6 mM, at different stages of the experiment were 100% biologically reduced in the form of Sb_2O_3 crystal precipitates. The Archaea community was widely enriched during the different operational stages, where *Methanosarcina* and *Methanolobus* genera were the two most abundant methanogens, with the former being predominant. Other studies suggested a key role of the methanogenic archaea *Methanosarcina* in AOM via reverse methanogenesis, as in Luo et al., 2017 where the electron acceptor was Br (V) in the form of BrO^{3-} [103]. A possible explanation is the close phylogenetic relationship between ANME-2a/b, ANME-2c, ANME-2d, and ANME-3 and *Methanosarcinales* [104]. The archaea family ANME-2d, also knowns as *Methanoperedenaceae* lineage, was found to be able to oxidise methane through Mn (V) reduction in a bioreactor supplied with CH_4 and pulse of birnessite operating for 480 days [105]. The inoculum originated from a bioreactor inoculated with freshwater sediment and performing AOM via Fe (III) reduction. The average Mn (II) production rate was 0.185 mmol L^{-1} day^{-1} with a methane consumption rate of 0.045 mmol L^{-1} day^{-1}.

3. EU-Founded Projects Involving Bioleaching for CRMs Recovery

In the last two decades, several projects aiming at applying bioleaching to mine waste (MW) recovery and e-waste recycling have been funded by the EU. An overview of both active and completed projects– currently a total of 12 to the best of our knowledge- is available in Table 2.

Ongoing projects such as BIORECOVER and RAWMINA, together with RUBICON and BIOCriticalMetals projects, target specifically the extraction of CRMs. BIORECOVER and RAWMINA projects aim at developing sustainable bioleaching processes for the selective extraction of CRMs from unexploited primary and secondary sources and MW up to a Technology Readiness Level (TRL) of 5 and 7, respectively [106–108]. Specifically, BIORECOVER targets the selective extraction of CRMs, REEs, magnesium and platinum group metals through the isolation of microbial consortia populating raw material and their application as pure or mixed culture [106,107]. RAWMINA aims at integrating bioleaching and nano-based materials in a continuous pilot-scale process for the recovery

of Sb, Co, Ge, and W from unexploited/underexploited metal-containing materials, to be employed in products, such as batteries, flame retardants, optical fibres, and industrial tools [106,108,109].

Similarly, RUBICON applied a novel bioleaching strategy for the exploitation of natural metal sources, such as laterites, polymetallic deep-sea nodules and sulfide ore deposits, using a downstream process for the specific recovery of cobalt, scandium, nickel and other solubilised metals (e.g., Cu, Zn, V and Mn) in a pilot-scale process at TRL 4-5 [110]. Finally, BIOCriticalMetals defined the bioleaching potential of a different microorganism isolated from a mining site consortium for critical high-tech metals, such as W, In, Ga, Te, and Mo, contained in the mine waste tailings. This enabled the creation of a microbial consortium that has been successfully tested at the reactor scale [111].

Among the 12 reported projects, only one, BiotaWEE, focused on the recycling of e-waste rather than the recovery of metals from mineral ores. BiotaWEE project aimed at the recovery of valuable metals (e.g., Cu, Ag, and Au) from the non-metallic fraction (NMF) of PCBs of different e-waste, applying an innovative 2-step bioleaching technology, combining an aerobic and an anaerobic process [112]. The project demonstrated the feasibility of this 2-step bioleaching process for the recycling of the suction dust from PCB mechanical treatment rather than the NMF. While a 2-step bioleaching technology, combining two aerobic steps, the first for base metal treatment and the second for precious metals, was successfully applied to both NMF and suction dust metal extraction [112]. During the 2-step bioleaching (anaerobic + aerobic) of suction dust, siderophilic (68% Co, 60% Ni, 40% Mn) and calcophilic (100% Sn, 75% Zn, 70% Cu, 46% Cd, 50% Tl) elements were extracted in the aerobic phase, together with CH_4 produced in the anaerobic phase (up to 62% in reactor headspace). Overall, recovery yields reached 182 Kg Cu, 0.24 Kg Ag and 0.021 Kg Au per ton of PCBs treated. Moreover, the implementation of this technology would avoid the incineration of 300 t/year of PCBs, with consequent reduction of waste and hazardous effluent generation (3.88 t/t PCBs, compared to a hydrometallurgical process), CO_2 production (45%) and operative costs (38%) [112]. Eventually, these projects will contribute to increasing the sustainability of European industry, decreasing its dependence on CRM import, and reducing production costs and environmental impacts. Along with enhancing applied research on bioleaching-related technologies, several companies have turned toward this area to make their production more sustainable through metal recovery and e-waste valorisation. The commitment to bioleaching technology has grown exponentially over the past two decades in several multinational industries, leaders in mining and precious metals extraction, with large investments dedicated to research and development in this biotechnology area and with the creation of new companies dedicated to this sector. An updated review on worldwide industrial applications, with a particular focus on copper bioleaching and gold recovery via biooxidation, has recently been published elsewhere [113].

As previously mentioned, also in Europe, this area of applied research is gaining increasing interest not only from the scientific community but also at the level of industrial applications. Some promising European industries that have implemented innovative applications of bioleaching are briefly described here as examples.

The first company in the UK to extract precious metals from e-waste using bioleaching was N2s [114], established in 2002. N2s is the UK's market leader in information technology (IT) lifecycle services. Their innovative technology solution for the recovery of metals from e-waste (i.e., printed circuit boards) was developed in partnership with Coventry University. N2s holds all relevant Environment Agency waste management licences and is fully e-waste-compliant for IT Recycling and Data Destruction services (accredited by the National Cyber Security Centre, NCSC).

Another example in Europe is represented by the company BiotaTec [115]. Based in Tartu, Estonia, BiotaTec was created in 2007 and formerly known as BiotaP, with the main focus on developing environment monitoring tools based on metagenomics, which since 2010 started to investigate different possibilities of employing microorganism-based

solutions for new innovative bioleaching technologies. Currently, they are developing and providing novel bioleaching approaches and biomining technologies for extracting different metals and increasing the availability of critical raw materials metals from low-grade ores and industrial waste streams, including e-waste and different metals/organics containing secondary waste.

A more recent case is that of Ekolive [116], which is a start-up founded in 2018 in Košice, Slovakia and is the first and leading provider of an EU/ETV-certified eco-innovative bioleaching method (InnoBioTech®) for processing waste/minerals/soil using bacteria. In 2019, in the first large industrial pilot project in Slovenia, they demonstrated the market maturity of bioleaching with heterotrophic bacteria for the upgrading of industrial minerals from mining waste.

Table 2. Overview of active and completed bioleaching projects founded by the EU in the last two decades. For each project, the funding program, project goals, budget and duration are reported.

Project Acronym	Funding Program	Project Goals	Project Budget	Duration	Ref.
Bioshale	Sixth Framework Programme (EU-70%)	Identification and development of innovative biotechnological processes for a safe, clean and viable exploitation of metal-rich black shale ores for metal production, and design of an innovative model of development of mining activities	€3,390,202	1 October 2004–30 September 2007	[117,118]
BioMinE	Sixth Framework Programme (EU-65%)	Development, improvement and integration of bioleach processes for the recovery of metals from primary and secondary metal-bearing materials	€17,442,380	1 December 2004–31 October 2008	[119]
ProMine	Seventh Framework Programme (EU-65%)	Development of new mineral-based nano-products and new technologies for strategic mineral supply, to stimulate the extractive industry to deliver new products to manufacturing industry	€17,232,739.10	1 May 2009–30 April 2013	[120,121]
SysMetEx (ERASysAPP)	Seventh Framework Programme (EU-90%)	Investigation of biofilm formation on the surface of the world's most abundant copper mineral, chalcopyrite, by acidophilic microorganisms interacting with the copper mineral and each other	€2,537,425	2015–2018	[122–126]
BioMOre	Horizon 2020 (EU-100%)	Development of a novel base metal mining technology coupling in situ leaching and bioleaching technologies to deep deposits, in order to reduce environmental and social impacts and operating costs of mining techniques	€8,564,961.75	1 February 2015–31 July 2018	[127]
BioFlex	EIT RawMaterials (EU co-funded)	Bringing together partners with infrastructure and expertise in biometallurgy including metals bioleaching from ores and waste, biosorption from liquid streams, bioprecipitation and bio-electrochemistry	NA	1 January 2016–31 December 2018	[128]
RUBICON	The European innovation partnership (EIP) on raw materials	Development of a novel biotechnical process for sustainable exploitation of laterites, polymetallic deep-sea nodules and weathered sulphide ore deposits in the EU. Definition of a downstream process for the specific recovery of the metal by-products cobalt and scandium, together with nickel and other solubilised metals (e.g., Cu, Zn, V, and Mn)	NA	1 March 2016–1 March 2020	[110]
BIOCriticalMetals (ERA-MIN)	Seventh Framework Programme (EU-95%)	Combining microorganisms having the potential to be used in the extraction of metals, with methods (bio and nano) to adsorb them to exploit potentially critical high-tech metals (W, In, Ga, Te, and Mo) tailings	€546,366	1 June 2016–31 December 2019	[111,129,130]

Table 2. *Cont.*

Project Acronym	Funding Program	Project Goals	Project Budget	Duration	Ref.
BiotaWEE	LIFE Programme (EU-60%)	Recovering of valuable metals (mainly Cu, Ag and Au) from the non-metallic fraction of the Printed Circuit Boards (PCB) of different Waste Electric and Electronic Equipment (WEEE) by the application of an innovative more efficient 2-step bioleaching technology, combining aerobic and anaerobic treatment	€932,377	1 July 2018–31 July 2022	[112]
BioLeach	EIT RawMaterials (EU co-funded)	Development and improvement of bioleaching technology for specific local deposits to obtain raw materials (RMs) appropriate for industrial utilization and broad the utilisation of local sources	NA	1 April 2019–31 March 2022	[131]
BIORECOVER	Horizon 2020 (EU-100%)	Research and development of a new sustainable and safe biotechnological process for the selective extraction of a wide range of Critical Raw Materials (CRMs)	€6,337,277.50	1 June 2019–31 May 2023	[106,107,132]
RAWMINA	Horizon 2020 (EU-85%)	Implementation and standardisation of a continuous pilot process integrating novel bioleaching and nano-based materials for Sb, Co, Ge, and W selective recovery from Mine Waste (MW) from unexploited/underexploited metal-containing materials	€10,857,402.68	1 May 2021–31 October 2024	[108,109]

4. Conclusions and Perspectives

This review provided a comprehensive overview of the main aspects of e-waste recycling by considering the economic, environmental, and social implications of a sustainable recovery system as ruled by EU legislation. Within this context, recycling is one of the fundamental values to be pursued to sustain the fast and increasing demand for raw materials from which emerging and renewable technologies (i.e., PV cells, turbines, batteries, catalysts, and H_2-based technologies) are dependent. Through recycling, the huge value held within the so-called *urban mine* of collected e-waste and by-products generated along the materials supply chain could be restored through different recovery strategies.

Despite being a well-known biotechnological process carried out by bacteria and fungi, bioleaching is less popular among archaea, especially methanogenic species populating anaerobic sludge, sediment, and wastewater. In this work, we addressed the relationships between metals and archaea and their potential as effective catalysts for metals recovery, reporting current status, experimental studies, possible benefits, and drawbacks of using them in combination with different metals, including heavy-, precious-, and critical- ones. Among the few works currently available in the literature, there are not many mentions of methanogens applications on end-of-life devices and old- and new scrap metals. That considered, further studies should aim to demonstrate the process's effectiveness or ineffectiveness. In this context, the choice of e-waste or other scrap metals from the refining process should be strategic, and the target should be selected on certain criteria, such as the following: (1) CAGR indicator for raw materials and corresponding application: a high value means high demand; (2) EOL-RIR indicator: a low value implies a low recycling rate; (3) illegal transboundary e-waste: a high risk for the environment and public health due to the release of toxic pollutants, as well as being an emerging social issue.

The potential advantage of the application of methanogenic archaea to bioleaching is to combine the ability to reduce CO_2 and leach metals from end-of-life devices or residues from their manufacturing. This would allow the coupling of two different technologies, gas-to-gas and metallurgy, with two main end products: CH_4 and dissolved metals. Although few studies have reported on both metals' requirements and their effects on methanogenic archaea, assessing the feasibility of this approach and defining process bottlenecks and strategies to overcome them is a priority. Given the level of readiness (TRL $\geq$ 5) and the versatility (e.g., integrated bioprocess rather than downstream process) of the CH_4

production technology, the application of a combined process with e-waste treatment can be strategic not only for the recovery of valuable metals per se but, as an example, also for the implementation of other existing processes, such as anaerobic digestion, in terms of biogas upgrading and anaerobic sludge exploitation as a source of bioleaching microorganisms.

Finally, further studies should also consider valuable strategies for the efficient separation of metal from microbial biomass to return them to the production chain.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/recycling8010020/s1, Table S1: List of elements found in e-wastes and categorised as CRMs or non-CRMs. Each element is identified on the basis of geographical distribution (first-three holders in the world), application, occurrence, and circular economy (CE) indicators, i.e., EOL-RIR, IR, and CAGR%.

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