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Urban Biomining: New Challenges for a Successful Exploitation of WEEE by Means of a Biotechnological Approach

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13.1 Introduction

The production of end-of-life equipment, known as waste of electrical and electronic equipment (WEEE) or e-waste, is a direct consequence of the modern revolution of the electronic industry and of the constant evolution of technology. According to the United Nations Environment Programme (UNEP), a dramatic increase in the illegal import of e-waste, 200%–400% in

South Africa and 500% in India, can be estimated for the period between 2017 and 2020 (Schluep et al. 2009). Organic and inorganic components in WEEE can cause environmental problems if not properly managed. Today, in the United States and Europe, the main WEEE management strategies are incinerators and landfills and these can represent a serious threat to the environment and human health, due to contaminant leaching in soils and ground waters, and to a release of potentially hazardous by-products in the atmosphere (Robinson 2009; Tsydenova and Bengtsson 2011). On the other hand, base (e.g. Cu) and precious metals (e.g. Au, Ag and Pd) in WEEE could be employed as raw materials, making WEEE an attractive secondary resource of valuable elements (Oguchi 2013). In the last decade, the scientific community and industrial research have made a significant effort in developing techniques for the recovery of metal components from WEEE, especially by pyrometallurgical and hydrometallurgical approaches. Nevertheless, such techniques may be extremely polluting and not environmentally sustainable (Korte et al. 2000; Mecucci and Scott 2002; Cui and Zhang 2008; Tsydenova and Bengtsson 2011). Biohydrometallurgical strategies, based mainly on the ability of microorganisms to produce leaching agents, are gaining increasing prominence in this field. For instance, the technique known in mining activities as bioleaching (i.e. biological leaching) is considered a novel approach for metal mobilisation from various types of solids (Beolchini et al. 2012). The main advantages of biohydrometallurgical methods would be low operating costs (due to a low energy input), reduced environmental impact and a general minimisation of the end product (Ehrlich 2001; C. L. Brierley 2010).

This chapter aims to define where we are in the application of biotechnological strategies for the exploitation of WEEE and the recovery of high-added-value metals. In the first part, WEEE is described within its regulatory scenario and within its potential as a source of important critical metals. Then conventional treatments for metals recovery are briefly described, with their corresponding advantages and disadvantages. Finally, the main scientific knowledge in this field about the exploitation of biotechnological strategies is summarised and discussed, with a particular focus on the potential of different microbial strains in the application of *urban biomining* for an efficient use of e-waste as a secondary resource.

13.2 Electric and Electronic Waste as an Important Source of Secondary Raw Materials

13.2.1 Waste Production

WEEE is defined by the EU directive 2002/96/EC (European Parliament, 2002) as electrical or electronic equipment that is a waste within the meaning

of Article 1(a) of Directive 75/442/EEC (Council Directive, 1975), including all its components, sub-assemblies and consumables, which are part of this product at the time of discarding (Li et al. 2013). In general, electronic waste comprises a broad range of electronic and electrical products, including large household appliances (e.g. washing machines and television sets) and hand-held equipment (e.g. cellular phones and personal computers). The economic growth increases the ownership of electronics, decreases simultaneously the life span of the electronic goods and eventually leads to rapid growth in the amount of unwanted and obsolete electronics, so that WEEE can be considered as one of the fastest-growing waste streams (Bigum et al. 2013). According to Huisman et al. (2008), the estimated rate of global WEEE generated is ~40 million tons per year; in 2007, India produced 439 kton of computers, printers, washing machines, mobile phones and TVs. Assuming an Indian population of ~1.12 billion in 2007, this equates to 0.4 kg per capita (Ongondo et al. 2011).

The composition of e-waste is extremely variable. Many studies have examined WEEE composition, dividing its components into five categories, namely, ferrous metals, non-ferrous metals, glass, plastics and other materials, but others classification could be made. A typical composition of WEEE is given in Figure 13.1 (full dataset in Ongondo et al. 2011). The metallic component itself can be very different due to the collected equipment; for instance, typical metals in printed circuit boards (PCBs) are copper (20%), iron (8%), tin (4%), nickel (2%), lead (2%), zinc (1%), silver (0.2%), gold (0.1%) and palladium (0.005%) (He et al. 2006). Depending on the type of equipment from which it originated, WEEE may contain substances that are particularly hazardous, such as Hg in fluorescent tubes, CFCs in freezers and refrigerators or Cd in mobile phones (Crowe et al. 2003). Owing to its content

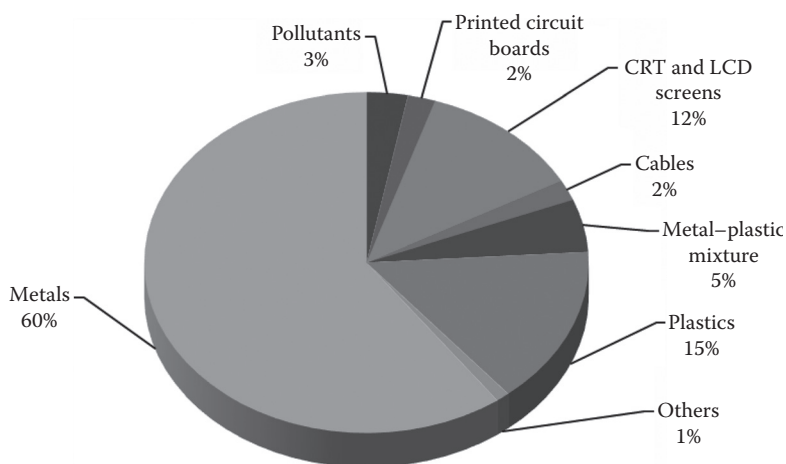


FIGURE 13.1
(See colour insert.) Typical material fractions in WEEE.

TABLE 13.1

Annual Import and Export of WEEE in the World

Country/Region	Annual Household Production (Million Tons)	Annual Export (Million Tons)	Annual Import (Million Tons)
USA	6.6	1.3	–
EU-25	7	1.9	–
Japan	3.1	0.62	–
China	3.1	–	2.0
India	0.36	–	0.85
West Africa	0.05	–	0.57
Total	20.21	3.82	3.42

Source: Adapted from Zoeteman, B. C. J., H. R. Krikke and J. Venselaar. 2009. *The International Journal of Advanced Manufacturing Technology* 47(5–8): 415–436.

in hazardous substances and to the volumes involved, the management of WEEE is an environmental issue of high priority; big amounts of WEEE are often exported from one country to another where disposal and treatment costs are lower or legislation is less restrictive (Table 13.1). The transboundary movement of WEEE has been particularly controversial. Initially, the main WEEE traffic routes were towards Asia (especially China) but, after the introduction of a tighter legislation in China, new destinations are chosen, such as Ghana, Nigeria, South Africa, Vietnam, India and the Philippines (Li et al. 2013). In addition, data about real waste flux among nations are very difficult to obtain because a very high percentage of WEEE disappear, leaving no official trace: a Greenpeace report (2009) states that 75% of European WEEE and 80% of American WEEE are illegally treated in developing countries. In order to control the mechanisms of waste import and export, some countries have adhered to the Organisation for Economic Co-operation and Development (OECD).

13.2.2 Metal and Rare Earth Elements

The diffusion of new technologies and economic growth causes a continuous request of raw material, especially metals, since they are key elements in the production processes of goods such as mobile phones, liquid crystal displays and other electrical and electronic equipment (EEE). Fourteen classes of raw elements have been identified as a very high risk of supply interruption for EU (Figure 13.2). The importance of these materials is connected to their growing demand, which, in turn, is driven both by the rate of production of new emerging technologies and by the growing rate of developing economies; on the contrary, geological scarcity is not considered in determining which raw elements are critical or not, at least within the next 10 years. Criteria for the identification of critical raw materials for EU have

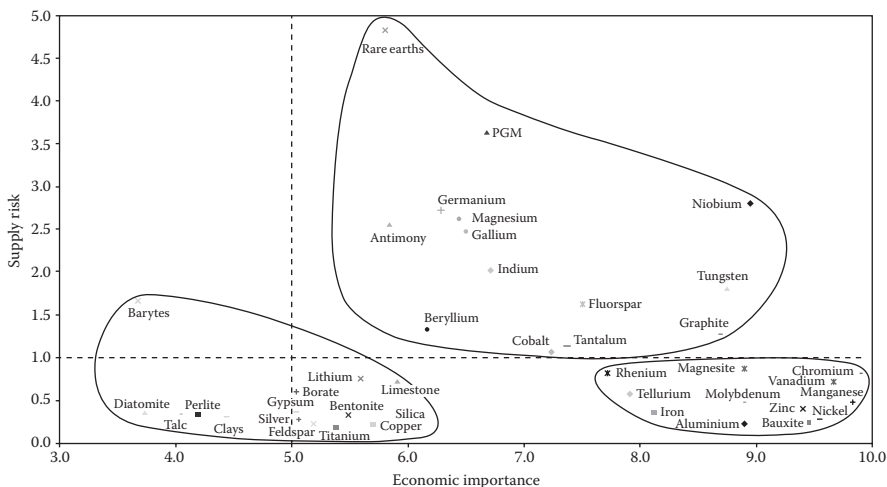


FIGURE 13.2
(See colour insert.) Critical raw materials in EU.

been recently established by the European Commission (2010); in particular, three aggregated indicators are used:

- Economic importance
- Supply risk (associated with political and socio-economic factors)
- Environmental risk (Figure 13.3)

According to such criteria, rare earth elements (REEs) are the most critical materials for EU. It is a group of 17 chemically similar metallic elements: the 15 lanthanides, plus scandium and yttrium. The demand for REEs derives from their key role in the production of electric and electronic equipment; for example, REEs are important constituents of permanent magnets, lamp phosphors, rechargeable NiMH batteries, catalysts and other applications. World demand for REEs has been estimated as around 133,600 tons per year, but the request is increasing and a demand of 210,000 tons is expected for 2015 (Humphries 2012). At the moment, China provides more than 90% of REEs and owns little less than 40% of the proven reserves (Binnemans et al.

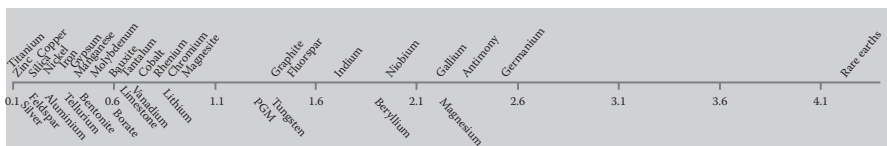


FIGURE 13.3
(See colour insert.) Critical raw materials according to their environmental country risk.

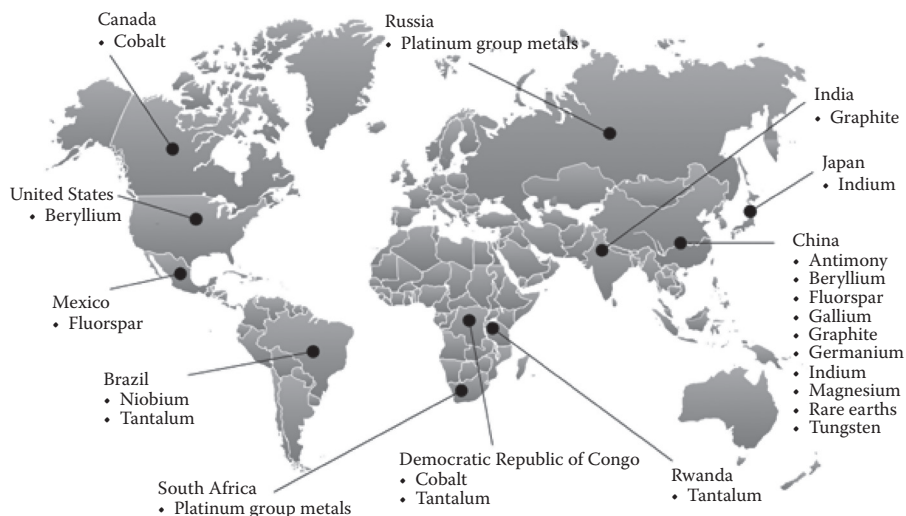


FIGURE 13.4

(See colour insert.) Production concentration of critical raw mineral materials.

2013). Moreover, China is the main provider in the market of other important elements, such as antimony, beryllium, fluorspar, gallium, graphite, germanium, indium, magnesium and tungsten (Figure 13.4).

The described situation is of concern for the whole world market and also for the environment. Using less critical metals as an alternative to critical REEs and investing in sustainable primary mining from old or new REEs deposits are two of the most promising strategies for facing the problems of resource depletion and the dependence of the European market on non-European countries (Binnemans et al. 2013). ‘Urban mining’ could be an additional alternative: the recovery of critical metals from a particular of type of waste, such as WEEE; this would allow to solve simultaneously both the economic issues, associated with the production of EEE, and the environmental issues, associated with their disposal.

13.2.3 Conventional Technologies for the Recovery of Metals

As mentioned above, the final disposal of electrical and electronic devices is a current issue of worldwide concern. WEEE management follows the principles set by waste hierarchy, reported within the Directive 2008/98/EC:

- a. Prevention
- b. Preparing for reuse
- c. Recycling

- d. Other recovery, for example, energy recovery
- e. Disposal

Landfill disposal and incineration are the main practices in waste management, despite some differences among countries, but they can pose serious threats to human health and the environment. Metals can be released from WEEE disposed in landfill sites by leaching processes, with consequences on the whole ecosystem, from the atmospheric to the aquatic and terrestrial compartments. Incineration reduces waste volumes but it is a source of very hazardous pollutants, such as dioxins and furans, which can be released into the environment if adequate flue gas cleaning systems are not implemented. In addition, incineration contributes significantly to the annual emissions of Cd and Hg (Crowe et al. 2003). In this context, the recovery of base valuable and precious metals (e.g. Au, Ag, Cu, Zn, Co, Y) from WEEE represents a valid alternative to landfills and incinerators. Indeed, WEEE can represent a concentrated source of metals, which were mined from ore minerals where their concentrations were lower. In addition, the extraction of such elements from ores consumes land and energy, produces wastewaters and releases huge amounts of sulphur dioxide and carbon dioxide, while metal reclamation from waste would reduce the phenomenon, with a substantial decrease in the environmental impacts. For example, the energy used to obtain 1 kg of Al by reclamation from waste is equal or less than 1/10 of that required for its extraction; recycling e-waste reduces the bauxite residues of about 1.3 kg and the emission of carbon dioxide and sulphur dioxide of 2 kg and 0.011 kg, respectively (Zhang et al. 2012). In this scenario, recycling policies are gaining more attention, and many countries have drawn up regulations for the management of WEEE. In particular, the European WEEE Directive (Directive 2012/19) aims at WEEE recycling to reduce the disposal of waste and 'to contribute to the efficient use of resources and the retrieval of valuable secondary raw materials'. Considering that WEEE can work as a source of 14 economically important raw materials identified by the European Commission, 'urban mining' looks more attractive than conventional management strategies.

The first step in recycling treatment schemes is the pre-treatment, by manual de-pollution, shredding, air classifiers/hoods, magnetic sorting and/or eddy-current separation, aimed at removing dangerous substances (CFCs, Hg, PCB) and optical systems. According to Bigum et al. (2012), the major pre-treatment outputs are manually sorted components (29%), a magnetic-iron (33%) and a residual plastic fraction (26%); any other fraction constitutes about 2%–3% of the flow. The overall output from a pre-treatment plant is (per 1000 kg of received high-grade WEEE):

- 114 kg of substances requiring special treatment according to regulation
- 165 kg of copper and precious metal fraction

- 381 kg of iron and magnetic steel
- 22 kg of aluminium
- 53 kg of fluff and residual waste
- 265 kg of plastic

Today, the subsequent treatment steps are based on pyrometallurgical, hydrometallurgical and electro-metallurgical processes. Pyrometallurgical processes require the heating of WEEE at high temperatures (often greater than 1000°C) to separate metals and impurities into different phases, so that valuable metals can be recovered. As a consequence, high energy requirements and the production of hazardous gases are associated with such strategy. Hydrometallurgical treatments are based on the use of leaching agents in aqueous solutions, such as strong acids and bases, often applied together with other complexing agents, such as oxalic acid, acetic acid, cyanide, halide, thiourea and thiosulphate. Compared to pyrometallurgical treatments, hydrometallurgical processes are less energy and cost demanding and require plants with relatively small capacities. Electro-metallurgy processes use electrical current to recover metals (e.g. electro-winning and electro-refining of copper, zinc and other elements), with high energy consumption. Recently, hybrid technologies have also been applied, which integrate the chemical approach (more efficient) with biological strategies (more environmentally friendly), thus taking advantage of the benefits of both chemical and biological leaching (Rocchetti et al. 2013).

13.3 Biohydrometallurgy: Main Mechanisms and Involved Microorganisms

Among the technologies for metal reclamation from WEEE, biohydrometallurgy is gaining increasing attention within scientific and industrial research. In biohydrometallurgy, the metabolic products of key microorganisms are the driving force in dissolving metal species into aqueous solutions, which are easier to manage or treat (Erüst et al. 2013). The specific knowledge about the metabolic mechanisms underlying biohydrometallurgical processes was first developed in the mining field, where microorganisms are exploited for large-scale operations of metal recovery from ores ('biomining'). Nevertheless, 'biohydrometallurgy' covers all the processes in which the mobilisation of elements from solid materials is mediated by microorganisms.

The first scientific evidence about the role of microorganisms in metal extraction (Colmer and Hinkle 1947) has led to the description and isolation from acid mine drainage (AMD) of the acidophilic, autotrophic,

iron- and sulphur-oxidising bacterium *Acidithiobacillus ferrooxidans* (formerly, *Thiobacillus ferrooxidans*; Temple and Colmer, 1951). Although it was not the first acidophilic strain with sulphur-oxidising activity to be described (namely, *Acidithiobacillus thiooxidans*; Waksman and Joffe, 1922), biohydrometallurgy originated from Colmer's discoveries, with the first industrial-scale extraction of copper from mine dumps through microbial action (early 1950s, Kennecott Copper Corporation; Zimmerley et al. 1958). Today, minerals/ores containing copper, gold and cobalt are processed on an industrial scale and promising results have been obtained for the industrial processing of sulphides of Ni, Zn, Mo Co, Ga, Pb and the platinum group metals (Ehrlich 2001; J. A. Brierley 2008; Ndlovu 2008; C. L. Brierley 2010; Lee and Pandey 2012).

In parallel with the increasing application of biohydrometallurgy, microorganisms and biochemical processes involved in metal mobilisation have been the object of growing investigations. The exploration of extreme environments (hot springs, volcanic regions, etc.) and the development of molecular biology techniques for detecting and identifying organisms have led to a broader knowledge of the microorganisms that interact with metals which that could be exploited in biohydrometallurgy. Today, the microorganisms that find real or potential application in biohydrometallurgy belong to all three domains of the tree of life: archaea, bacteria and eucarya (Ehrlich 2001; Olson et al. 2003; Norris 2007; Schippers 2007; Vera et al. 2013). An overview of the main strains involved (or potentially involved) in the biological treatment of WEEE for metal recovery and their mechanisms are provided in the next sections.

13.3.1 Fe/S-Oxidising Bacteria and Archaea

The transformation of base metal sulphides into water-soluble metal sulphates mediated by extremely acidophilic microorganisms (i.e. bioleaching) is the most known and commercially applied biohydrometallurgical process. Another successful and extensive commercial application of biohydrometallurgy is the bio-oxidation pre-treatment of refractory sulphide gold ores, where microorganisms are used to oxidise pyrites and other sulphides to expose gold occluded within the mineral matrix (Olson, Brierley, and Brierley 2003). Microorganisms involved in such processes belong to a non-phylogenetic group of acidophilic, aerobic and chemolithotrophic strains in the bacteria domain, often known as Fe/S-oxidising bacteria, due to their metabolism based on the oxidation of reduced sulphur compounds (and/or elemental sulphur) and ferrous ions. The main products are sulphuric acid (and a list of sulphur oxidation intermediates) and ferric ions. The most known and studied strains in this group are *At. ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans* but many other species are known today. In addition, iron-oxidising and sulphur-oxidising strains among archaea have been identified, so the microbial diversity in bioleaching and bio-oxidation processes is much wider than previously hypothesised (Johnson 2012; Dopson and Johnson 2012; C. L. Brierley and Brierley

2013; Vera et al. 2013). Indeed, leaching bacteria are distributed among α -, β - and γ -proteobacteria (*Acidithiobacillus*, *Acidiphilium*, *Acidiferrobacter*, *Ferroplasma*), Nitrospirae (*Leptospirillum*), Firmicutes (*Alicyclobacillus*, *Sulfobacillus*) and Actinobacteria (*Ferrimicrobium*, *Acidimicrobium*, *Ferrithrix*); leaching archaea belong mostly to Crenarchaeota phylum (*Sulfolobus*, *Acidianus*, *Metallosphaera*, *Sulfurisphaera*), although within Euryarchaeota there are two acidophilic iron(II)-oxidising strains (*Ferroplasma acidiphilum* and *Ferroplasma acidarmanus*; Vera et al. 2013).

These microorganisms can be roughly organised into three broad groups based on the temperature ranges for iron and sulphur oxidation: mesophiles (below 40°C), moderate thermophiles (about 45°C or more) and thermophiles (about 70°C or more). Obviously, there is not a precise temperature threshold that divides these groups; for example, *Acidithiobacillus caldus* and *Acidimicrobium ferrooxidans* are quite active from about 25°C to almost 55°C. The group of mesophiles is constituted by Gram-negative strains within bacteria domains: *Acidithiobacillus*, *Thiobacillus* and *Leptospirillum* are the main genera. *At. ferrooxidans* has been an object of particular interest, owing to its remarkably broad metabolic capacity: it can live aerobically either on the oxidation of iron(II) or reduced inorganic sulphur compounds (RISCs) to elemental sulphur, but the anaerobic growth is possible by the oxidation of hydrogen or sulphur coupled with iron(III) reduction (Pronk et al. 1992; Johnson 2012). The other species within the *Acidithiobacillus* genus can oxidise sulphur but not iron. Mesophilic iron oxidisers are mainly affiliated with the *Leptospirillum* genus (*L. ferrooxidans*, *L. ferriphilum*). Among moderate thermophiles, sulphur oxidisers are affiliated with Gram-positive genera within Firmicutes (*Sulfobacillus* and *Alicyclobacillus*), while iron oxidisers belong to Actinobacteria (*Ferrimicrobium*, *Acidimicrobium* and *Ferrithrix*). The thermophile group is formed by strains belonging to the archaea domain. Sulphur-oxidising archaea are affiliated mainly with the genera *Sulfolobus* and *Metallosphaera*, while iron oxidisers are affiliated with the genus *Ferroplasma*. Although relatively few works have been published about the thermophile group, the number of archaea known to be directly involved in mineral sulphide oxidation appears to be comparable with that of mesophiles and moderate thermophiles (Norris 2007). Indeed, novel isolates have been shown to grow on pyrite and chalcopyrite at about 90°C (Plumb et al. 2002). No psychrophilic sulphur-oxidising acidophiles have been described, although the sulphur- and iron-oxidising proteobacterium *Acidithiobacillus ferrivorans* is psychro-tolerant, and grows at 4–35°C (Dopson and Johnson 2012).

Sulphur oxidation follows different pathways in the three groups. Curiously, *At. ferrooxidans* can be considered as the most studied acidophile, but its sulphur-oxidising pathways are not so well known. Owing to difficulties in developing genetic techniques in acidophiles, a large proportion of the hypotheses regarding RISCs metabolic pathways in these prokaryotes are based on systems biology: putative genes have been assigned in several microorganisms. As best described by Dopson and Johnson (2012) and other

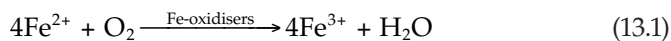
review works (Rohwerder and Sand 2007; Barrie Johnson and Hallberg 2008; Jones et al. 2012), the oxidation of elemental sulphur and RISCs in Gram-negative bacteria (such as *At. ferrooxidans* and related species) appears to be due to a dioxygenase enzyme (rather than a dehydrogenase as observed in neutrophilic bacteria), termed sulphur oxygenase reductase (SOR). The production of ATP is due to an F_0F_1 ATPase; the process includes the final electron acceptors cytochrome bo_3 and cytochrome c oxidases. In contrast, many enzymes as well as their encoding genes, have been characterised in *Acidianus ambivalens* (archaea), so our image of sulphur metabolism for archaeal acidophiles is much clearer than that in the bacterial acidophiles. In spite of this, little is known about RISC metabolism in Gram-positive acidophiles (e.g. *Sulfobacillus* spp.). The primary difference between archaea and Gram-negative bacteria is that S^0 is disproportionated in the former, as opposed to being oxidised (Dopson and Johnson 2012).

While many sulphur-oxidising acidophiles appear to be obligate aerobes (e.g. *At. thiooxidans* and *At. caldus*), others (e.g. *At. ferrooxidans* and *Acidiferrobacter thiooxydans*) can use ferric iron as an alternative electron acceptor and grow in anoxic environments. Very interesting mutualistic interactions have been described. For example, neither the sulphur-oxidising autotroph *At. thiooxidans* nor the iron-oxidising heterotroph *Ferrimicrobium acidophilum* can oxidise pyrite when grown in pure culture, but they can do so when grown in co-culture: ferric iron is generated by bio-oxidation of ferrous iron by *Fm. acidophilum*, which uses organic carbon (as C source) provided by *At. thiooxidans*; the dissolution of pyrite occurs for abiotic oxidation by Fe(III) ions produced (Okibe and Johnson 2004).

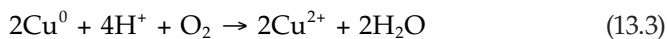
Although involved molecules are often not well characterised, the overall mechanisms of metal solubilisation can be considered as well established. Nevertheless, such knowledge refers almost exclusively to sulphide ores (Sand et al. 2001; Rohwerder and Sand 2007). Although metals in PCBs are in metallic form (zero-valence, with the exception of ferric iron), there is no reason to suppose that Fe/S-oxidising bacteria can mobilise metals from WEEE by a sort of direct mechanism. Owing to its unique ability to use both sulphur and iron, *At. ferrooxidans* has been broadly used as a model microorganism to study and describe the mechanisms mediated by leaching bacteria in solubilising metals. The solubilisation of metal sulphides by *At. ferrooxidans* and related strains has long been described as a process based on two independent mechanisms: a 'direct mechanism' (i.e. the direct enzymatic oxidation of the sulphur moiety of the metal sulphide) and an 'indirect mechanism' (i.e. the non-enzymatic metal sulphide oxidation by Fe(III) ions combined with enzymatic (re)-oxidation of the resulting Fe(II) ions; Sand et al. 2001). However, it is now completely accepted that the 'direct mechanism' of biological metal sulphide oxidation does not exist: the 'indirect mechanism' has been singled out as the sole mechanism and can be more appropriately defined as 'the non-enzymatic metal sulphide oxidation by Fe(III) ions combined with the enzymatic (re)oxidation of the resulting

Fe(II) ions' (Rohwerder et al. 2003). Anyway, a microbe attachment to the ore really occurs and enhances the rate of mineral dissolution, so both 'contact' and 'non-contact' mechanisms occur (Sand et al. 2001; D. E. Rawlings 2002; Rohwerder et al. 2003; Rohwerder and Sand, 2007). Fe/S-oxidising bacteria approach the mineral surface by creating a biofilm, whereas the majority of cells attach to the sulphide surface, and planktonic bacterial cells remain floating in the bulk solution. The attachment process is predominantly mediated by the extracellular polymeric substances (EPS) that create a complex slime and fill the space between the cell wall and the surface. In bioleaching with Fe/S-oxidising bacteria, metal dissolution occurs for acid leaching and/or oxidation attack; the main role of leaching bacteria consists of the generation and regeneration of leaching agents, mainly Fe(III) ions and protons.

Some authors have proposed reactions describing the main mechanisms of copper bioleaching from PCBs (Choi et al. 2004; Cui and Zhang 2008; Xiang et al. 2010; Zhu et al. 2011; Ilyaset al. 2013). In the presence of iron as the energy source for leaching strains, the bio-oxidation of Fe(II) produces Fe(III), which is responsible for the oxidation of Cu^0 (insoluble) in Cu^{2+} (soluble), according to Equations 13.1 and 13.2:



Cu leaching was also observed in the absence of iron, with elemental sulphur as the energy source. This suggests that part of zero-valence copper is solubilised by protons, although in such cases, molecular oxygen is involved:



During the dissolution of copper, Fe(III) ions are reasonably released from the PCB metallic component; Fe(III) ions will participate in the reaction (Equation 13.2) producing new protons by hydrolysis, and enhance copper solubilisation in Equation 13.3. The solubilisation of Zn, Ni and Al would follow the same mechanisms, according to their thermodynamic feasibility reactions:



13.3.2 Cyanogenic Microorganisms

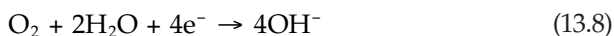
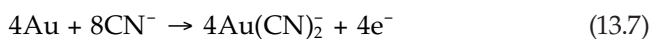
In the mining industry, bio-oxidation by Fe/S-oxidising bacteria is used to oxidise sulphides surrounding gold in refractory ores, in order to improve its accessibility for subsequent steps in the gold extraction circuit. Bio-oxidation is usually followed by chemical extraction of gold by complexation with cyanide ions (cyanidation) in alkaline conditions. The application of the cyanidation process for the extraction of gold and silver from ores has been known since 1898 (Smith and Mudder 1991). Cyanide is one of the few compounds (e.g. chlorides, other halides and thiourea) able to form water-soluble complexes with gold, with high extraction efficiencies even in gold amounts as small as 0.25% (Smith and Mudder 1991; Syed 2012). Recently, the biological production of cyanide by microorganisms and its potential application are gaining new attention by the scientific community. The use of biogenic cyanide (i.e. biocyanidation) may offer a valid alternative to conventional gold extraction techniques; the expressions 'alkaline bioleaching' or 'heterotrophic bioleaching' are often used to indicate the solubilisation of metals by cyanogenic microorganisms (G M Gadd 2000; Cui and Zhang 2008; Hennebel et al. 2013; Mishra and Rhee 2014), although the latter is also used for bioleaching by fungi (Aung and Ting 2005; Santhiya and Ting 2006; Sabra et al. 2011). Moreover, Au is not the sole target in biocyanidation, since other metals (such as Ni, Fe, Ag and Zn) can form stable complexes with CN^- (A. Smith and Mudder 1991). The use of cyanogenic strains would allow the mobilisation of metals from solids under alkaline conditions and this might be advantageous in view of commercial-scale operations. For instance, copper mobilisation from carbonate-rich rocks is known to be very acid consuming (Krebs et al. 1997; Dopson and Johnson 2012), so, the application of autotrophic Fe/S-oxidising microorganisms or heterotrophic microorganisms forming organic acids is very likely unfeasible. Moreover, environmental problems due to a potential release of cyanide in natural waters may be significantly reduced by exploiting the capability of cyanogenic bacteria in detoxifying cyanide by the enzyme β -cyanoalanine synthase (Macadam and Knowles 1984).

Hydrocyanic acid (HCN) is formed by a variety of heterotrophic bacteria (e.g. *Chromobacterium violaceum*, *Pseudomonas fluorescens*, *P. aeruginosa*) and fungi (e.g. *Marasmius oreades*, *Clitocybe* sp., *Polysporus* sp.); the production of HCN by *Pseudomonas plecoglossicida* was just known in 2006 (Brandl and Faramarzi 2006). In general, cyanide formation (i.e. cyanogenesis) by strains in bacteria domain has attracted larger attention, compared to fungi (Campbell et al. 2001; Faramarzi et al. 2004). Although archaea-gold interactions are known (Reith et al. 2007), cyanogenesis in archaea has been not reported yet.

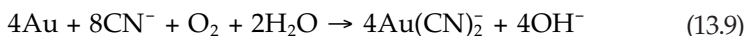
C. violaceum is a mesophilic, Gram-negative β -proteobacterium, described at the end of the nineteenth century, and it is probably the most studied microorganism for potential application in low environmental impact processes of Au recovery from ores (Lawson et al. 1999; de Vasconcelos et al. 2003). HCN is produced as a secondary product of the oxidative decarboxylation

in the catabolism of glycine, the main precursor for cyanide. The production of HCN occurs in a short period of the growth curve: between the end of the exponential phase and the early stationary phase in *C. violaceum*, just at the beginning of the stationary phase in the genus *Pseudomonas*. The involved enzyme is called HCN synthase (encoded by the *hcnABC* operon in *C. violaceum*); the amount of cyanide produced typically ranges around 2–50 mg/L (Brandl et al. 2008; Pham and Ting 2009). In the late stationary phase, *C. violaceum* detoxifies cyanide by converting it to β -cyanoalanine (Knowles and Bunch 1986).

Au dissolution by (bio-)cyanidation consists of an anodic reaction (Equation 13.7) and a cathodic one (Equation 13.8):



The overall reaction is known as Elsner's equation, as shown in Equation 13.9 (Hedley and Tabachnick, 1958; Smith and Mudder, 1991; Kita et al. 2006):



Cyanidation of other metals follows very similar reactions (Campbell et al. 2001; Faramarzi et al. 2004). HCN pK_a is 9.3 but optimal pH for cyanogenesis in *C. violaceum*, and other cyanogenic bacteria, ranges between 7 and 8 (Lear et al. 2010). At physiological pHs, the main compound is HCN, of which a large amount is lost via volatilisation and little cyanide is available for metal complexation; despite this, cyanide anions are the most available form at a pH equal or greater than 10.5, values that compromise bacteria activity and life (Knowles and Bunch 1986). Another issue is related to oxygen availability. In the growth phases in which cyanogenesis occurs, *C. violaceum* rapidly consumes dissolved oxygen for respiration; therefore, little molecular oxygen is available for Au dissolution (Equations 13.6 to 13.8). In congruence, Kita and co-authors (2006) have described a decrease in Au dissolution from solids due to low dissolved oxygen concentrations.

13.3.3 Potential of Fungi in Metal Recovery from WEEE

Many fungal species can survive and grow in adverse conditions (e.g. low pH, low temperature) and show high tolerance levels to various types of contaminants (Gadd 2010). Bioremediation strategies based on the exploitation of fungi have also been proposed. In particular, bioleaching of metals by fungi (i.e. 'fungal leaching' or 'heterotrophic leaching') has been mostly investigated for metal extraction from low-grade ores and mine tailings (Mulligan et al. 2004), industrial waste (e.g. spent refinery catalysts; Aung and Ting 2005; Santhiya and Ting 2006) and wastewater

sludge (More et al. 2010). Considering the ability of fungi to modify metal speciation and mobility, such microorganisms could also offer a potential alternative in the recovery of critical metals from WEEE, where base valuable and precious metals are in zero-valence form (with the exception of ferric iron). Fungi can solubilise metals through several mechanisms, but the production of weak organic acids is considered to be the most important mechanism of metal solubilisation. Indeed, citric, oxalic and gluconic acids can solubilise metals by forming water-soluble complexes (complexolysis; Burgstaller and Schinner 1993; Bosecker 1997). In addition, carboxylic acids produced by fungi can attack the mineral surface by their protons and lead to a release of associated metals (acidolysis; Gadd 2007). Metal complexation can occur also with functional groups on the cell wall surface (e.g. carboxyl, carbonyl, amine, amide, hydroxyl and phosphate groups; Baldrian 2003).

The main scientific contribution of fungal leaching on WEEE is by Brandl and co-authors (2001), where *Aspergillus niger* and *Penicillium simplicissimum* were investigated. *A. niger* showed the best efficiency in Cu mobilisation. Compared to lithotrophic bacterial leaching, fungal leaching offers the advantage of operating at mildly acidic conditions, which may minimise the eventual phenomenon of H₂S production that results from the addition of strong inorganic acids (Sabra et al. 2012).

13.4 State of the Art of Biohydrometallurgy in Metal Extraction from WEEE

The early works about the recovery of metals from e-waste by means of biological strategies have involved the exploitation of either of leaching bacteria/archaea (*Sulfolobus* sp., *At. ferrooxidans* and *At. thiooxidans*; Bowers-Irons et al. 1993; Brandl et al. 2001) or of bacteria and fungi that are able to produce 'biological surface active compounds' (BSAC; *Bacillus* sp., *Saccharomyces cerevisiae* and *Yarrowia lipolytica*; Hahn et al. 1993). These studies have demonstrated that biomobilisation of metals from WEEE is possible and may be advantageous in view of commercial applications. New research has been carried out subsequently and the number of papers in this field is increasing; nevertheless, the number of studies is still limited and new research is needed to reach a full-scale application. The biohydrometallurgical studies, published in international scientific journals between 2001 and early 2014, have focussed on the recovery of base valuable and precious metals from PCBs (also known as PWBs, printed wire boards): Fe/S-oxidising bacteria (i.e. leaching bacteria) can be exploited to recover base valuable metals such as Cu and Zn (Table 13.2), while cyanogenic strains are suitable for the recovery of precious metals, such as Au (Table 13.3).

TABLE 13.2

Summary of the Main Scientific Paper within Base-Valuable Metal Recovery by Bioleaching

Type of E-Waste	Microorganisms	Scale ^a	Energy Source	Temperature	Metals	References
PCBs (Tv)	<i>At. ferrooxidans</i> + <i>At. thiooxidans</i> + <i>L. ferrooxidans</i>	Flask (10 g/L)	Pyrite, Fe(II), S ⁰	35°C	Cu	Bas et al. (2013)
PCBs (unspecified)	AMD ^b (<i>Acidithiobacillus</i> spp., <i>Galianella</i> spp., <i>Leptospirillum</i> spp.)	Flask (20 g/L)	Fe(II)	30°C	Cu	Xiang et al. (2010)
PCBs (unspecified)	<i>S. thermosulfidooxidans</i> + <i>T. acidophilum</i>	Reactor (150 g/L)	S ⁰ (biogenic)	45°C	Cu, Zn, Ni, Cd, Al, Cr, Pb	Ilyas et al. (2014)
PCBs (unspecified)	<i>S. thermosulfidooxidans</i> + <i>T. acidophilum</i>	Column (10 kg)	Fe(II), S ⁰	45°C	Cu, Zn, Ni, Al	Ilyas et al. (2010)
PCBs (unspecified)	Municipal activated sludge	Flask (6.7 g/L)	S ⁰	22°C	Cu, Zn, Ni, Cd, Al, Cr, Pb	Karwowska et al. (2014)
PCBs (unspecified)	<i>At. ferrooxidans</i> + <i>At. thiooxidans</i> isolated from sewage treatment plant (China)	Flask (18 g/L)	Fe(II), S ⁰	32°C	Cu, Zn, Ni, Pb	Liang et al. (2010)
Poly-metallic concentrate (PCBs)	AMD ^b (China)	Flask (12 g/L)	Fe(II)	30°C	Cu, Zn, Ni, Al	Zhu et al. (2011)
Poly-metallic concentrate (unspecified WEEE and end-of-life vehicles)	<i>At. ferrooxidans</i> + <i>At. thiooxidans</i> + <i>L. ferrooxidans</i>	Reactor (10 g/L)	Fe(II)	35°C	Cu, Zn	Lewis et al. (2011)
Unspecified WEEE	<i>At. ferrooxidans</i> + <i>At. thiooxidans</i>	Flask (50 g/L)	Fe(II), S ⁰	30°C	Cu, Zn, Ni, Al	Brandl et al. (2001)

Note: Additional studies are discussed in the main text.

^a Optimal pulp density for Cu recovery in brackets.

^b Bacteria enriched from acid mine drainage (AMD).

TABLE 13.3

Summary of the Main Scientific Paper within Gold Recovery by Cyanogenic Bacteria

Type of E-Waste	Microorganisms	Scale ^a	Energy Source	Temperature	Metals	References
PCBs (PC)	<i>C. violaceum</i> + <i>P. aeruginosa</i>	Flask (10 g/L)	Luria Broth	30°C	Au, Cu, Fe, Zn, Ag	Pradhan and Kumar (2012)
Unspecified WEEE (mainly PCBs)	<i>C. violaceum</i> (mutation for alkaline conditions)	Flask (5 g/L)	Luria Broth	30°C	Au, Cu	Natarajan and Ting (2014)
Unspecified WEEE (mainly PCBs)	<i>C. violaceum</i> (engineered strains)	Flask (5 g/L)	Luria Broth	30°C	Au	Tay et al. (2013)
Unspecified WEEE	<i>C. violaceum</i> , <i>P. fluorescens</i> <i>migula</i>	Flask (5 g/L)	n/a	n/a	Au, Cu	Pham and Ting (2009)

Note: Additional studies are discussed in the main text.

^a Optimal pulp density for Cu recovery in brackets.

13.4.1 Recovery of Cu and Other Base Valuable Metals from WEEE by Bioleaching with Fe/S-Oxidising Strains

A first study by Brandl and co-authors (2001) has compared the capability of two microbial groups in the recovery of base valuable metals (i.e. Al, Cu, Ni, Pb and Sn) from powdered e-waste: mesophilic Fe/S-oxidising bacteria (*At. thiooxidans* and *At. ferrooxidans*) and fungi (*Penicillium simplicissimum* and *A. niger*). Their results have suggested that Al and Cu biomobilisation efficiencies are highly affected by the concentrations of solids to be treated (i.e. pulp density), while Ni and Zn are more easily recovered by bioleaching processes: with 100 g/L pulp density, the solubilisation decreased from >90% to 0%–5% and from 65 to 20% for Al and Cu, respectively, while Zn and Ni removal efficiencies were still high (>95% for Zn, about 60% for Ni). The same study showed that bioleaching with adapted fungi allows operating with very low amounts of e-waste (1–10 g/L). We have found references to another scientific work dealing with the application of fungi in the recovery of metals from WEEE (Hahn et al. 1993). Other works have confirmed the problems associated with a high solid concentration and have also dealt with other important constraints. According to the scientific literature, the main aspects that need a deep investigation and optimisation are particle size, solid concentration, kind and dosage of energy source, bacterial inoculum size and style, initial pH and temperature. Mainly, elemental sulphur and/or ferrous ions have been tested as suitable energy

sources for leaching bacteria, although the use of pyrite (FeS_2), as a source of both iron and sulphur, has been recently reported (Ilyas et al. 2010; Bas et al. 2013). Studies have reported that the lower the particle size, the higher the metal removal rate, unless the particle size is too small (Wang et al. 2009; Zhu et al. 2011; Ilyas et al. 2014). In this case, particle–particle collisions would lead to bacterial cell damage. Nevertheless, it is not possible to fix a specific limit because such a phenomenon also depends upon shaking conditions and reactor type.

The majority of the papers about the recovery of base valuable metals from WEEE by bioleaching address the application of mesophilic Fe/S-oxidising bacteria, such as *At. ferrooxidans*, *At. thiooxidans* and *L. ferrooxidans* (11 papers out of 16), while a smaller number deals with the exploitation of moderate thermophilic strains, such as the bacterium *Sulfobacillus thermosulfidooxidans* or, among archaea, *Sulfolobus*-like organisms (4 papers out of 16; see also Table 13.2). Cu is one of the most studied metals; some papers have addressed other metals, too, but optimisation studies have focussed mainly either on Cu or on the total amount of metals (Liang et al. 2013; Ilyas et al. 2014). According to the scientific literature, Zn, Cu, Cd and Ni can be removed with high efficiency, although precipitation and re-complexation phenomena can occur (in the presence of either S^0 or Fe^{2+} , as energy source). The metal precipitation phenomenon may be favoured when Fe(II) is used as the energy source, since jarosite is produced in acidic environments (Fonti et al. 2013). Zhu and co-authors (2011) have observed that a longer incubation time determines a high jarosite precipitation, with slowdown in Cu removal. Indeed, solubilised metals can co-precipitate with Fe(III) in jarosites, and/or jarosite may form a layer on the surface of PCB crumbs and cause passivation (Choi et al. 2004; Zhu et al. 2011). Ni and Cd appear to be removed in a shorter time than Zn and Cu, which could require several days of treatment. In addition, the solubilisation of Cu, Pb, Ni and Cr improves when the temperature is increased to 37°C ; air bubbling appears to be insignificant (Karwowska et al. 2014). Very interestingly, the solubilisation of Cr is favoured in the co-presence with microorganisms able to produce BSAC, such as *Bacillus subtilis* and *Bacillus cereus* (Karwowska et al. 2014).

The analysis of the scientific literature indicates that the direct growth of microorganisms in the presence of e-waste is not advisable because of toxic effects on living cells that would allow processing just small concentrations of waste materials (i.e. 1–5 g/L). Although Fe/S-oxidising bacteria are known to tolerate high metal concentrations (Tuovinen et al. 1971; Das et al. 1997; Leduc et al. 1997; Nies 1999; Dopson et al. 2003; Watkin et al. 2009), a solid concentration equal/greater than 10 g/L can cause an activity depletion in *At. ferrooxidans* and *At. thiooxidans* (Brandl et al. 2001; Vestola et al. 2010). Such a toxic effect occurs not only due to the high metal concentration but also due to the non-metallic component of PCBs and other e-waste (i.e. plastic and organic compounds, such as isocyanates, acrylic and phenolic resins, epoxides and phenols; Ludwig et al. 2002). The majority of the authors have

tackled this problem using various strategies (one of the following or more than one): (1) two-step bioleaching, in which the biomass is produced in the absence of electronic scraps and the solid to be treated is added after growing (Brandl et al. 2001; Liang et al. 2010; Xiang et al. 2010; Karwowska et al. 2014); (2) pre-adaptation of microorganisms (Ilyas et al. 2007; Yang et al. 2009; Ilyas et al. 2010; Liang et al. 2010; Ilyas et al. 2014); (3) pre-treatment for removing the non-metallic component or to stabilise the pH at optimal values for bacteria (Ilyas et al. 2010; Zhu et al. 2011); and (4) high inoculum size (Yang et al. 2009; Zhu et al. 2011). A study by Liang et al. (2010) have demonstrated that in a bioleaching treatment with a two-step strategy, multiple PCB additions (4–8 g/L) at different times can improve the feasibility of Cu, Ni, Zn and Pb removal.

The use of indigenous Fe/S-oxidising bacteria appears to be more advantageous than type or purchased strains, and mixed cultures are shown to be more efficient than single cultures (Ilyas et al. 2007; Wang et al. 2009; Liang et al. 2010; Zhu et al. 2011; Karwowska et al. 2014). In an interesting study by Karwowska and co-authors (2014), Zn, Cu, Cd, Ni and Cr have been solubilised from crumbled PCBs (two main fractions were considered: $\phi = 0.1 \div 0.25$ and $\phi > 2$ mm) by a mixture of municipal activated sludge and municipal wastewater (containing *Thiobacillus thioparus*, *Thiobacillus denitrificans*, *At. thiooxidans* and *At. ferrooxidans*, according to PCR-based techniques). A maximum efficiency (of about 70%, 90%, 100%, 60% and 15%) has been obtained in the presence of S⁰ 1% and solid 6.7% (w/v). Bacteria enriched from AMD (identified genera *Acidithiobacillus*, *Galionella* and *Leptospirillum*) have been reported to remove about 95% Cu from PCB powder in 5 days without the adaptation step, under the initial conditions of pH 1.5 and 9 g/L Fe(II) (Xiang et al. 2010). Similarly, Zhu and co-authors (2011) reported that unidentified leaching bacteria enriched from AMD can solubilise about 90% Cu from metal concentrates (PCBs pre-treated to remove the whole non-metallic component), in the presence of ferrous ions (optimum: 9–12 g/L) and with a solid concentration up to 12 g/L. At higher solid concentrations, Cu extraction efficiencies decreased, but considering that this study was performed only on the metallic part of PCBs, the actual metal content was very high. Lewis and co-authors (2011) have carried out studies on the recovery of Zn and Cu from polymetallic concentrates (obtained by physical pre-treatment of a mixed feed of metallic scraps, WEEE and end-of-life vehicles) by bioleaching with a copper-adapted consortium of mesophilic Fe/S-oxidising bacteria (*At. ferrooxidans*, *At. thiooxidans* and *L. ferrooxidans*). Pure cultures of *At. ferrooxidans* can remove about 100% Cu from PCBs powder in 36 h, in the presence of Fe(II) 9 g/L and pulp density 25 g/L (Yang et al. 2009); a mixed culture of non-indigenous *At. ferrooxidans*, *At. thiooxidans* and *L. ferrooxidans* can remove about 90% Cu from powdered Tv PCBs in the presence of Fe(II) 9 g/L (80% Cu with 50 g/L pyrite) and solid 10 g/L (Bas et al. 2013). Nevertheless, no information about the mobilisation of the other metals is given in such papers. In addition, many studies with type/wild strains and/

or monocultures have reported that the pH was maintained low (around 2 or less) by adding concentrated sulphuric acid. It is not always clear if these operations for pH controlling were carried out also for the abiotic control tests as this could significantly change the scientific impact of the results and should be taken into account when bioleaching studies with different characteristics are compared.

According to Ilyas and co-authors, moderate thermophilic Fe/S-oxidising bacteria (working at 45°C or more, vs. 32–35°C optimal for mesophiles) would determine higher rates of metal solubilisation than mesophilic and extremely thermophilic strains (Ilyas et al. 2007; Ilyas et al. 2010; Ilyas et al. 2013; Ilyas et al. 2014). Mixed cultures of chemolithotrophic (e.g. *S. thermosulfidooxidans*) and heterotrophic acidophiles (e.g. *Thermoplasma acidophilum*) would offer greater bioleaching potential, although to gain high metal bioleaching efficiencies (about 70%–80% for Ni, Al and Zn, 90% for Cu, with 150 g/L pulp density; Ilyas et al. 2014) a preadaptation step for the microorganisms involved is needed. Mesophilic Fe/S-oxidising strains have been tested at 50°C and an increase in the Cu solubilisation rate was observed, but it was confirmed to be due to abiotic processes (Lewis et al. 2011). Ilyas and co-authors have also demonstrated that sources of biogenic S⁰ from desulphurisation refinery plants can be a suitable growth substrate (greater sulphur oxidation in a shorter time period). This could contribute to making the bioremediation process more economical, and avoid the addition of ferrous ions in the medium (Ilyas et al. 2014).

Bioleaching strategies are not suitable when the main objective is the recovery of Pb and Sn, since the bioleaching approach appears to suffer of Pb and Sn precipitation phenomena (Pb would precipitate as PbSO₄, Sn as SnO). All the scientific articles have reported Pb and Sn precipitation, when these two metals were investigated, there were just two exceptions; some authors had also reported a high level of Cu precipitation (Choi et al. 2004; Cui and Zhang 2008). Low iron concentrations could reduce the phenomenon, but concerns about the application of bioleaching strategies are still reasonable: further steps of treatment could be required, with an increase in cost and environmental impact. Although the study by Brandl and co-authors, which we discussed above, would suggest that bioleaching with Fe/S-oxidising bacteria (i.e. 'lithotrophic leaching') could represent a more advantageous strategy, the exploitation of fungi allowed the mobilisation of Sn and Pb, which usually precipitate in bioleaching (Sn: 60% with *P. simplicissimum*, 40% with *A. niger*; Pb: 100% with *P. simplicissimum*, 40% with *A. niger*; 1 g/L pulp density). In addition, bioleaching techniques may be characterised by a high environmental impact, if a high amount of acid and a long period of treatment are needed (Beolchini et al. 2013), so a combined approach could improve the feasibility of an eventual bioleaching process; live fungi could be substituted by a direct use of organic acids produced in optimal conditions (gluconic, oxalic, citric and ascorbic acids). For instance, commercially available gluconic acid (Naglusol™) is known to solubilise >97% of Pb, Ni, Sn and Zn with

a scrap concentration of 100 g/L (Raimann 1996). However, in this regard, we have to mention that studies of toxicity assessment by leaching procedures (e.g. toxic characteristic leaching procedure (TCLP), synthetic precipitation leaching procedure (SPLP) and waste extraction test (WET)) have shown that environmental risks due to Pb appear to be relatively low (Ilyas et al. 2014). A similar approach has been studied by Choi et al. (2004) to improve copper solubilisation and increase the feasibility of a bioleaching process by adding citric acid during the process.

Solid concentration is a very important constraint in the development of commercial-scale processes. At present, among the papers analysed, only three have attempted to scale up from flask experimentation. 150 g/L of powdered PCBs (pre-treated by acid leaching to stabilise pH and reduce toxic effects) can be processed in a 2.5 L stirred-tank reactor (STR) with biogenic S^0 as a sole energy source for bacteria, while 10 kg can be treated by column bioleaching (about 1.2 L volume) in the presence of both Fe(II) and S^0 powder, after removing non-metallic components by a high-density saturated solution of NaCl (Ilyas et al. 2010; Ilyas et al. 2014). The recovery of more than 95% of Cu and Zn is possible in 50 h with 100 g/L pulp density and in the presence of Fe(II) 3 g/L as the sole energy source, by a two-step bioleaching strategy in a batch-mode reactor with ceramic rings as biofilm carriers (Lewis et al. 2011): in these conditions, mesophilic bacteria have been demonstrated to favour the solubilisation of Cu (Zn solubilisation was probably abiotic) and regenerate ferric ions.

13.4.2 Recovery of Au and Other Metals from WEEE by Cyanogenic Bacteria

A relevant interest in the potential utilisation of WEEE as a secondary source of raw materials is related to its content in gold and other precious metals. Au is used in electronics for its excellent resistance to corrosion and high electrical conductivity. Existing processes for recovering Au from electronic waste are based on a pyrometallurgical or hydrometallurgical approach and are extremely polluting and not environmentally sustainable (Korte et al. 2000; Mecucci and Scott 2002; Cui and Zhang 2008; Tsydenova and Bengtsson 2011). Although bacterial cyanide production has been known for many years and gold mobilisation by *C. violaceum* has already been reported (A. D. Smith and Hunt 1985; Campbell et al. 2001; Faramarzi et al. 2004; Faramarzi and Brandl 2006; Kita et al. 2006; Lear et al. 2010), very few works in the scientific literature address the extraction of gold (and/or other metals) from WEEE by cyanogenic strains. Among these, just four papers have addressed the topic with a quantitative approach (Table 13.3).

An analysis of the scientific literature available has revealed that the efficiency of gold recovery from WEEE usually ranges from 5% to 30%, with the exception of the study by Pradhan and Kumar (2012) that has reported 10 times higher efficiencies; on the contrary, in the early reports about the

exploitation of cyanogenic microorganisms from minerals and other solids (e.g. gold powder) gold solubilisation efficiencies reached even 100% (Campbell et al. 2001; Faramarzi et al. 2004; Kita et al. 2006; Brandl et al. 2008). The rate of Au dissolution is known to depend upon several factors, including cyanide concentration, particle size, dissolved oxygen concentration, T, pH and competition with other metals for cyanide complexation. In biological cyanidation processes, pH is probably the most important constrain because it significantly affects both the bacterial growth rate and the availability of cyanide ions. Indeed, the recovery of gold by cyanidation requires very alkaline conditions (Windom et al. 1989), which are disadvantageous for the growth of *C. violaceum* (Knowles and Bunch 1986). In turn, a depletion of bacterial activity determines low or no cyanogenesis; therefore, low or no CN⁻ ions are available for gold complexation. Researchers have faced this issue by two-step bioleaching strategies (all the studies in Table 13.3) or by using strains adapted to high pH values. Natarajan and Ting (2014) induced a strategic mutation in *C. violaceum* by exposing cells to *N*-nitroso-*N*-ethyl urea (100 mM), which allowed the selection of *C. violaceum* cells capable of growth in alkaline media (pH 9–10). Without mutation, pH 10 was inhibitory for the wild strain to grow. In 2013, Tay and co-authors have reported on Scientific Reports (*Nature*) the construction of two metabolically engineered *C. violaceum* strains, able to produce more cyanide than wild-type bacteria. The two engineered strains were allowed to recover from WEEE more than twice the gold dissolved by wild-type strains.

The best gold recovery efficiencies were obtained with a very low e-waste pulp density. In the study by Natarajan and Ting (2014), cells of *C. violaceum* (mutated for alkaline growth) solubilised about 5% of gold in the presence of 5 g/L of non-pre-treated powdered WEEE; an acid pre-leaching allowed to increase the efficiency up to 22.5% of total Au. Pham and co-authors (2009) have obtained slightly higher yields (about 10%) from non-pre-treated e-waste, either by wild strains of *C. violaceum* or by *P. fluorescens*. However, all the studies considered here have reported a decrease in Au solubilisation efficiency with a higher solid concentration. Biocyanidation of powdered WEEE previously treated by bioleaching with *At. ferrooxidans* has allowed working with solid up to 40 g/L, with gold extraction values comparable to those with un-pre-treated solid 5 g/L (Pham and Ting 2009). Pradhan and Kumar have obtained very high values of gold extraction from powdered PCBs in the presence of mixed culture of *C. violaceum* and *P. aeruginosa*; with a 10 g/L pulp density, about 85%, 75% and 50% of Cu, Au and Zn, respectively, were solubilised. Nevertheless, in the presence of the sole *C. violaceum*, extraction yields were comparable or just slightly lower. However, observed differences in gold solubilisation efficiencies appear to be closely related to the property and composition of the e-waste to be treated, but no study has addressed this point.

C. violaceum appears to be the most suitable strain for gold recovery by biocyanidation. Brandl et al. (2008) compared *C. violaceum* and *P. fluorescens* for

the production of cyanide and subsequent formation of dicyanoaurate complex and reported a higher solubilisation of gold for *C. violaceum*. Other information about the performances of different cyanogenic microorganisms has been obtained from research on minerals or pure metal powder (Campbell et al. 2001; Faramarzi et al. 2004; Faramarzi and Brandl 2006; Kita et al. 2006).

In general, the identification of the best operating conditions is the main challenge to face toward the real application of biocyanidation to WEEE. Another important issue to face is related to the presence of other metals than Au. Cu and other base valuable metals in WEEE compete with Au in the complexation with CN^- anions. For this reason, some authors suggest to pre-treat powdered WEEE in order to remove most metals. For instance, Natarajan and Ting (2014) used 6 M nitric acid, which resulted in a removal of nearly 80% of Cu, between 55% and 70% for Ag, Zn, Ni, Pb, Fe and Al, and about 90% for Sn. Moreover, there is a need for studies that deal with the effect of WEEE heterogeneity in structure and composition. No study has addressed a scale-up of the process yet.

13.5 New Challenges for the Development of Sustainable Processes Based on a Biotechnological Approach

Incineration or landfill disposal of WEEE represent both an environmental threat and an important economic loss. Today, it is clear that WEEE is a very promising secondary source of critical metals. Various chemical and physical techniques have been proposed for the reclamations of these elements; nevertheless, high costs and environmental impacts are often associated with such techniques, due to metal speciation and other characteristics of e-waste. Bacteria, archaea and eucarya domains offer a variety of microorganisms that can find (or have already found) real application in biohydrometallurgy. This could offer alternative, cost-efficient and environmentally sustainable strategies for the recovery of base valuable and precious metals from WEEE. Despite the wide heterogeneity in WEEE composition, microorganisms with particular metabolic capabilities (e.g. Fe/S-oxidising strains, cyanogenic bacteria) have shown to be successfully applied for the extraction of various metals from this waste. In the case of bioleaching by Fe/S-oxidising strains, scale-up attempts have already been performed. In general, however, full-scale applications require further investigations; an optimisation of the operating conditions is needed and many aspects still must be addressed, such as the evaluation of costs and actual environmental impacts. At the same time, various insights and possible solutions have recently been suggested. The application of more than one biological strategy could lead to high solubilisation efficiencies for many more elements compared to a singular approach, and it could also offer the possibility to face issues highlighted by researchers

and still just partially solved, such as the acid-neutralising power of WEEE and the toxicity of its components. Biohydrometallurgical extraction of metals from WEEE could be coupled with biological strategies for metal recovery from the derived leachates, such as biosorption, bioprecipitation and extraction by biogas. In addition, the use of other types of waste as an energy source for the microorganism involved has been demonstrated as a valid option and could offer the possibility to face two environmental problems contemporaneously, with several advantages.

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