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Study and optimisation of copper bioleaching process for electronic waste valorisation

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STUDY AND OPTIMISATION OF COPPER BIOLEACHING PROCESS FOR ELECTRONIC WASTE VALORISATION

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“Caminante, no hay camino,

se hace camino al andar”

Antonio Machado

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CONTENTS

CHAPTER 6. ADAPTATION OF THE MINERAL BIOLEACHING PROCESS FOR METAL RECOVERY FROM E-WASTE

CHAPTER 7. DEVELOPMENT OF A BIOLEACHING PROCESS IN A CONTINUOUS STIRRED-TANK REACTOR (CSTR) TO RECOVER METALS FROM E-WASTE

Summary

In the current economical context, the use of waste material with economic potential should be a priority. In this sense, the increasing production of electrical and electronic equipment waste (WEEE) makes these materials a potential source for valuable and scarce metals. For this reason, it is important to develop new metal recovery methodologies economically that are more profitable, sustainable and environmentally friendly. A possible solution to this problem is to take advantage of the metabolic activity of certain microorganisms, mainly bacteria, to regenerate the responsible agents for the extraction of metals from the matrix in which they are contained once the useful life of them has ended. This process is known as bioleaching or biological leaching.

In this thesis, a study of this biotechnological process for metal recovery from WEEE has been carried out. Firstly, bioleaching to recover copper from low-grade chalcopyrite was studied to establish the bases of the methodology, already applied in the biomining field, as well as to check the feasibility of the technique in this field. Subsequently, bioleaching was extended to be applied in the field of the electronic waste, thus recovering metals from printed circuit boards (PCB) based on their high metal content and the limited availability of metals in nature. Given the interest of this process, not very studied in the field of electronic waste, an adjustment of those parameters that allow optimizing the operation is necessary. For this reason, the effect of several parameters has been studied such as pH effect, PCB concentration or particle size, as well as the most appropriate system to perform the process (flasks, bioreactor or column).

After bioleaching, the extracted metals remain in the leaching solution, so a last step to obtain the metals in their metallic state again and separated from the initial matrix should be performed which closes the recovery cycle. The study to recover the bioleached copper has been carried out more superficially in this thesis, focusing on cementation as a simple and cheaper alternative to other more complex processes such as electrowinning.

In addition to the metal extraction through bioleaching, this thesis has been also focused on studying the limits of the technology due to the complex and varied composition of the waste, such as the toxic effects that bioleached metals could cause to the microorganisms involved in the process or the evaluation of possible substrate inhibition. The measurement of the biological activity may be the solution when there are limitations of quantifying biomass in systems where the formation of precipitates can be habitual, as in bioleaching. For this reason, a microrespirometry-based procedure has been developed that allows to directly measure the oxygen consumption and, thus, the microbial activity at real time. In microrespirometry, the formation of precipitates does not

interfere with the measurement, which allows obtaining a reliable result of the microbial activity.

Thus, after affirming the feasibility of bioleaching as a simpler, cheaper and environmentally friendly alternative to traditional physical-chemical processes, this thesis establishes the most favourable conditions to obtain the greatest possible copper recovery through bioleaching. These bases are the previous phase to scale-up the technology to be implemented in an industrial environment.

Resumen

En el actual contexto económico, el provecho de materiales residuales con potencial económico debería ser prioritario. En este sentido, la creciente producción de residuos eléctricos y electrónicos (REES) convierte estos materiales en una potencial fuente de metales muy valiosos y escasos. Por este motivo, es importante desarrollar nuevas tecnologías de valorización de metales que sean económicamente más rentables, sostenibles y respetuosas con el medio ambiente. Una posible solución para este problema consiste en aprovechar la actividad metabólica de determinados microrganismos, principalmente bacterias, para regenerar los agentes responsables de la extracción de metales de la matriz donde se encuentran inmovilizados una vez finalizada la vida útil del aparato eléctrico que los contiene. Este proceso es conocido como biolixiviación o lixiviación biológica.

En esta tesis se ha llevado a cabo el estudio de este proceso biotecnológico para la recuperación de metales procedentes de REES. En primer lugar, se estudió la biolixiviación para recuperar cobre a partir de calcopirita de baja ley para establecer el procedimiento de la metodología, ya aplicada en el campo de la biominería, y comprobar la viabilidad de la técnica en este campo. Posteriormente, la biolixiviación fue aplicada al campo de los residuos electrónicos, realizando así la extracción de metales de placas de circuito impreso (PCB, del inglés, printed circuit boards), basándose en la gran cantidad de metales que éstos contienen y su limitada disponibilidad en la naturaleza. Ante el interés de este proceso, no muy estudiado en el campo de los residuos electrónicos, es necesario ajustar aquellos parámetros que permitan optimizar la operación. Por este motivo, se ha estudiado el efecto de varios parámetros que afectan al proceso como el pH, la concentración de residuo o el tamaño de partícula, así como también el sistema más adecuado para llevar a cabo el proceso (matraz, biorreactor o columna).

Tras la biolixiviación, los metales extraídos permanecen en solución por lo que es necesario realizar una última etapa para llegar a obtener los metales en su estado metálico nuevamente, aunque separado de la matriz inicial en este caso, y cerrar así el ciclo de la recuperación. En esta tesis el estudio para recuperar el cobre lixiviado se ha realizado de forma más superficial, centrándose en la cementación como alternativa simple y económica a otros procesos más complejos como la electrólisis.

Además de la extracción de metales mediante biolixiviación, esta tesis también se ha centrado en estudiar factores que limitan la tecnología debido a la compleja y variada composición de los residuos, como es el efecto tóxico que pueden provocar los metales biolixiviados sobre los microorganismos involucrados en el proceso, así como la

evaluación de la inhibición por sustrato. La medición de la actividad biológica puede ser la solución cuando haya limitaciones de cuantificar la biomasa en sistemas donde la formación de precipitados suele ser habitual, como es el caso de la biolixiviación. Por este motivo se ha desarrollado un procedimiento basado en la microrespirometría que permite obtener de forma directa el consumo de oxígeno y, por tanto, la actividad a tiempo real de una muestra biológica. En las microrespirometrías la formación de precipitados no interfiere en la medición por lo que permite obtener un resultado fiable de la concentración microbiana.

Así pues, tras afirmarse la viabilidad de la biolixiviación como alternativa más simple, económica y medioambientalmente sostenible a los procesos físico-químicos tradicionales, esta tesis establece las condiciones más favorables para obtener la mayor recuperación de cobre posible mediante biolixiviación. Estas bases son la fase previa para escalar la tecnología a implementar en un entorno industrial.

Chapter 1

Motivations and thesis overview

1.1. Motivations

The present thesis has been developed in the Department of Mining, Industrial and ICT Engineering of the UPC, in the research group of Biological Treatment of Odours and Gaseous Pollutants (BIOGAP). The thesis has been developed within the projects "Monitorización, modelización y control para la optimización de biofiltros percoladores de desulfuración anóxicos y aerobios" (CTM2012-37927-C03-02), "Valorització econòmica i sostenible de residus electrònics" (2016LLAV00034) and "Optimization and validation of a bio-based prototype for valuable metals recovery from electronic wastes" (2018PROD00097). These projects have been funded by "Ministerio de Ciencia, Innovación y Universidades" from the Spanish Government and co-funded, the "Agència de Gestió d'Ajuts Universitaris i de Recerca" and the European Union through the European Regional Development Fund. Whereas the first project (CTM2012-37927-C03-02) has developed different techniques to monitor biomass at different conditions, the other projects propose the use of a biotechnological process for the valuation of electronic waste, as a more sustainable and profitable alternative for the recovery of valuable metals. Specifically, the second project (2016LLAV00034) proposes to enhance the basic research carried out in relation to the recovery of valuable metals from electronic waste using biotechnological techniques while the third project (2018PROD00097) is focused on the demonstration of the technical and economic viability of the biorecovery process previously developed. Moreover, it is noteworthy that part of the experiments carried out in this thesis (in particular part of the experiments performed in Chapter 7) have been developed in collaboration with the research group Environmental Microbiology from the Technische Universität Bergakademie Freiberg (Freiberg, Germany). This collaboration was carried out through a research stay of the thesis author, which was funded by the destination university within the project 30110017 DAAD Young GEOMATENUM International.

Following these projects, this thesis is developed to answer the research topics raised in them. Hence, the present thesis is focused on the development of a technology that allows recovering metals from wastes with low operation and investment costs besides being more environmentally friendly in comparison to the traditional methods. It must be pointed out that this thesis directly drives to a further knowledge and optimization of the bioleaching technology in order to establish the bases of the process and thus be able to be applied on an industrial scale in the future. It is noteworthy that the bioleaching process developed in this thesis have been registered in the patent P201830406 "Método para la recuperación biológica de metales en residuos eléctricos y electrónicos", obtaining the maximal punctuation. Moreover, the international patent "Method for the biological recovery of metals in electric and electronic waste" has been requested, but it is still under evaluation.

1.2. Thesis overview

The thesis is organized into twelve chapters, each one focusing on specific aspects of bioleaching technique as explained below.

In this first Chapter, the motivations and the thesis overview are presented. In Chapter 2, a general introduction describes the relevant background information about the extraction of metals from metal-containing materials. This information facilitates the understanding of the following chapters, since many basic topics and concepts are explained. In Chapter 3, the general and the specific objectives of the thesis are stated. In Chapter 4 all the general materials and methods employed during the experimental phase of the thesis are presented. In this chapter, the setups corresponding to suspended biomass cultivation, culture conditions, as well as the methodologies and the analytical techniques used during bioleaching experiments are described in detail.

Chapters 5, 6, 7, 8, 9 and 10 contain the most relevant results and discussion obtained during the thesis. In Chapter 5, copper extraction from ores by means of bioleaching is presented, developing the methodology and testing its effectiveness in this field. Due to the growing production of electronic waste in the world and the large amount of discarded valuable metals that this implies, the methodology studied in the previous chapter is adapted in Chapter 6 to study the extraction of copper from this kind of wastes, specifically for end-of-life mobile phones. In particular, the bioleaching process is studied at different operational conditions to optimize the metal recovery in flasks. In Chapter 7, the process is developed on a larger scale, using a continuous stirred tank reactor (CSTR). The aim is to evaluate the effectiveness of bioleaching in a pilot plant in order to research how an increase of the volume in the system affects the process. In this chapter, fluorometric measurements are also done to observe biomass evolution during the process, since the biological activity is essential for the correct operation. Since the biomass can be exposed to other metals, apart from the target one, during the bioleaching, this fact could affect the microbial activity. Hence, in Chapter 8 a toxicity study is conducted to evaluate the effect of some bioleached metals to the microorganisms' growth, which is made by means of microrespirometry tests. In addition, the microrespirometry technique is also applied in this chapter to observe biomass growth and its evolution at different concentrations of iron (II) to assess whether there is substrate inhibition. Chapter 9 is focused on the development of the methodology to extract metals in continuous mode to improve the efficiency of the process. For this purpose, a column for continuous extraction is presented and different parameters such as pH, particle size, solid concentration and contact time, among others, and different strategies are studied in order to increase the metal extraction from the waste. Moreover, from all the previous knowledge obtained, in Chapter 10, a pilot plant to recover metals continuously by means of bioleaching is proposed as a previous step to their application

at industrial scale. This pilot plant includes all steps from the biological regeneration of the leaching agent to obtaining the metal powder, going through the separation of the biomass to avoid its inactivation as well as the continuous leaching of metals from the electronic waste.

In Chapter 11 the conclusions extracted from the results obtained in previous chapters are exposed and future research topics are recommended. Finally, Chapter 12 contains the references used along the thesis.

Chapter 2

Introduction

2.1. Minerals and electronic wastes as source of valuable metals

In human progress, metal extraction has been a fundamental activity since Bronze and Iron times. The global increase of human population and the development of several nations have increased the demand of all natural resources, including metals. The rapid advance of technology development causes a high mineral product consumption which eventually produces the reduction of high-grade ore reserves (Figure 2.1). For this reason, in the last decades more attention has been paid to low-grade ores and industrial and mining wastes (Sajjad et al. 2018).

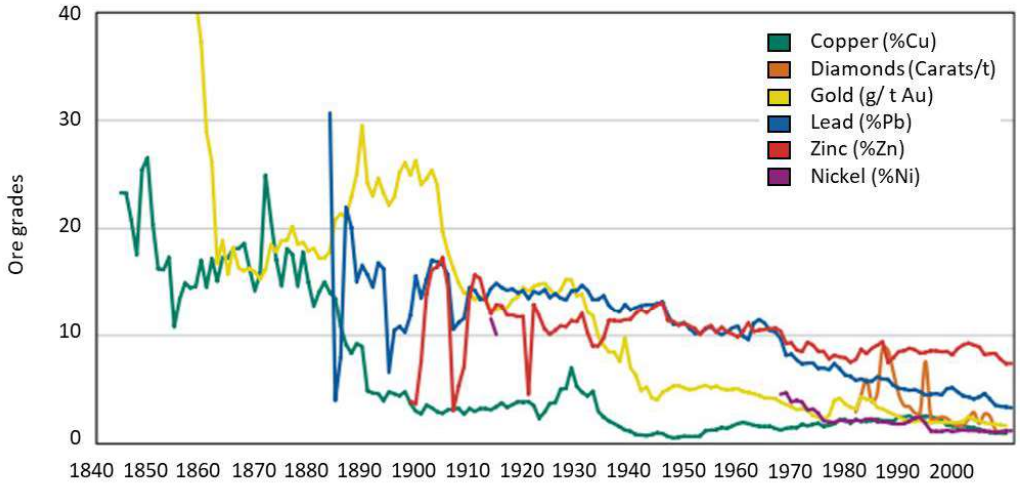

Figure 2.1. Evolution of the ore grade of mines from 1840 to 2005 (Adapted from UNEP (2011).

According to SNL Metals Economics Group (2013), ore head grades decline over time since companies initially mine high-grade zones to recoup capital costs and other early expenditures. In addition, significant deposits are found at greater depths or in more remote areas which also increases the cost of mineral exploitation. This fact combined with the increasing energy costs is pushing up the copper mining industry's capital and operating costs. For this reason, the European Copper Institute (2018) affirmed that it is possible to focus on obtaining copper from the huge amount that it is in use today. The main premise is that valuable materials can be recovered from waste in a way that is analogous to mining to produce high value and secondary raw material (Xavier et al. 2019). In this sense, a profitable urban mining can result from the e-waste management.

The urban mining concept was firstly used in a research publication in 1993 (Savage, Golueke, and Stein 1993) and it describes a form of closed-loop supply chain management, offering a sustainable way to exploit mineral resources by reducing

primary material intake and stimulating the circularity in the supply chain (Xavier et al. 2019). Hence, the urban mining concept is also related to the circular economy, since the basis of its concept is the reuse and recirculation of products and materials. In this regard, the circular model tries to integrate the finiteness of resources and proposes the reintroduction of materials from secondary sources in a regenerative system in line with the concept of the 3Rs which are reduce, reuse and recycle. An important advantage of urban mining is that it allows to recover up to ten times greater valuable metals in comparison to the amount extracted from primary mineral deposits (Xavier et al. 2019). What is more, the European Copper Institute (2019) affirms that more than 40% of the copper demand from the member countries of the European Union is covered with recycling. Therefore, the urban mining denotes the systematic reuse and recycling of anthropogenic resources from urban areas. Originally, this concept concentrates on waste electric and electronic equipment utilization as modern urban ore. However, currently urban mining also includes all kind of anthropogenic stocks like landfills, buildings, infrastructure and industries (Avarmaa et al. 2019). At the same time with the consumption of natural ore resources, the urban mines are storing significant and increasing amounts of valuable metals. Table 2.1 shows the amount of various metals found in nature as mineral deposits and the range in content of the same metals in e-waste. It can be appreciated that the concentrations of copper, tin, nickel and lead found in the e-waste are higher than those in natural deposits. This fact demonstrates the importance of electronic waste as a possible source of raw material and the motivation to develop processes to recover these metals from it.

Table 2.1. Metals content in ores and PCBs (adapted from Bizzo, Figueiredo, and De Andrade (2014))

Metal	Ores (%)	PCBs (%)
Cu	0.5 – 3.0	12.0 – 29.0
Fe	30.0 – 60.0	0.1 – 11.4
Ni	0.7 – 2.0	0.3 – 1.6
Zn	1.7 – 6.4	0.1 – 2.7
Sn	0.2 – 0.9	1.1 – 4.8
Pb	0.3 – 7.5	1.3 – 3.9
Au	$0.5 \cdot 10^{-3}$	$2.9 \cdot 10^{-3}$ – 0.1
Ag	$0.5 \cdot 10^{-3}$	$0.1 \cdot 10^{-1}$ – 0.5

2.1.1. Electronic waste in the world

Waste electrical and electronic equipment (WEEE) and electronic waste (e-waste) are the two more frequently used terms for discarded devices and appliances that use electricity. According to Robinson (2009), e-waste alludes to discarded electronic goods as computers or mobile phones, whereas WEEE also includes non-electronic appliances as refrigerators, air conditioning units or washing machines. Nowadays, this distinction is not clear due to the increasing use of electronics like microprocessors in electrical equipment. National e-waste policies and legislation play an important role to set standards and controls to govern the actions of the e-waste stakeholders in both public and private fields. Since these policies and legislation must be sustainable and function properly, it is crucial to establish a financial model including the collection sites and logistics along with the physical recycling itself. Nevertheless, the types of e-waste covered by legislation are different from one country to another, making the coordination to collect and recycle this kind of waste difficult. In particular, 67 countries have national e-waste management laws. This means that 66% of world population are covered by them (Figure 2.2) (Balde et al. 2017).

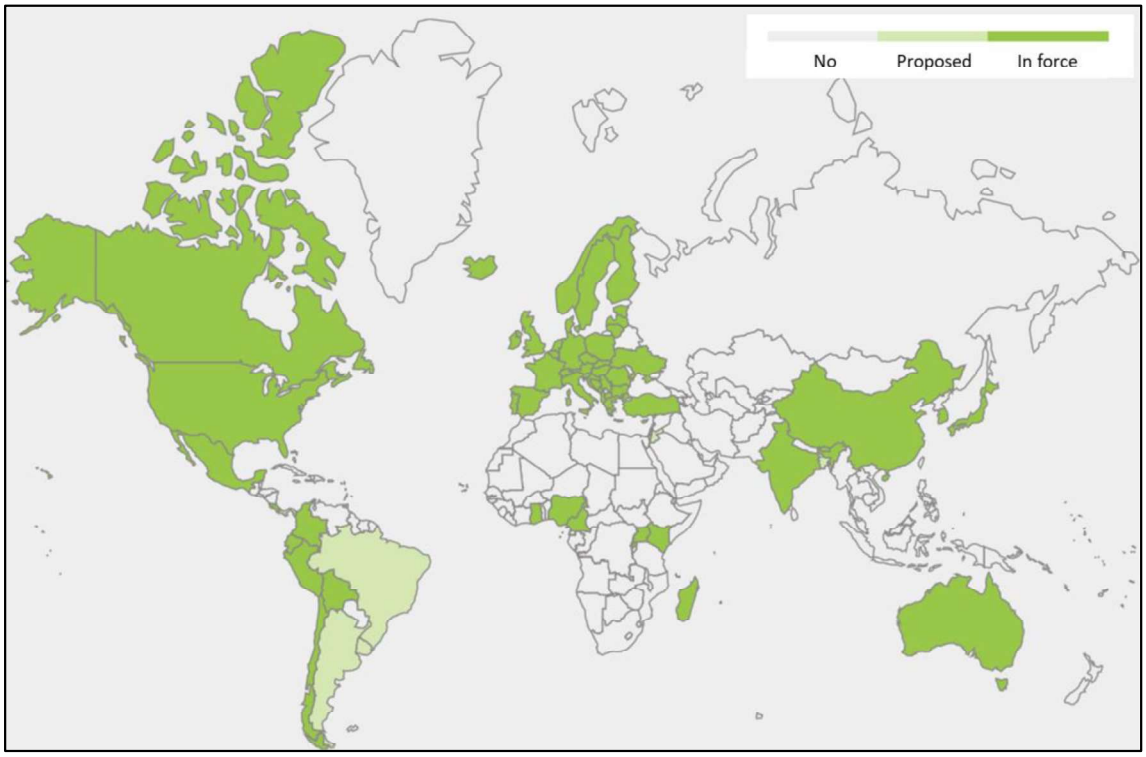

Figure 2.2. Countries regulated by national environmental protection laws specifically designed for e-waste (Balde et al. 2017).

Additionally, the countries that already have e-waste policies should contribute to the development of circular economy models not only to favour collection and recycling, but also to change the direction of policy measures towards reusing, refurbishing and remanufacturing the end-of-life e-waste. It means that legislation on e-waste should encourage a better product design during their production. In this sense, the recycling and repair of e-waste might be easier or might make the products more durable. Most legislation and policies currently refer to the principle "Extended Producer Responsibility" (EPR) which was firstly described in academic circles in 1990 (Balde et al. 2017). This principle requires manufacturers to accept responsibility for all the stages in the product lifecycle which is from their production to their end-of-life management (Figure 2.3). The EPR principle is implemented in several legislations and policies.

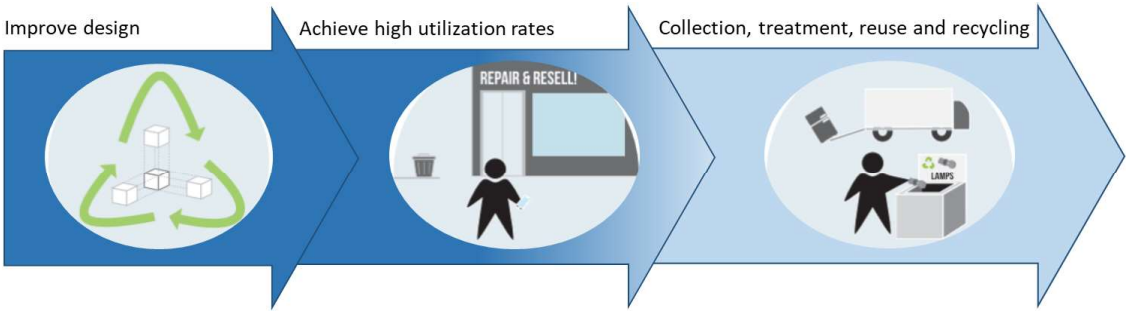

Figure 2.3. Scheme of the primary objectives of the Extended Producer Responsibility (EPR) principle (Adapted from Balde et al. (2017)).

In addition, in order to control the transboundary movements of hazardous wastes and their disposal, in which e-waste is included due to the hazardous elements it has, *The Basel Convention* was signed by 186 countries. It consists of a multilateral treaty to abolish the environmentally and socially detrimental hazardous waste trading patterns. The Convention affirms that hazardous waste should not be treated freely like ordinary waste and thus it establishes written notifications and approval for all the cross-border movements of hazardous wastes. Although the Convention does not include a regulation for the equipment destined to be reused, this statement is in accordance with its main environmental objective to prevent waste generation, since the reuse extends the lifecycle and thus reduces the generation of hazardous waste. Nevertheless, the distinction of whether something is waste or not is a long-standing discussion under the Basel Convention and the most recent Conference-of-Parties (COP13) could not reach a final consensus (Balde et al. 2017). Additionally, the lack of collection infrastructure that channels e-waste is usual in developing countries due to the difficulties to comply with international standards. Hence, it results in a lack of treatment facilities in these

places. Despite of that, according to Ogunseitan et al. (2009), since the mid-1990, e-waste has been recognized as the fastest-growing component of the solid-waste streams. It has been calculated that more than 40 million tons of e-waste are generated each year (Figure 2.4) (Li et al. 2015; Zeng, Mathews, and Li 2018) and the United Nations Environmental Program estimated that the volume of e-waste increases by a minimum of 3-5% per year, which is nearly three times faster than the growth of municipal waste (Schwarzer et al. 2005). In this way, it is estimated to grow to 51 million tons by 2021 (Hsu et al. 2019), while only 12.5% of e-waste in the world is recycled (The World Counts 2020).

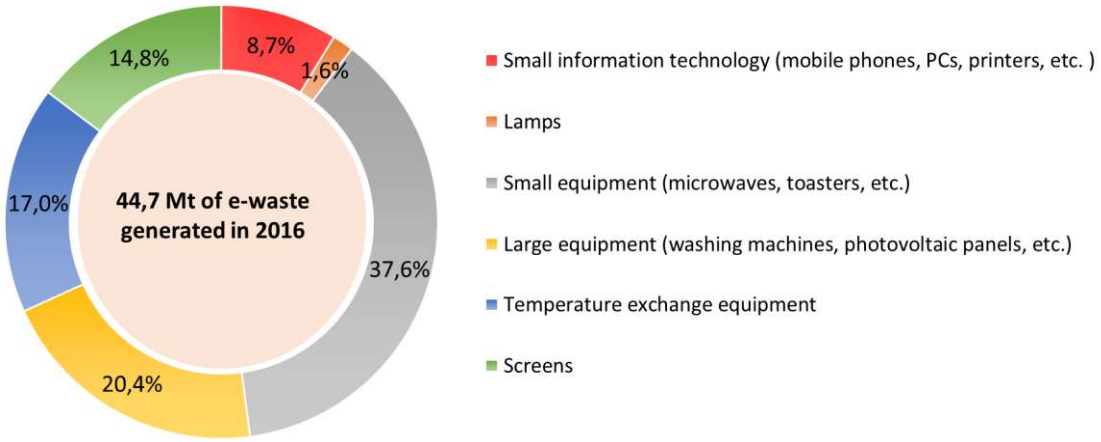

Figure 2.4. Estimation of the e-waste total generation per category (Adapted from Balde et al. (2017)).

It is noteworthy that the distribution of the e-waste generation is quite different between regions (Table 2.2). Asia is the largest producer in the world, generating 40.7% of the total e-waste in the world. The main countries of Asia that contributes to this amount of e-waste generation are China and India (Figure 2.5). Nevertheless, only 15.0% of the total e-waste generated in Asia are collected. The most efficient e-waste collector is Europe, collecting 35.0% of the total generation. Moreover, in Northern Europe the collection rate is 49.0%, the highest in the world. This high percentage of e-waste collection in comparison to the rest of the regions is related to the strict legislation imposed by the European Union which requests its members to collect 45% of the amount placed on the market. On the contrary, Oceania is the lowest e-waste producer with 1.6% of the total e-waste in the world. It is noteworthy that Africa does not collect any of the e-waste generated, which is related to the fact of not having legislation on this type of waste in practically any of the countries (see Figure 2.2). In the special case of America, although the United States are one of the largest producers (Figure 2.5), this

region does not have the highest percentage of e-waste generation due to the relatively low amount of e-waste generated in the rest of the American region. According to Li et al. (2015), China and United States are the largest producers of e-waste in the world, generating more than 3 million tonnes per year each which is twice the level of production achieved by other industrialized countries.

Table 2.2. Regional e-waste generation and collection rate (Adapted from Balde et al. (2017)).

Region	E-waste generation (Mt)	E-waste generation in the world (%)	Collection rate (%)
Asia	18.2	40.7	15.0
Europe	12.3	27.5	35.0
America	11.3	25.3	17.0
Africa	2.2	4.9	0.0
Oceania	0.7	1.6	6.0

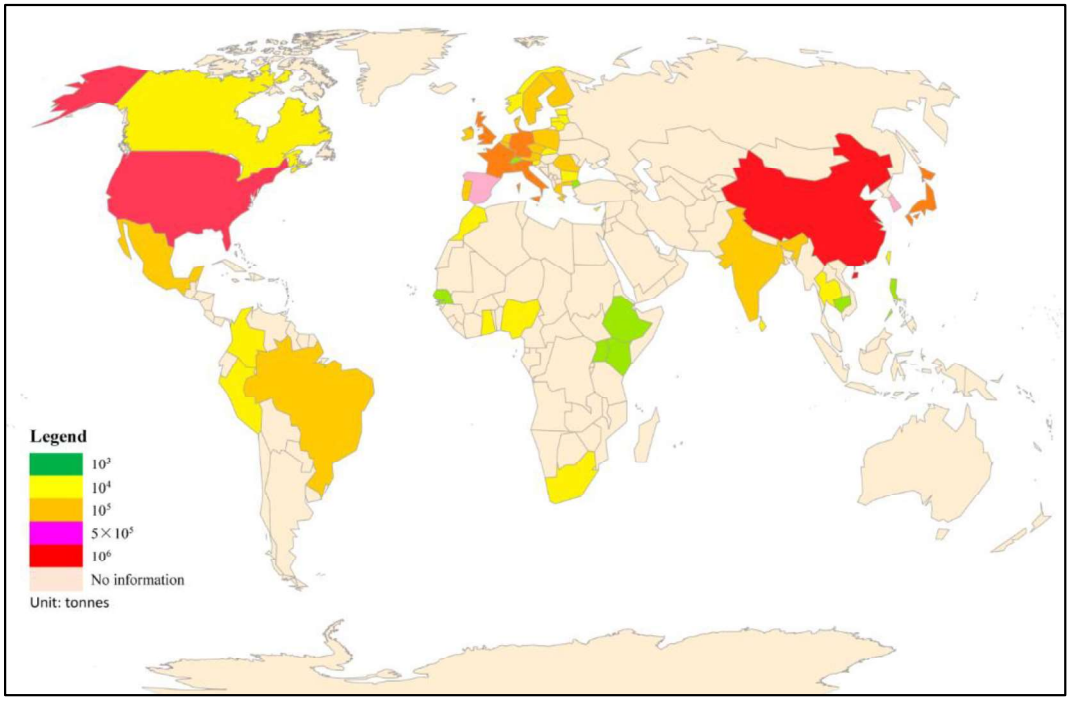

Figure 2.5. E-waste production in the world by country (Li et al. 2015).

Despite the existence of the Basel Convention (UNEP 2018) on the control of the transboundary movements of hazardous wastes and their disposal and other conventions, the transfer of e-waste from one country to another remains relatively high (Iqbal et al. 2015). As shown in Figure 2.6, the major flow of e-waste enters into major

countries in Asia such as China, India and Pakistan. It has been reported that the e-waste imported to China comes from the United States, the European Union, Japan, South Korea and several other countries in the world (Iqbal et al. 2015). The associated export of e-waste from developed to developing regions has been ongoing for years. Moreover, there is still very limited information about these exportations due to its illicit character, so hidden flows of them are estimated to be typically highly variable.

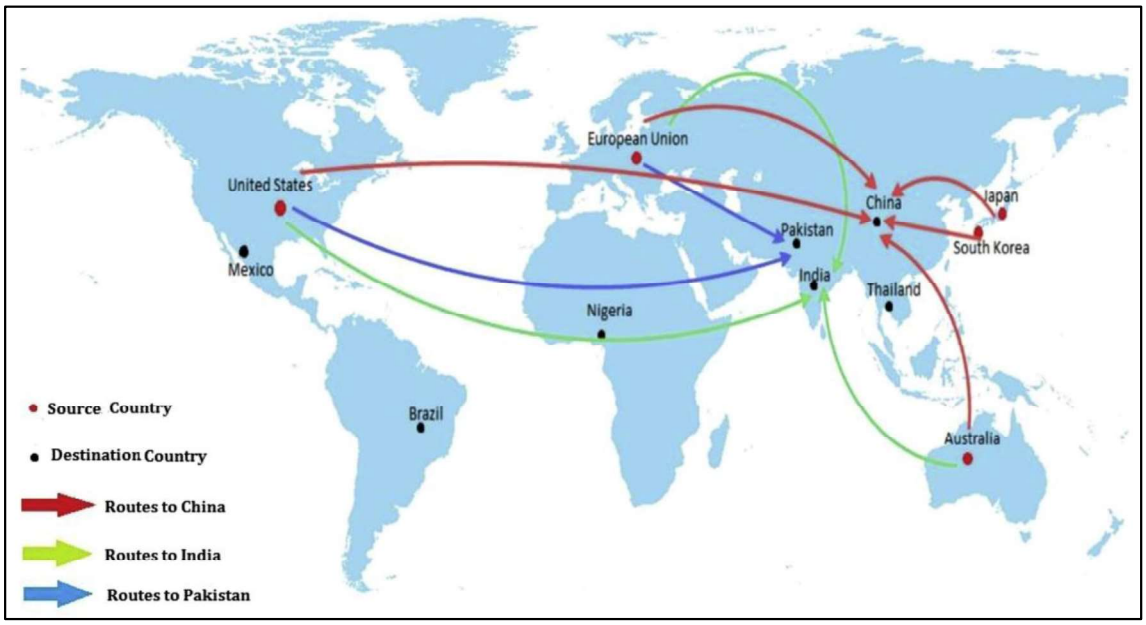

Figure 2.6. Transboundary movements of e-waste from developed countries to developing Asian countries (Iqbal et al. 2015).

2.1.2. Electronic waste composition

The main components of the electric and electronic equipment are the printed circuit boards (PCB), which are used to achieve mechanical and electrical connections. Electronic components in PCBs are assembled by conductive pathways etched on a non-conductive substrate laminating Cu sheets. However, the PCBs of different devices always have different structures, which makes the recycling of this kind of waste so complicated. The basic structure of the PCBs is known as “copper-clad laminate” and its consists of an organic medium substrate and a number of metallic components to realize the electrical connections inside the board (Alwaidh, Sharp, and French 2014). Depending on the structure, PCBs can be divided into single-sided, double-sided, multi-layer, rigid, flexible and flex-rigid. The application of each is described in Table 2.3.

Table 2.3. Classification of PCBs based on their structure and the main application of each one (Adapted from Hao et al. (2020)).

Type of PCBs	Main applications
Single-sided	Televisions and household appliances
Double-sided	Instrumentation, computers, LED lighting, etc.
Multi-layer	For complicated designs like medical equipment and satellite systems
Rigid PCB	With single, double or multi-layer application same as them
Flexible PCB	With single, double or multi-layer used for special requirements
Flex-rigid PCB	Using in the case when space or weight are prime concerns

In general, PCBs contain more than 60 kinds of elements, consisting of 40% metals, 30% organic materials and 30% ceramics (Figure 2.7) (Ogunniyi, Vermaak, and Groot 2009). The main organics that are contained in the PCBs include acrylonitrile butadiene styrene (ABS), polycarbonate (PC), polyvinyl chloride (PVC), polytetrafluoroethylene (PTFE), polyethylene (PE), polypropylene (PP) and high impact polystyrene (HIPS) (Hsu et al. 2019). The ceramic components of PCBs generally consist of silica, titanates, alumina and alkaline oxides (Hsu et al. 2019). The metals include base and precious metals, such as copper, silver or gold, which could be retrieved to be used again (Fornalczyk et al. 2013), making waste PCBs an important metal resource. In addition, waste PCBs are also packed with toxic chemicals as arsenic, lead, mercury and poly-brominated flame retardants among others. Unfortunately these metals do not have as much economic value as the precious or basic metals.

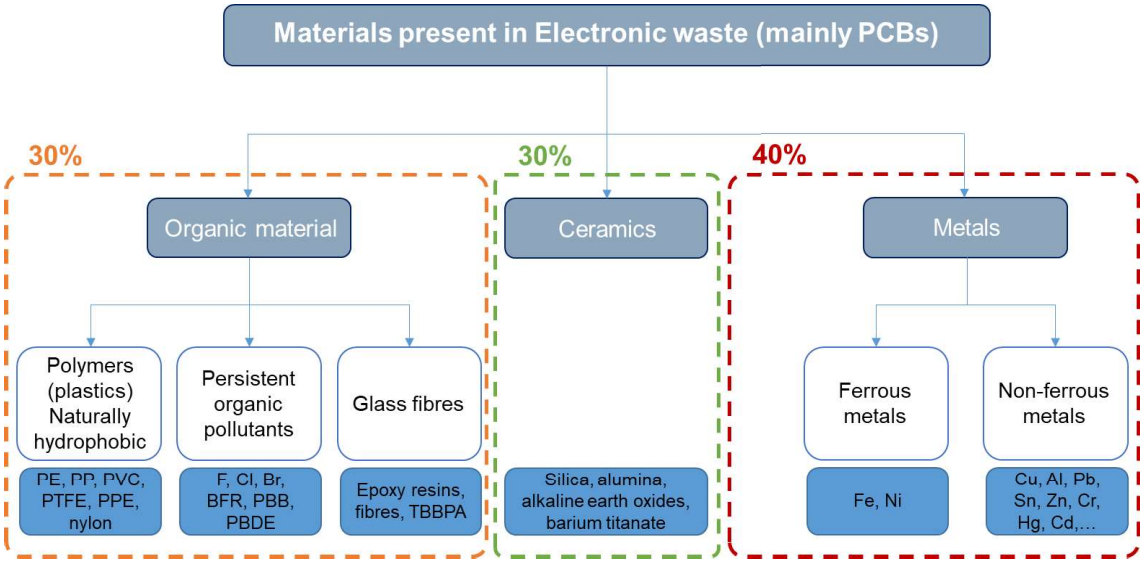

Figure 2.7. Composition of the electronic waste in general terms. Abreviations: PE (Polyethilene), PP (polypropylene), PVC (polyvinylchloride), PTFE (polytetrafluoroethylene), PPE (polyphenyl ether), BFR (brominated flame retardand), PBB (polybrominated biphenyl), PBDE (polybrominated diphenyl ethers), TBBPA (tetrabromobisphenol A) (Adapted from Chauhan et al. (2018)).

Although 40% of PCBs are generally metals, the specific content of them depends on the type of device, the age of the equipment or the process of the manufacturer (Hao et al. 2020). As an example, Table 2.4 summarizes the composition of different PCBs from reported studies in which a large variation of metal composition can be observed among PCBs of several devices. It is noticed that the highest amount of precious metals (Ag, Au and Pd) are found in mobile phone PCBs whereas the PC board, the DVD board and the TV board have the highest concentration of Cu, Fe and Al, respectively.

Table 2.4. Composition of PCBs from different electronic devices (Fornalczyk et al. 2013).

Type of device	Contents (%)				Contents (ppm)		
	Fe	Al	Cu	Plastic	Ag	Au	Pd
TV board	30	15	10	28	280	20	10
PC board	7	5	18	23	900	200	80
Mobile phone	7	3	13	43	3000	320	120
DVD	62	2	5	24	115	15	4
Calculator	4	5	3	61	260	50	5

As Hao et al. (2020) affirmed, the age of the device and the manufacturer also affect the metal concentration of PCBs even being the same device. This fact was corroborated by Chen et al. (2018a), who analysed the metal concentration of 36 different mobile phones' PCB from different companies, models and years of production from 2002 to 2013. They found that, for instance, the copper concentration varies from 20438 mg/kg to 37472 mg/kg or from 25 mg/kg to 4304 mg/kg in the case of silver. This fact, added to the inhomogeneous and composite nature of the materials of PCBs, makes it quite difficult to generalize a recycling process to recover metals from different e-waste devices (Khaliq et al. 2014).

Nevertheless, according to the Environmental Protection Agency (2014), approximately more than 142000 computers and 416000 mobile phones are discarded every day. Moreover, from one million computers, it is possible to recover nearly 24 kg of gold, 250 kg of silver and more than 9000 kg of copper and every million mobile phones could contain 34 kg of gold, 350 kg of silver and 15875 kg of copper (Environmental Protection Agency 2020).

2.1.3. Copper and its importance in the world

Archaeological evidence demonstrates that copper was one of the first metals used by humans at least 10.000 years ago (International Copper Study Group 2019). From the end of the Stone Age it was present in inventions and is a key element for the future that remains to come. Its importance has been such that even historians have called the Copper Age and the Bronze Age two periods of yesteryear. Although its use decreased by the evolution of the steel industry, after 1831 when the electrical generator was discovered by Faraday, the use of copper increased, becoming a strategic metal again. This metal is the third most used metal in the world after iron and aluminum (Diaz 2016) and its global demand continues to grow, tripling in the last 50 years the world refined usage (International Copper Study Group 2019). This is, in part, due to copper having established numerous important uses in almost all branches given its malleability, ductility, conductivity of both heat and electricity and its capacity to withstand corrosion (Radetzki 2009). Among the different uses of copper, the main usage was and is to produce electric and electronic equipment, whereas industrial application was the lowest copper user in 2018 (Figure 2.8). With respect to regions, Asia was the major user by using 68% of the total copper used in the word during 2018, followed by Europe (17%) and America (13%) (International Copper Study Group 2019).

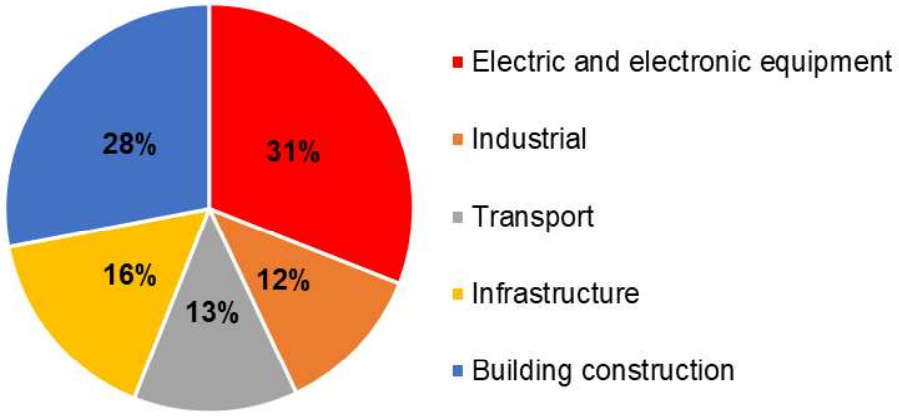

Figure 2.8. Usage of copper by end use sector in 2018 (Adapted from International Copper Study Group (2019)).

Copper occurs naturally in the Earth's crust in variety forms. It can be found as a pure "native" copper, which is an uncombined form of copper that occurs as a natural mineral, but this mineral is found in much lower proportion than other ores which also contain copper. Hence, it is usually found in deposits of different minerals, such as

cuprite (Cu_2O), malachite ($Cu_2CO_3(OH)_2$), azurite ($Cu_3(CO_3)_2(OH)_2$), chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4) and chalcocite (Cu_2S) (Figure 2.9).

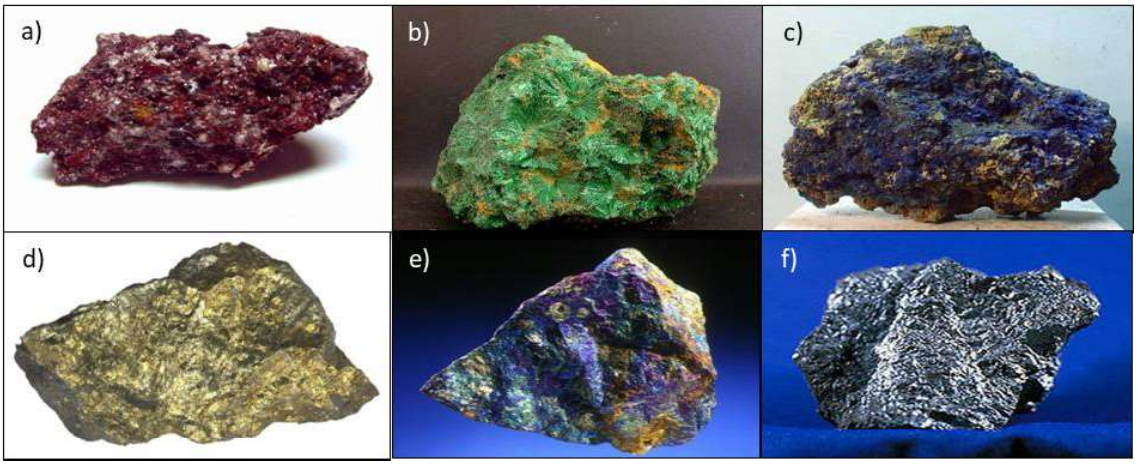

Figure 2.9. Pictures of the most common ores containing copper: a) cuprite; b) malachite; c) azurite; d) chalcopyrite; e) bornite; and, f) chalcocite (Fundación integra 2014; Mundo mineral 2013).

As stated above, these minerals are found in throughout the earth's crust. In particular, it is calculated that the outer 10 km of earth's crust contain 67 parts per million of copper (European Copper Institute 2019). In some places, volcanic activity or hydrothermal processes, among others, deposited molten copper in specific locations millions of years ago. In these areas the deposits of copper are exploited since they contain medium-grade or high-grade ores, containing enough copper to obtain a profitable exploitation. The main natural deposits of copper are found in Chile, Peru, United States and Democratic Republic of the Congo (DRC) (Figure 2.10). However, the main companies that focus their activity on the exploitation of copper are Freeport in USA, Codelco and Minera Escondida Ltda. in Chile and BHP Billiton in Australia (Radetzki 2009).

In terms of weight, copper is the third most important metal with a total refined consumption of 23.5 million tons in 2017, China being the country that consumes the most (11.8 million tons) followed by the United States (1.8 million tons) and Germany (1.2 million tons). In the same year copper production from mines was 20.2 million tons (Cochilco 2018) and during the period 2008-2018, 197 million tons of copper have been mined (International Copper Study Group 2019).

Refined copper production derived from mine production is referred to as "primary copper production" which is obtainable from a primary raw material. However, there is another important source of raw material which is scrap, and the process classified as "secondary copper production". The recovery of copper from scrap recycling is possible,

since this metal is one of the few raw materials that can be recycled repeatedly without any loss of its chemical or physical properties in the recycling process. Moreover, closing metal loops through increased reuse and recycling enhances the overall resource productivity. It is estimated that the recycling of copper from the old scrap provided the equivalent to 8% of apparent consumption (U.S. Geological Survey 2019). Taking into account that copper remains one of society's most widely used metals and it also plays a vital role in electronics, vehicles and electrical power generation, among many other applications (Mudd and Jowitt 2018), increasing recycling rates is a central strategy for dealing with the e-waste problem as well as for assuring the copper supply in the world.

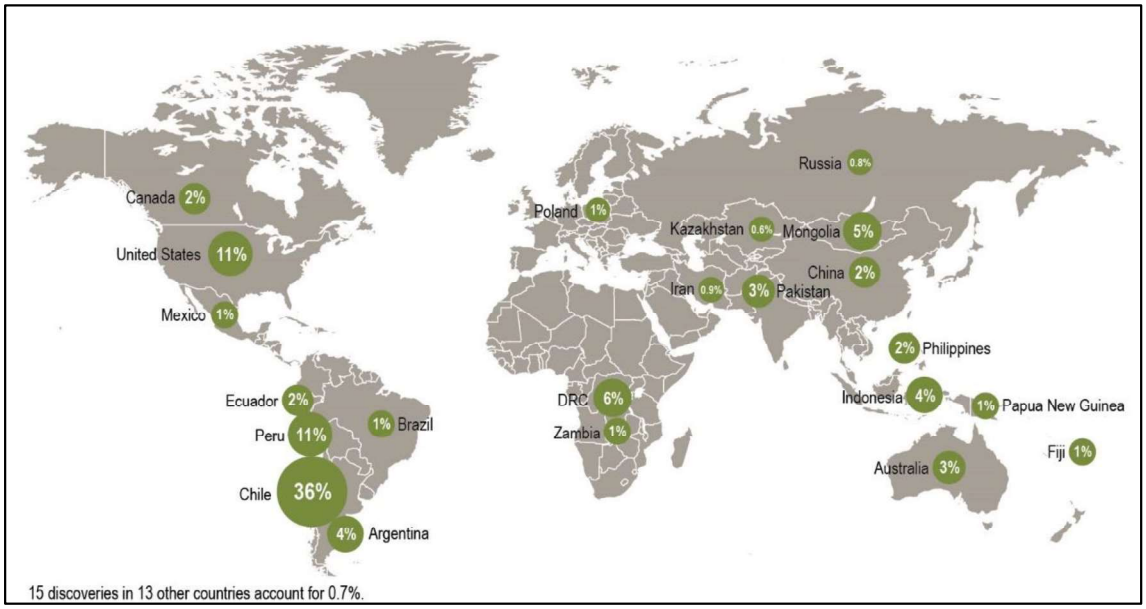

Figure 2.10. Location of the natural deposits of copper in the world (SNL Metals Economics Group 2013).

Hence, it is important to increase the recovery and recycling levels in order to ensure that there is enough copper to respond to the future demands of society. One way that favors the reduction of the price and increases the recovery and recycling is to find cheaper extraction methods as an alternative to the traditional ones that allows to treat low-grade mineral or electronic waste. In this way, it will also reduce the amount of generated wastes.

2.1.4. Obtaining of metal-containing materials from their origin

Before metals can be recovered from ores and PCBs, these must previously be extracted from the mining deposits or the electronic waste, respectively. The processes are completely different since their origins are also different.

2.1.4.1. Extraction of ores from mines

There are two basic ways to extract minerals from the ground which are underground mining and surface or open pit mining (Figure 2.11). The selection of the method depends on the location and shape of the deposit, strength of the rock, ore grade, mining costs and also the current market price of the commodity.

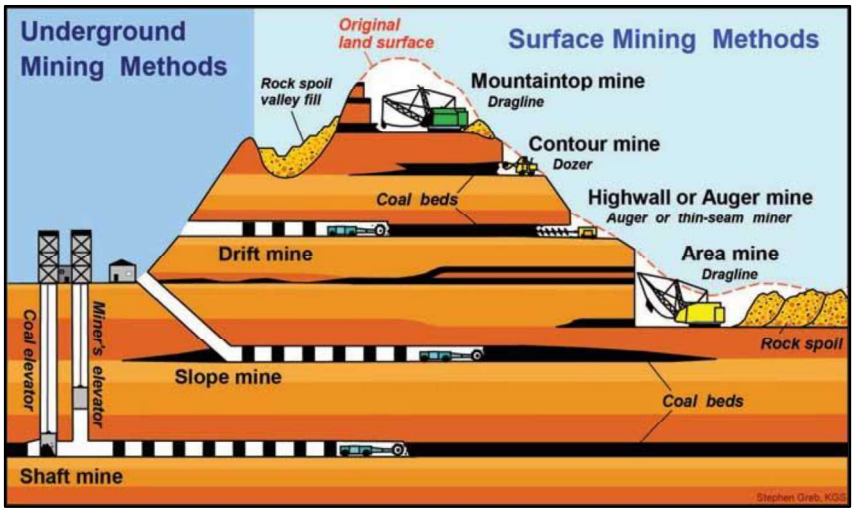

Figure 2.11. Diagram of the surface and underground mining (Voolstra 2013).

Underground mining is used in high-grade metallic ores, since they are usually found in veins deep under the Earth's surface. Although this kind of mining tends to be more expensive, the exploitation results profitable due to the amount of metal that can be recovered. First, the rock is drilled and blasted, and then, the material is moved to the surface by truck, conveyor belt or elevator. Once on the surface, the material is sent to a crusher and then to a mill. After milling, a flotation or other benefication step are done to separate the ore from the waste rock.

Surface or open pit mining is used when the ores are found closer to the surface. This method can be used in lower grade metal ores, since it generally costs less than underground methods, resulting in a profitable mine. Many industrial minerals are mined in this way as these ores are usually low in value and were deposited near the Earth's surface. In surface mine, hard rock must be drilled and blasted as in underground mining, but sometimes in surface mining some minerals are soft enough to be mined without blasting. Although surface mining is used in low grade ores, there is a method included in this kind of mining, that is placer mining, which is used to recover valuable minerals, since it is usual to find this type of minerals in sediments from river channels, beach sands or ancient stream deposits which are found on the Earth's surface. In placer operations, the mined material is washed and purged to concentrate the heavier

minerals. This kind of process may be done by open-pit or by various surface excavating equipment or tunnelling equipment.

2.1.4.2. Extraction of PCBs from e-waste

As it has been described before, PCBs are the main part of the e-waste and they contain around 40% of metals. Therefore, since the metals in the electronic scrap are mainly found on PCBs, proper separation of this part of the waste is essential to recover the metal of interest. The process basically consists of three stages: dismantling, crushing and mechanical separation treatment.

Dismantling consists of the separation of the motherboard from the rest of the components and, thus, makes a first differentiation of the elements that may be more conflicting such as capacitors or plastic covers. Sometimes, heat treatment at not very high temperature can be performed to separate the solder pond, also facilitating disassembly (Duan et al. 2011). But more recent studies incorporate bioprocesses for PCB dismantling (Monneron-Enaud et al. 2020). Elements extracted in this stage can then be reused for new uses or properly disposed (Ghosh et al. 2015).

After the separation of the motherboard, the particle size has to be reduced. To do this, a wide variety of equipment can be used to crush, cut or pulverize PCBs. In general, metallic particles are different from the plastic or ceramic ones (Kaya 2016), so the crushed material is then subjected to a separation process that aims to separate the metallic fraction from the non-metallic one. This process can be performed using many different techniques that are classified intro three main groups according to the principle on which they are supported: magnetic, electrostatic and mechanical methods.

Magnetic methods can be performed in dry or aqueous systems such as a vibratory table with magnetic separator or permanent magnet followed by the use of Eddy or Foucault currents. Electrostatic methods are based on high voltage systems as a rotating crown separator. When the process of separation starts from a fine powder, the electrostatic methods allow to obtain a powder with high metal content. Finally, the mechanical methods are based on particle differences as shape, size, mass or density. Some examples of this kind of separation would be flotation, which depends on the hydrophobicity, cyclones, vibratory sieves and so on. Nevertheless, the most common is to use more than one physical method to achieve better separation rates (Huang et al. 2009).

2.2. Physico-chemical recovery of copper from ores and e-waste

Once the ore has been extracted from the mine and/or the PCBs have been separated from the electronic devices, they have to be treated to recover the copper that they contain. The recovery of metals from ores has been done since many years and very similar processes have been adapted to recover metals from e-waste. The recovered metals are used as raw materials to produce different devices such as the electronic ones. In general, there are two strategies to recover copper from ores and PCBs which are named pyrometallurgy and hydrometallurgy. The first method is based on physico-chemical processes (pyrometallurgy), and the second, in contrast, on the use of chemical reagents (hydrometallurgy). Depending on the waste to treat (ores or PCBs), the steps in each method change a little, but they are based on the same principles.

2.2.1. Pyrometallurgy

Pyrometallurgical processing, including incineration, melting and gas-phase reactions at high temperatures, among others, is the most traditional method to recover metals from ores. Moreover, it has also become a traditional method to recover non-ferrous metals as well as precious metals from e-waste in the past two decades. Although pyrometallurgical processing to recover metals is always based on the use of high temperature to smelt metals, the specific process used to treat ores and PCBs can vary since the chemical reactions that take place are quite different in each case.

In the mining field, pyrometallurgy is performed in five different steps (Pascual and Nadal 2008). In the first one, the copper-bearing ores are crushed, milled and sieved to the desirable particle size and then the inert material (gangue) is removed by flotation. The second step consists on the roasting of the mineral at high temperatures (over 600 ºC) using air. The purpose is that oxygen from the hot air is combined with sulphur and with metals from the mineral. In the case of treatment of chalcopyrite, one of the minerals mostly extracted for copper production, the chemical reaction that takes place is described in Eq. (2.1).

$$2\,FeCuS_2 + 4\,O_2 \rightarrow 2\,FeO + Cu_2S + 3\,SO_2 \qquad (2.1)$$

After the roasting of the chalcopyrite, the concentrate is introduced into an oven at 1200 ºC to melt it. At this stage, two different layers are formed due to differences in the material densities. On the one hand cuprous sulphur is formed and, on the other hand, the ferrous silicate slag. Since the slag is not important for the final product, it is removed and the rest of the melted mineral is oxidized by air again, following Eq. (2.2).

$$Cu_2S + O_2 \rightarrow 2\,Cu + SO_2 \quad (2.2)$$

The copper obtained from chalcopyrite with this method is called "blister copper" and its purity is around 98 and 99.5%. Moreover, a final step may be also performed in order to remove even more impurities or to recover other metals from the ore such as silver or gold. In general, for this purpose electrolytic methodologies are the most used. These methodologies include arranging at least one anode of copper material to be refined and one cathode, both in contact with the electrolyte solution. The anode and the cathode are connected electrically to an electrical source, which is operated under potential-controlled conditions. The electrical potential at the cathode causes the deposition of electrorefined copper on it. By this methodology it is possible to obtain copper with purities up to 99.98%.

In the e-waste field, the crushed particles of PCB obtained after the pre-treatment are smelted in furnaces and coarse metal ingots can be obtained. For this purpose, three different pyrometallurgical processes have been developed: Noranda, Rönskar and Umicore (Cui and Zhang 2008).

The Noranda process (Figure 2.12) consists of introducing the materials into a molten metal bath at 1250 ºC. Inside it, metals are mixed with supercharged air that contains up to 39% of oxygen. The energy cost of this process is reduced by the combustion of plastics and other flammable materials in the feeding. As a result of the mixture that takes place in the bath, impurities such as iron, lead or zinc are converted into oxides which become attached to a silica-based slag. The slag is then cooled and crushed to recover more metals before disposal. The copper matte obtained in this way still contains precious metals saturated to its surface, so it is transferred to the converters. After an upgrading in the converters, a liquid blister copper is obtained which is refined in anode furnaces and cast into anodes. The purity of the copper obtained at this step is 99.1%. The remaining 0.9% contain precious metals such as gold, silver, platinum and palladium along with other recoverable metals. Therefore, these marketable precious metals can be later recovered from the anodes with electrorefining. In the Noranda process, about 100.000 tons of e-waste can be treated per year, which represents 14% of total throughput (the balance being mostly mined copper concentrates) (Cui and Zhang 2008).

The Rönnskar process (Figure 2.13) consists of the introduction of the electronic waste into different stages depending on its purity. Scrap with high copper content is introduced into the converting process directly, whereas scrap with low copper content is introduced into the Kaldo Furnace before going to the converter. In the Kaldo reactor

(patent US 4415360) blended feed material with e-waste and lead concentrates is charged by skip hoist. The oxygen needed for combustion to take place in the Kaldo furnace is provided by an oxygen lance and the off-gases derived from combustion are subjected to an additional combustion with air at 1200 ºC. In this reactor a mixed copper alloy is produced which is sent to the copper converting for recovery of metals such as silver, gold, palladium, nickel, or copper itself. In this stage, ash is also produced which is sent to other operations of the process in order to recover the metals it contains (e.g. lead, antimony, indium, and cadmium). This process can also treat up to 100000 tons of e-waste per year, as in the case of the Noranda process (Cui and Zhang 2008).

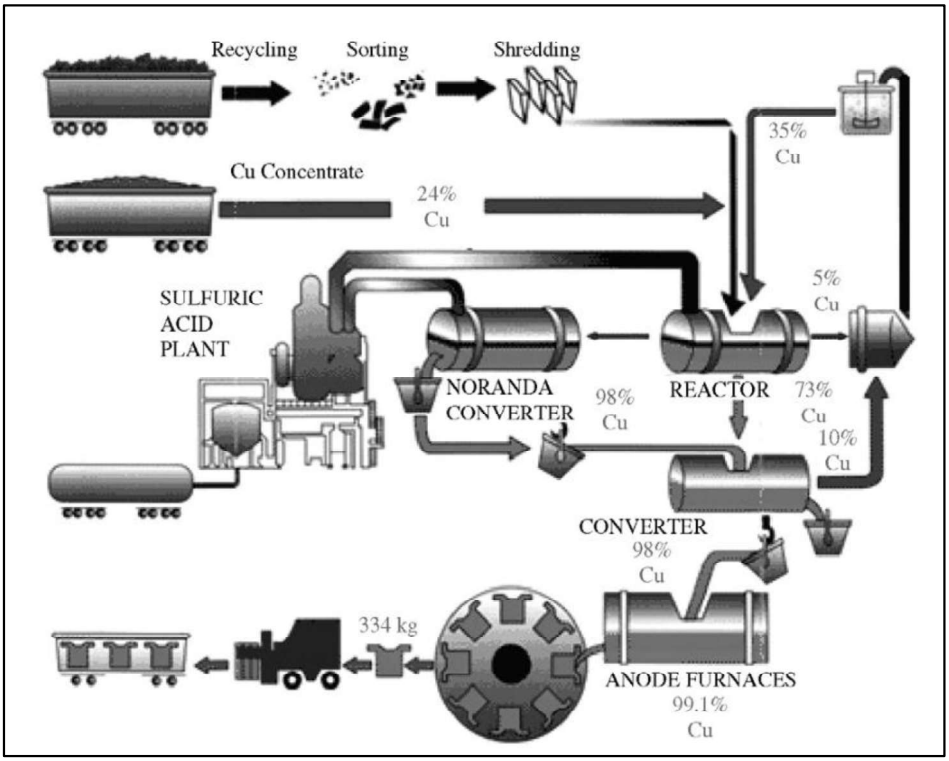

Figure 2.12. Diagram of the Noranda process to recover copper from e-waste (Cui and Zhang 2008).

The Umicore process (Figure 2.14) is focused on the recovery of precious metals although many other metals can be recovered. The e-waste is fed into an oven at 1200 ºC in which it is dissolved under an oxygen-rich air atmosphere. Plastics or other organic substances in the feed are used as a source of energy when burned. In this process precious metals and copper remain in the metal phase, whereas lead and other metals are concentrated in the slag. The metal phase is treated, so that, the precious metals and copper are recovered by leaching and electrolysis. The purity of the copper obtained in this process is 91%. The gases produced in the process are cooled with the energy recovered from the process and they are cleaned using techniques such as filtration,

electrofiltration or scrubbing. The sulphide formed is converted to SO_2, which is then transformed to sulphuric acid.

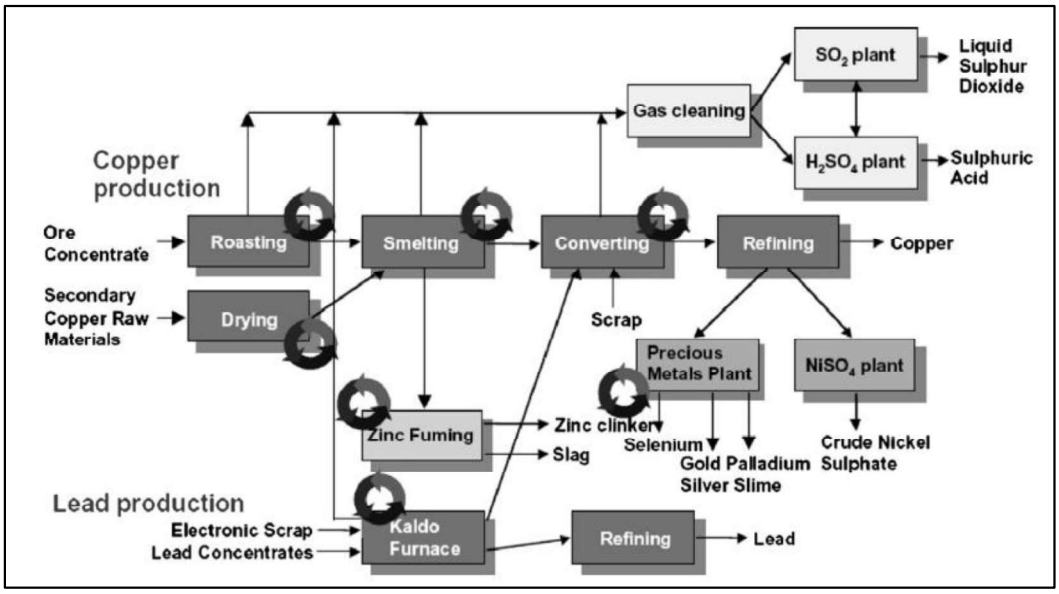

Figure 2.13. Diagram of the Rönnskar process to recover copper from e-waste (Cui and Zhang 2008).

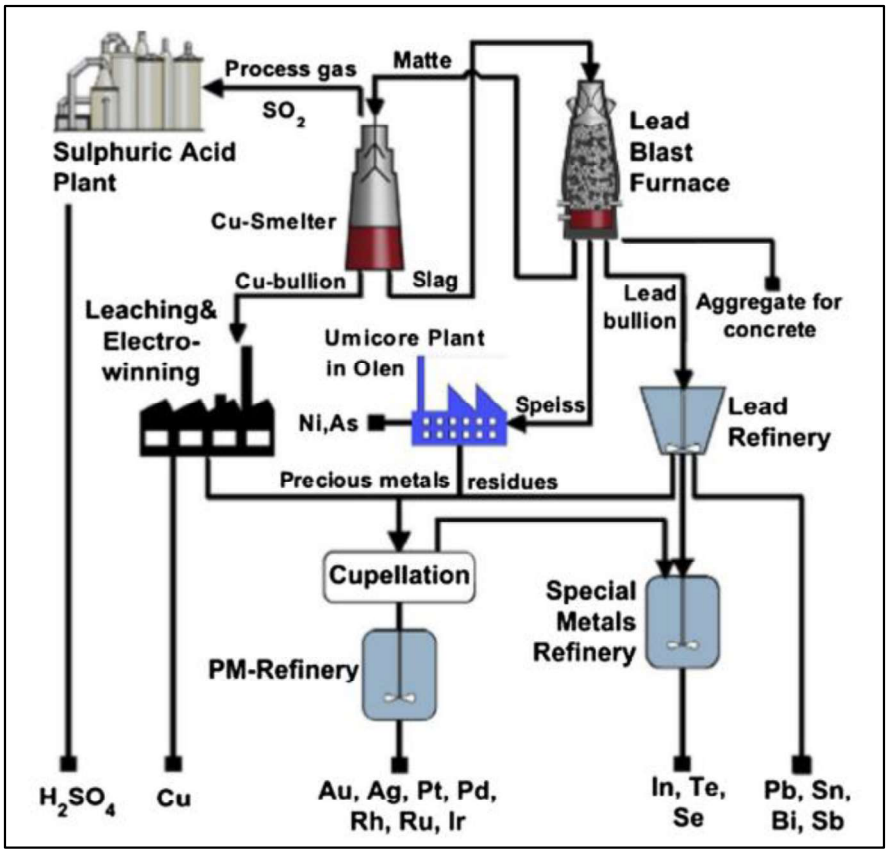

Figure 2.14. Diagram of the Umicore process to recover metals from e-waste (Yu-Gong et al. 2016).

In all three cases, an oven at 1200 ºC approximately is used into which the electronic waste is introduced to be melted. Although the three methods are quite similar, the Umicore process is more complex than the others, since it also includes hydrometallurgical steps in the procedure. Nevertheless, the Noranda process allows to obtain a higher purity of copper (99.1%) in comparison to the purity obtain by the Umicore (91%). Despite availability of these methods, current research is aimed at pyrometallurgical processes utilizing thermal plasma in conjunction with the combustible organics contained in e-waste (Shuey and Taylor 2005). In particular, this process is performed in a plasma reactor (Figure 2.15) vertically located to allow a continuous direct contact of the samples with the plasma gas, which is a mixture of CH_4 and CO_2. The samples are fed from the opening at the top of the reactor, opposite the off-gas fume hood. At the bottom of the reactor the temperature varies from 385 to 570 ºC at the moment of charging and the highest recorded temperature is achieved when the material is treated (around 840 ºC). It is noticed that temperatures inside the reactor before feeding are contributed by plasma gas, whereas temperature increases after feeding are mainly due to degradation of the organic components of the PCBs. In this sense, the thermal plasma process to recover metals from e-waste minimizes the energy requirements at the same time that the complete destruction of organic compounds during the recovery of entrained metal values is assured (Shuey and Taylor 2005).

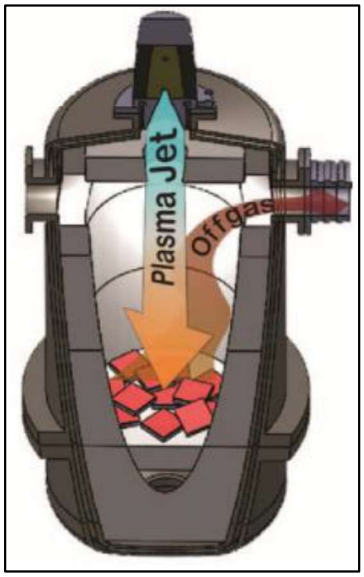

Figure 2.15. Reactor used for the recovery of metals from e-waste by thermal plasma treatment (Mitrasinovic et al. 2011).

2.2.2. Hydrometallurgy

Hydrometallurgical processing consists of the extraction and recovery of metals using different chemicals as strong acids or organic solutions. In any hydrometallurgical process two main steps are required. On the one hand, the leaching or lixiviation step in which the metals from the solid matrix are transferred into an aqueous phase. It is usually in this stage that not only the metals of interests are transferred into solution, but also some undesirable constituents present in the material. On the other hand, the separation step in which the metals of interests are separated from the undesirable elements presents in the solution.

There are different hydrometallurgical techniques depending on the type of leachate used: chemical leaching and hydrometallurgical etching (Figure 2.16).

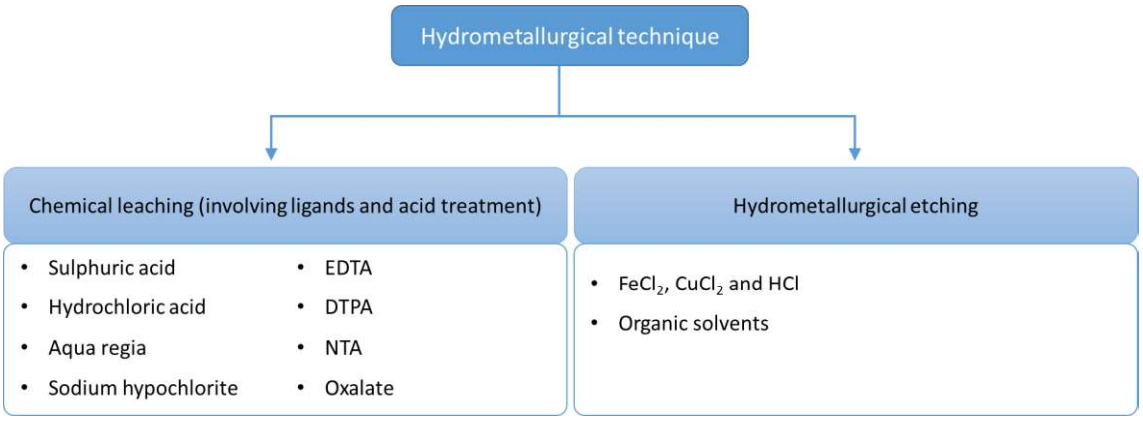

Figure 2.16. Hydrometallurgical techniques used to recover metals from ores and e-waste. Abreviations: EDTA (Ethylene diamine tetraacetic acid), DTPA (diethylene triamine pentaacetic acid), NTA (nitrilotriacetic acid) (Adapted from Pant et al. (2012)).

Currently, acid leaching is the most popular leaching method among hydrometallurgical processes. Although it is quite corrosive, the use of acids to leach metals from ores or e-waste allows high leaching rates and fast kinetics. On the contrary, methods like cyanide leaching for the standard gold recovery are being phased out due to their high toxicity. There are some other methodologies less hazardous than the previous ones such as thiourea and thiosulfate leaching, but they are not as economically feasible since both require considerable amounts of reagents due to the poor stability of the thiourea and the slow kinetics of the thiosulfate (Hsu et al. 2019).

In the mining field, the most common methods are the leaching in place, the heap or dump leaching, the percolation or vat leaching, the agitation or pulp leaching and the high pressure leaching.

The *leaching in place or in situ* is used for very low grade ores for which the transport expenses are not reasonable (Watling 2015). Even so, this methodology is also carried out for higher ore grades due to the good results obtained. For its application, the ore body must be enclosed between impermeable strata and also permeable to the leaching solution. The process takes place at ambient temperature and pressure. Using this methodology to recover metals usually takes years to be completed (e.g. chemical in situ leaching of uranium).

The *heap or dump leaching* is used for low grade ores because of its low cost and it is the mainly used hydrometallurgical process to recover metals at industrial scale (Ghorbani et al. 2015). The dump leaching consists of a dump between 10 and 15 m high formed by the ore, which is placed over a compacted soil that is leveled at a slight inclination. For preventing the liquid to go into the ground a liner can be also used. The dump is irrigated with a dilute leaching solution that percolates through the ore to dissolve valuables, collecting the solution at the base of the dump. Heap bioleaching is similar to dump leaching, but the main difference is that uncrushed material is used in dump leach whereas crushed and/or agglomerated material is used in heap leach (Ghorbani et al. 2015) (Figure 2.17). This fact has the consequence that the dump leaching needs one to two years to extract 50% of the desirable metal due its slow kinetics. In the case of the heap leaching, since small particles are used, the kinetics can range from two months to two years depending on the leaching facility of the ore treated. Moreover, in heap bioleaching the extraction can also range from 30% to over 90% for the ores easiest to leach. As leaching in place method, the heap and dump leaching are performed at ambient temperature and pressure. However, it is noted that when sulphides are treated with this methodology for example, bioleaching of sulphides releases a lot of heat, so that within the heap there may be 60 or 70ºC.

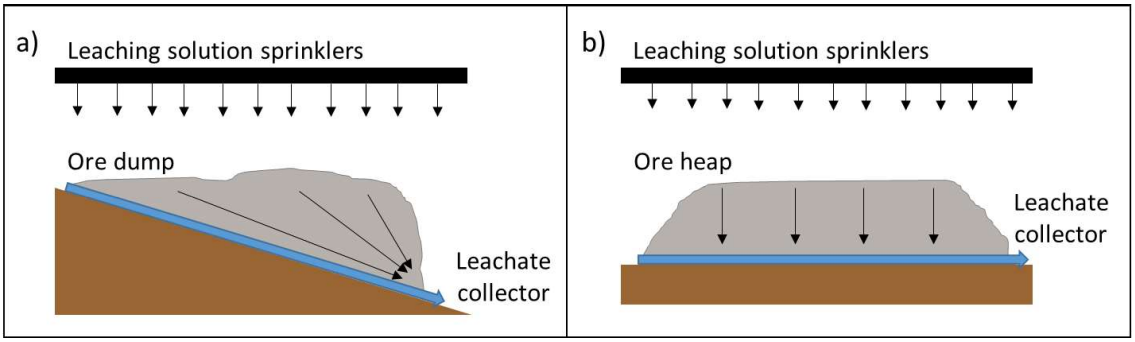

Figure 2.17. Diagram of (a) dump leaching and (b) heap leaching used in the mining field (Adapted from Abhilash and Pandey (2013)).

The *percolation or vat leaching* is suited for porous and sandy material and cannot be applicable to material which tends to pack into impermeable masses (Cope 1999). The material is placed in a vat with a false bottom covered with a filtering medium and the leaching solution is added on the top of the vat. The solution percolates through the material, collecting the dissolved metals at the bottom. In this methodology the regularity of the particle size is important for the good percolation. This leads to slow extraction and channeling of solutions through the bed. The process takes place at ambient temperature and pressure.

The *agitation or pulp leaching* consists of mixing the leaching solution with the finely ground raw material, forming a pulp which has to be continuously agitated in order to reduce the time required for the extraction (Gupta and Mukherjee 2000). This agitation can be mechanical like motor-driven impellers or pneumatic like compressed air. In this methodology the material treated must have moderate or high grade.

The *high pressure leaching* is performed in closed tanks or autoclaves (Xu et al. 2010). The process can take place in presence or absence of oxygen/air. In absence of oxygen/air, the rate of leaching is low whereas in the presence of oxygen, the oxygen partial pressure can control the leaching rate, increasing the rate with increasing partial pressure. The autoclave is the main part of the pressure leaching plant and when an autoclave is used, agitation is required. Autoclaves can be applied for continuous or batch leaching.

When the PCBs have been crushed into small particles, they can be treated as fine ore particles. Therefore, all the leaching configurations applied in the mining field can be adapted for metal recovery from e-waste, excepting the leaching in situ configuration since the e-waste is not found underground as it occurs with ores.

According to Tuncuk et al. (2012), hydrometallurgical processes offer relatively low capital cost, reduced environmental impact and high metal recoveries in comparison to the pyrometallurgical ones. This fact makes hydrometallurgical processes more suitable for small scale applications. Nevertheless, hydrometallurgical processes require the use of hazardous chemicals as strong acids which implies a high-cost process. Hence, to adopt effective and eco-friendly recycling technologies is essential to prevent environmental pollution, landfill disposal (in the case of e-waste) and to save energy and natural resources. For this reason, the main goal for metal recycling is to develop a proper technology to reduce the harmful environmental impact. This should also be economically attractive to compensate recycling cost and, thus, appealing to the interest of governments and private investors (Khaliq et al. 2014). In this sense, bio-

hydrometallurgical processes have been developed as an alternative technology for recovering metals from ores and concentrates (Watling 2006; Zhang and Xu 2016).

2.3. Biological recovery of metals from ores and e-waste

Biological leaching, also called bioleaching, offers a promising technology to recover metals from metal ores or e-waste. It is defined as the extraction of metals by the metabolic activity of bacteria (or metabolic compounds), and it is applicable to recover metals from low-grade ores, to removal of toxic metals and to recovery of metals from waste materials as e-waste (Pollmann et al. 2018). According to Valix (2017), biologically assisted degradation of waste has a high potential as a recycling technology due to its low environmental impact, low operational cost and low energy requirements. Rawlings (2002) affirmed that, almost without exceptions, the procedures of microbial extractions are more environmentally friendly than the traditional physicochemical ones. This is because biological recovery is usually performed under ambient conditions which significantly reduced the required energy in comparison to the pyrometallurgical extraction, and also reduced the harmful gas emissions. Moreover, the metabolic products formed during bioleaching are not usually harmful, hence avoid expensive palliation to prevent environmental pollution and processing risks, so that the biological recovery results in a lower operating investments (Valix 2017).

The first scientific evidence for the role of microorganisms in metal solubilisation was evident in the middle of 20th century when *Thiobacillus ferrooxidans* (later reclassified as *Acidithiobacillus*) was identified for the first time, being isolated from acid mine drainage by Colmer and Hinkle in 1947 (Dave et al. 2018). Since then, research activities on the use of such bacteria picked up and commercial application of bioleaching to recover metal from ores began to emerge (Natarajan 2018). In particular, the commercial application of bio-hydrometallurgy was initiated in 1980 for copper leaching from heaps and numerous copper heap bioleach operations have been set up since then (Mishra et al. 2005). Nevertheless, the recovery of metals by bioleaching is considered one of the most promising technologies tested in the last decades to obtain metals (Kasper et al. 2015). So many researchers are still focusing on improving and optimizing the technique today (Kaksonen et al. 2018; Sajjad et al. 2018; Zhao et al. 2019; Zhou et al. 2019). Due to the promising results obtained in the recovery of copper from ores by bioleaching throughout history, this technique has been adapted to be applied in the field of the e-waste. In this way, metals can be recycled from the scrap, thus reducing the environmental impact of its disposal, which is an emerging worldwide problem today.

Although several studies have been conducted in recent years to test the use of such technique to obtain metals from ores, there are still relatively few studies focused on the possibility of using this technique to recover metals from e-waste (Kasper et al. 2015), thus, the research is still under development. Moreover, its practical application continues to face many challenges on a scale that allows to market the process and be competitive, such as the toxicity of the e-waste elements or the scaling of the process (Valix 2017).

2.3.1. Bioleaching mechanisms

The mechanism of the bioleaching process has been widely studied in the recovery of metals from minerals, whereas it has been little studied in the recovery of e-wastes. Nevertheless, some authors affirmed that the mechanism of e-waste bioleaching is similar to the mineral one (Choi et al. 2004; Willner and Fornalczyk 2013).

The mechanisms of the bioleaching technique can be classified in two different categories: the direct and the indirect leaching. In the first one, there is a physical contact between the microorganisms and the metal or the mineral by means of extracellular polymeric substance (EPS) synthesized by the microorganisms and the microorganisms are directly involved in redox processes of the metal or mineral (Fig. 2.18a). In the second one, the microorganisms do not necessarily act on the metal or mineral surface, but they regenerate the chemical agent that leaches the metal (Fig. 2.18b). In fact, the indirect leaching may take place in contact with the metal or the mineral, but it may also take place by planktonic cells.

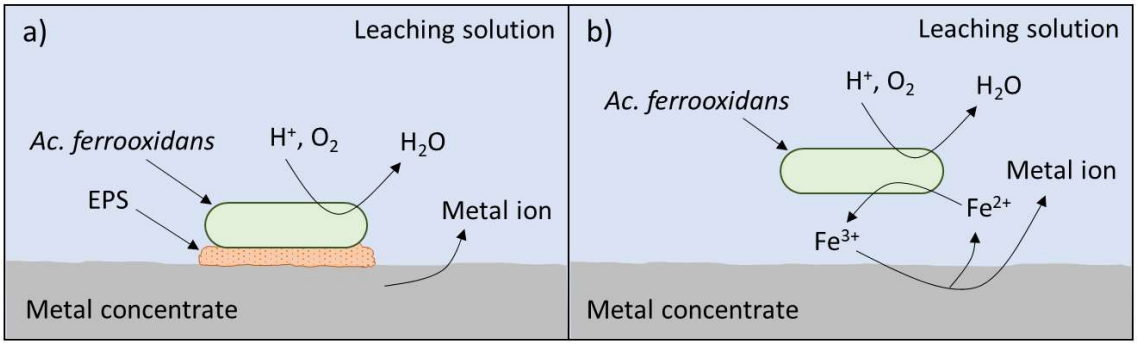

Figure 2.18. Diagram of bioleaching mechanisms for metals extraction: (a) direct mechanism and (b) indirect mechanism (adapted from Watling (2006)).

In the direct leaching mechanism, the mineral is directly attacked by the microorganisms to oxidize the sulphidic sulphur to elementary sulphur, releasing the metal as a metal sulphate as it is shown in Eqs. (2.3) and (2.4) (Pant et al. 2012).

$$MS + H_2SO_4 + 0.5\,O_2 \rightarrow MSO_4 + S^0 + H_2O \quad (2.3)$$

$$S^0 + 1.5\,O_2 + H_2O \rightarrow H_2SO_4 \quad (2.4)$$

where *M* is the metal to be extracted from the metal ore or concentrate.

There is some evidence explaining that intimate contact between the bacteria and the mineral surface is needed (Bosecker 1997). However, the mechanism of attachment and the initiation of metal solubilisation are not completely understood. Some authors affirmed that the bacteria do not attach to the whole mineral surface, but prefer specific sites of structure imperfections and the solubilisation of metals is produced by electrochemical interactions (Bennett and Tributsch 1978; Rodriguez-Leiva and Tributsch 1988).

In the indirect mechanism, the bacteria generate a lixiviate which chemically oxidizes the metal-containing material. Hence, the microorganisms do not necessarily act on the mineral or metal surface, but they regenerate the chemical agent. In fact, during the indirect mechanism the bacteria may be in contact with the mineral. In acid solutions, the leaching agents are iron (III) cations, which are in turn reduced to iron (II). Therefore, the reaction depends on the metal and mineral extracted but, in general, it is based on a redox reaction in which the mineral is oxidized to its soluble form and the iron is reduced. The role of the microorganisms is the oxidation of iron (II) to iron (III), to regenerate the leaching agent as it is shown in Eqs. (2.5) and (2.6) (Pant et al. 2012).

$$MS + 2\,Fe^{3+} \rightarrow M^{2+} + 2\,Fe^{2+} + S^0 \quad (2.5)$$

$$2\,Fe^{2+} + 0.5\,O_2 + 2\,H^+ \rightarrow 2\,Fe^{3+} + H_2O \quad (2.6)$$

where *M* is the metal to be extracted from the metal ore or concentrate.

Depending on the intermediates formed in the process, the indirect mechanism is also divided in two different sub-mechanisms when metal sulphides are treated, which are the thiosulphate and the polysulphide mechanisms (Schippers and Sand 1999). As Figure 2.19 depicts, in the thiosulphate mechanism the oxidative attack is performed exclusively by the iron (III), whereas in the polysulphide mechanism the attack can be performed by the iron (III) and/or by protons.

It is noteworthy to point out that these sub-mechanisms have been extensively described in the mining field. Depending on the ore treated, the indirect mechanism can be the thiosulphate or the polysulphide one (Crundwell 2003; Klaus 1997; Mishra et al. 2005; Sand et al. 2001). For instance, the thiosulphate mechanism occurred when acid-insoluble metal sulphides are treated like pyrite or molybdenite, whereas the

polysulphide mechanisms occurred when acid-soluble metal sulphides are treated like chalcopyrite or sphalerite.

Despite the description of the direct and indirect mechanisms, a universal theory about the mechanism of metal leaching is still to be revealed. Nevertheless, most of the important contributors to the direct versus indirect leaching discussion agree that the mechanism of mineral solubilisation is the indirect one, taking into account the observation of kinetics, stoichiometry and other considerations (Crundwell 2003; Rawlings 2002). In the e-waste field, Lilova et al. (2006) affirmed that both direct and indirect mechanism can be performed, but the direct oxidation of the metal is relatively slow compared to the indirect mechanism. Therefore, the last one is the predominant one in e-waste bioleaching processes. Depending upon the special needs of particular bacteria, a large variety and types of culture media are employed in the isolation of bacteria according to their biochemical and physiological properties.

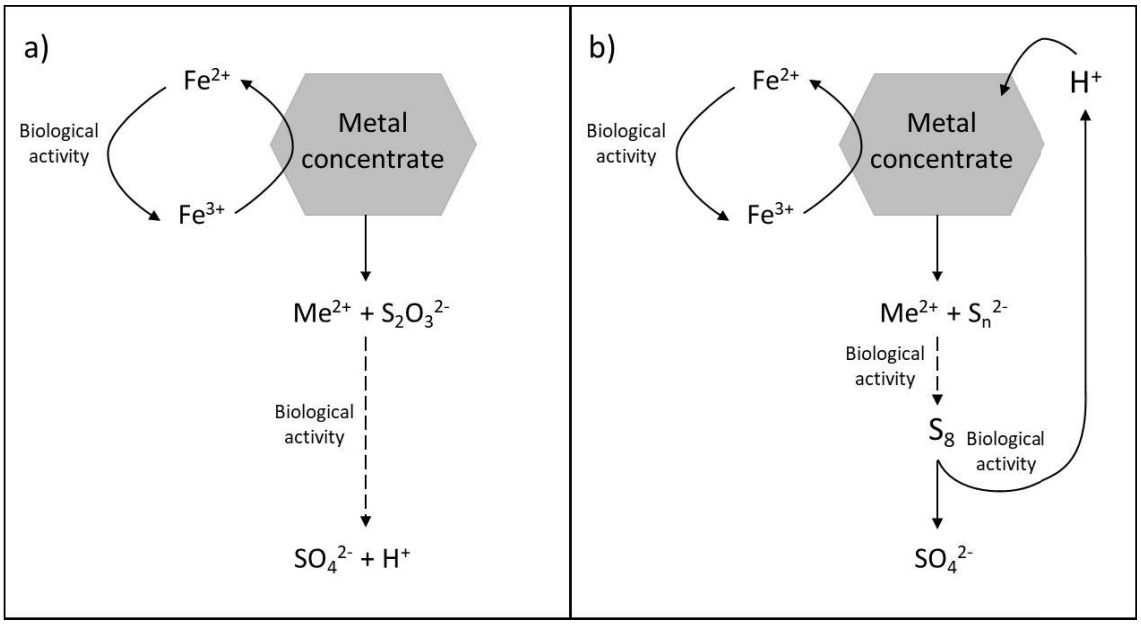

Figure 2.19. Indirect bioleaching mechanisms proceed via (a) thiosulphate or (b) polysulphide mechanisms. Dashed lines indicate the presence of intermediate sulphur compounds (adapted from (Schippers and Sand 1999)).

2.3.2. Microorganisms involved in bioleaching processes

Microorganisms and their metabolites play a pivotal role for the solubilisation of metals into an aqueous phase from ores, concentrates and e-waste (Dave et al. 2018). The main characteristic of the microorganisms used to recover metals is their capacity to grow in very aggressive environments. The major microorganisms involved in

bioleaching processes can be broadly grouped as iron and sulphur oxidizers, cyanogenic organisms and organic producers (Table 2.5).

Table 2.5. Major microorganisms involved in bioleaching processes (Brandl 2001; Dave et al. 2018; Li et al. 2020; Riveros et al. 1989; Zhang et al. 2010a).

	Name of organisms	Optimal pH	Temperature (ºC)	Source of energy	Metabolic product as lixiviant	Metal extracted
Iron oxidizers	*Acidithiobacillus ferrooxidans*[1]	2.0	28-35	Ferrous sulphate	Ferric sulphate	Cu, Zn, Ni, Pb, Cd
	Leptospirillum ferrooxidans	1.8	30			
	Leptospirillum ferriphilum	1.6	40			
Sulphur oxidizers	*Acidithiobacillus thiooxidans*	2.0-3.5	10-37	Sulphur and reduced sulphur compounds	Sulphuric acid or oxidized form of sulphur compound	Cu, Zn, Ni, Al
	Sulfobacillus thermosulfidooxidans[2]	1.7-2.4	40-55			
	Sulfolobus spp.	2.0-3.0	55-85			
Cyanogenic organisms	*Chromobacterium violaceum*	7	28	Glycine	HCN	Au, Ag, Pd, Pt
	Pseudomonas aeruginosa	7	37			
	Pseudomonas fluorescence	7	30			
Organic acids producers	*Aspergillus niger*	4.5	30	Carbohydrate (Glucose or sucrose)	Citric, oxalic, gluconic and malic acid	Cu, Zn, Ni, Pb, V, Mo, Al, Co, Li
	Penicillium simplicissimum	5.5	22-30			

[1]*Acidithiobacillus ferrooxidans can also oxidize sulphur compounds.*

[2]*Sulfobacillus thermosulfidooxidans can also oxidize iron.*

As shown in Table 2.5, the most commonly used microorganisms to bioleach metals are aerobic chemoautotrophic acidophiles. Moreover, the bacteria most active in bioleaching belong to the genus *Leptospirillum* and *Acidithiobacillus*. In particular, *Acidithiobacillus ferrooxidans* (Figure 2.20) is the most investigated organism, being present in more than 30% of bioleaching studies (BIOMOre 2018). They have been of particular interest since they belong to the few microorganisms known to obtain energy from iron oxidation in acidic environments (Zhan et al. 2019). In fact, this microorganism was first isolated from acid mine wastewater by Colmer and Hinkle (1947), although they can be found in different natural environments such as soil, sea and fresh water or volcanic ash (Zhan et al. 2019).

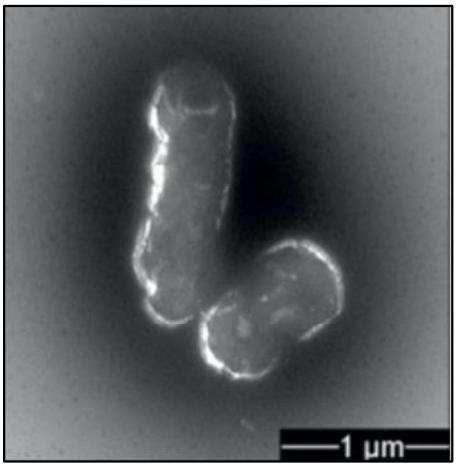

Figure 2.20. Microscopic observation of *Acidithiobacillus ferrooxidans* cells through scanning electron microscope (SEM) (Díaz-Tena et al. 2013).

Specifically, *Acidithiobacillus ferrooxidans* is an aerobic bacterium, gram-negative and *γ*-proteobacterium (Barron and Luecking 1990; Valdés et al. 2008). As chemoautotrophic bacteria, they use carbon dioxide as a carbon source while they obtain energy from the oxidation of ferrous ions or of sulphur compounds using oxygen or ferric ions as final electron acceptor (Nemati et al. 1998). *Ac. ferrooxidans* are classified as acidophilic and mesophilic microrganisms, since their optimal growth is achieved at ca. pH = 2 and 30 ºC, although they can also grow at pH = 1 or lower (Valdés et al. 2008). They may not affect human health, since their properties do not allow them to colonize and harm the human body. Therefore, the *Technical Rules for Biological Agents* classified these microorganisms in the risk group 1, which comprises those microorganisms for which it is improbable that they cause an infectious disease in humans. With respect to their physical characteristics, *Ac. ferrooxidans* are rods which are up to 1.0 µm long and 0.5 µm wide (Johnson et al. 2007).

2.3.3. Bioleaching performance

The design of a bioleaching process depends on the microbial activity, and on the chemical or mineral composition of ores or solid wastes. For this reason, an optimization of the process conditions is needed prior to the technical application of the technology to recover metals from a particular solid matrix.

2.3.3.1. Reactor configurations

The first bioleaching experiments performed at laboratory scale were carried out in airlift percolators (Bosecker 1997). They consist of a glass tube with a sieve-plate at the bottom, which is filled with the particles of the metal ore or concentrate. The packing is irrigated by the leaching solution inoculated with bacteria and the liquid collected at the bottom is pumped to the top of the column to recirculate it again by compressed sterile air. In this sense, the air is also useful for the proper aeration of the system. The airlift percolator can take between 100 and 300 days to recover the metals, since the oxygen supply is often inadequate in this system and, moreover, the surface ratio unfavourable. For this reason, the airlift percolator has been replaced by the submerged bioleaching.

The submerged bioleaching consists of placing the metal ore or concentrate as fine particles in the leaching solution in a container, which is kept stirring. The container can be an Erlenmeyer flask or even a bioreactor, which is more sophisticated. In this system, the more accurate control of the process as well as the improvement of the conditions to favour the microorganisms' growth allows to obtain higher metal recovery rates in considerably shortened times in comparison to those obtained with the airlift percolator. This technique is the most used bioleaching technique in laboratory investigations since 1990 (Kaksonen et al. 2018). Nevertheless, column bioleaching is also used at laboratory scale to simulate heap or dump leaching as previous experiments before the implementation of the process at industrial scale.

The column leaching involves the percolation of the leaching solution through a solid stationary phase, which is placed inside the column by a support. Hence, it is quite similar to the airlift percolator, but in this case, the liquid is not pumped by compressed air but by a liquid pump itself. Depending on the size, the columns can be made of glass, plastic, lined concrete or steel and they can be used to treat from several kilograms to few tonnes (Bosecker 1997). Most column systems have devices to take samples or to measure parameters like temperature or pH. This gives information about what is to be expected in the dump or heap leaching and how it has to be optimized to increase the efficiency of the process.

2.3.3.2. Industrial bioleaching

The most simple form to conduct a bioleaching to extract metals is to collect the material in heaps, irrigating the heap with the nutrient solution and the microorganisms and collecting the liquid (leachate) at the bottom of the heap (Bosecker 1997). The leachate is recirculated due to the slow velocity of the bacterial oxidation, although the recirculation of the leachate is usually subjected to solvent extraction and it is the reffinate from the solvent extraction which is put on the heap again. There are different methodologies to extract metals biologically from ores or solids at industrial scale, including dump and heap bioleaching, underground bioleaching and tank bioleaching.

The dump and heap bioleaching are performed in the same way as in dump and heap leaching explained in section 2.2.2. The difference is that the leachate passes through an oxidation vessel in bioleaching process. In this vessel, the microorganisms regenerate the iron (II) ions of the leachate to iron (III) ions before being recirculated to the top of the heap or the dump again. It is also possible to install the aeration at the bottom of the heap avoiding the oxidation vessel, so the re-oxidation takes place in the heap itself. In both cases, the microorganisms regenerate the leaching agent unlike the chemical leaching in which the iron (III) is constantly added to the heap or the dump to extract metals.

The underground leaching is used to recover metals in mines. It consists of the introduction of the liquid containing bacteria inside galleries or mine waste, which filters through the stratum and leaches the target metals. After a sufficient time for reaction, the solution is collected and pumped to another gallery or zone. This methodology is quite similar to in-situ leaching, but underground bioleaching is more appropriate in those cases in which the ores are too low-grade or the deposit too small. Nevertheless, as it occurred in in-situ leaching, the procedure requires sufficient permeability of the ore body and impermeability to the gangue in order to prevent any percolation of the bioleaching solution, although barriers have to be applied to not distributed the solution outside the intended area.

Considering the high extraction rates obtained with the submerged bioleaching in laboratory investigations, these bioreactors were tested at industrial scale. Although this technique allows to have much higher reaction rates than heap, dump and underground bioleaching, tank bioleaching is more expensive to construct and to operate. Even so, this technique is used to treat refractory gold ores at industrial scale for instance (Bosecker 1997). In this sense, several bioreactor-based bio-hydrometallurgical technologies have been commercialised such as BIOX™, the first and most widely used in commercial applications for the bio-oxidation of refractory sulphidic gold ores (Kaksonen et al. 2018). The BIOX™ plant (Figure 2.21) consists of six reactors where three of them are configured in parallel whereas the rest are operated in series. The

process involves the continuous loading of a flotation concentrate slurry with the solid content usually at 20% (w/w), although some recent studies found that the solid content can be increased up to 30% without affecting the process efficiency (Mahmoud et al. 2017). The reactor temperature is maintained around 40-45 ºC and the retention time inside them varies from 4 to 6 days. The operating pH is between 1.2 and 1.6 and dissolved oxygen concentration in the slurry is maintained over 2 mg/L (van Aswegen, van Niekerk, and Olivier 2007).

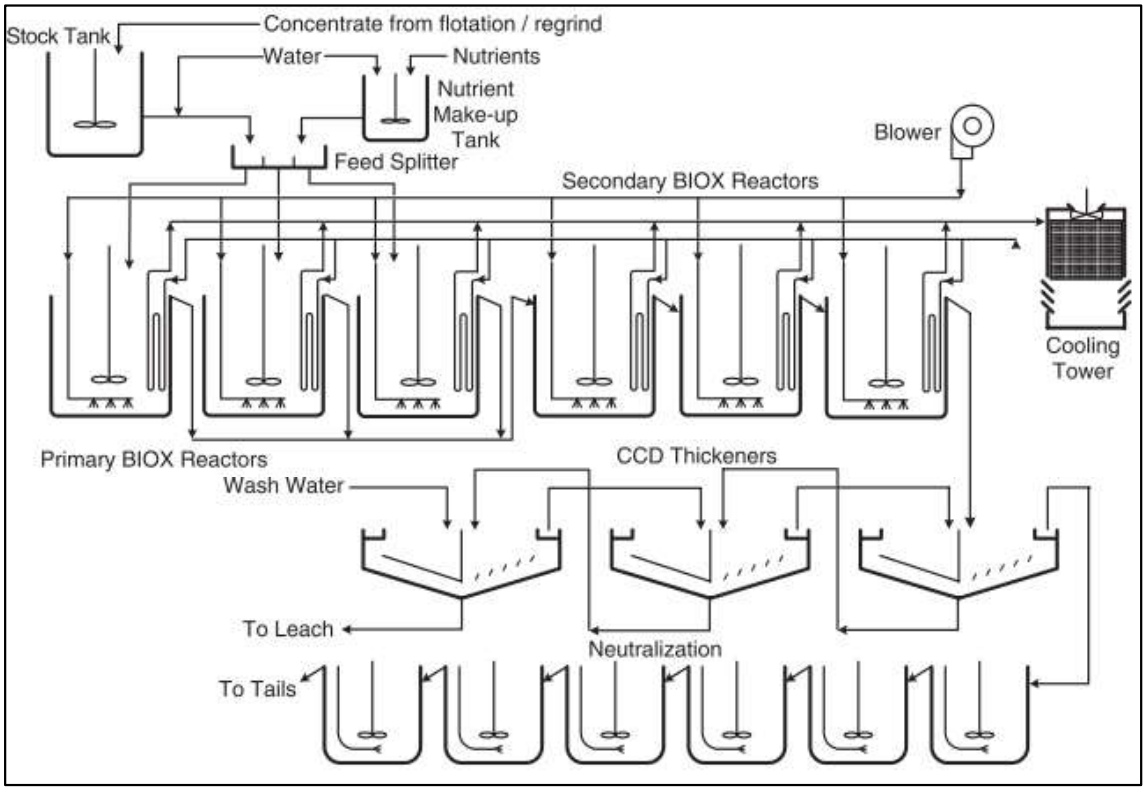

Figure 2.21. Typical flow sheet of the BIOX™ plant for the treatment of sulphide gold minerals (van Aswegen et al. 2007).

2.3.4. Factors influencing the bioleaching process

The bioleaching effectiveness depends mainly on the microorganisms and the chemical composition of the metal containing material to be treated (ores, concentrate or e-waste). In this sense, in order to obtain the maximum yields of metal extraction it is important to achieve optimal conditions for the bacterial growth as well as a good contact between the leaching agent and the solid treated. Therefore, there are many biotic and abiotic factors that can influence on the bioleaching efficiency and on the activities of the microorganisms. The factors can be categorized as physicochemical parameters, microbiological parameters, properties of the metal-containing material and type of process or conditions. Most of these parameters have been studied by different authors in recent years, as shown below.

The physicochemical parameters include pH (Dorado et al. 2012; Liang et al. 2013; Mishra et al. 2008; Mousavi et al. 2008; Xiang et al. 2010; Yang et al. 2009), temperature (Hong and Valix 2014; Lambert et al. 2015), redox potential (Vilcáez, Suto, and Inoue 2008; Zhao et al. 2015) and oxygen content and its availability (Giebner et al. 2016; Mazuelos et al. 2017; Thurston, Mandernack, and Shanks 2010), among others. Redox potential (ORP) is a very important physicochemical parameter in bioleaching processes. Similar to how the concentration of hydrogen ions determines pH, the tendency of electron transfer between chemical species and electrodes determines the ORP of an electrode couple. Therefore, ORP represents how easily electrons are transferred to or from species in the solution. In bioleaching reactions (see Eqs. 2.5 and 2.6), the bio-oxidation of iron (II) to iron (III) changes the ORP. For this reason the activity of the microorganisms can be monitored by ORP, since the presence of oxidizing agents in the medium such as iron (III) or oxygen has a positive correlation (Jafari et al. 2018). This occurs because the more oxidized species in the medium, the higher the redox potential. Hence, biological activity increases the redox potential, which in turn favours the metals extraction from ores or solid wastes in general (Gu et al. 2018).

Microbiological parameters of bioleaching are also important, since they can highly affect the effectiveness of the process. This group of parameters includes microbial diversity (Brandl, Bosshard, and Wegmann 2001; Fu et al. 2008; Hallmann et al. 1992; Klink et al. 2016; Mražíková et al. 2013; Qiu et al. 2005; Wang et al. 2009), population density (Bas et al 2013; Third et al. 2000; Willner 2013; Willner and Fornalczyk 2013), microbial activity and oxidation ability of microorganisms (Esquivel-Rios et al. 2014; Meruane and Vargas 2003; Nemati et al. 1998b; Owen and Laybourn-Parry 1987; Sampson and Phillips 2001; Song et al. 2011), and metal tolerance (Benzal et al. 2020a; Cho, Ryu, and Choi 2008; Das et al. 1997; David et al.as 2008; Magnin et al. 1998).

Properties of the metal-containing material to be leached also influence the bioleaching effectiveness. Main factors are particle size (Adhapure et al. 2014; Joshi et al. 2017; Shah et al. 2015; Wang et al. 2009; Zhu et al. 2011) and metal composition or type of metal-containing material (Agate and Khinvasara 1986; Arshadi and Mousavi 2015; W. a. Bizzo, Figueiredo, and De Andrade 2014; Dong et al. 2013; Madrigal-Arias et al. 2015; Wang et al. 2014).

Finally, the type of process or conditions may also influence the process. This group includes the effect of the leaching method used, for instance, performing the process in one or two steps (Benzal et al. 2020b; Fomchenko and Muravyov 2017; Heydarian et al. 2018; Isildar et al. 2016; Wang et al. 2017), the amount of solid treated (Brandl et al. 2001; Xiang et al. 2010; Yang et al. 2014; Zhu et al. 2011) and the mode of operation including heap, column or bioreactor operations (Benzal et al. 2020a; Chen

et al. 2015; Couillard and Mercier 1991; Ghorbani et al. 2015; Ilyas et al. 2010; Ilyas, Lee, and Kim 2014; Jujun et al. 2015; Qiu et al. 2011; Rivera-Santillán, Patricio-Ramírez, and Olvera-Pérez 2013; Rodrigues et al. 2015; Rossi, Trois, and Visca 1986; Silva et al. 2015; Tipre and Dave 2004; Vakylabad et al. 2012).

All of these parameters can affect the process, so they have to be optimized in order to achieve the highest metal recovery. However, depending on the metal-containing material as well as on the system used to bioleach, the operational conditions will vary which implies that the optimization has to be carried out for each waste and system.

2.4. Recovery of copper from leaching solution

Once the copper has been bioleached from the ores or the electronic waste, it remains in solution, so it has to be separated from the aqueous phase. There are different methodologies to transform copper ions in aqueous solution to its metallic form as solvent extraction followed by stripping and electrowinning, ion exchange, electrolysis and cementation (Agrawal and Kapoor 1982; Benzal et al. 2020b; Khattab et al. 2013; Zhang et al. 2010b). Among these methodologies, one of the most simple and low-cost methods is cementation. Cementation is a process in which metal ions precipitate by a more reducing agent. Iron, zinc and aluminium are some choices which supply the electrons for copper ion reduction in the cementation process, but iron is considered to be the best for this purpose (Jhajharia et al. 2016) (Eq. 2.7).

$$Cu^{2+} + Fe^{0} \rightarrow Cu^{0} + Fe^{2+} \qquad (2.7)$$

Thus, cementation allows to recover copper as a metallic particles with the physical characteristics that are necessary for metallurgical companies to take advantage of them (Stefanowicz et al. 1997).

It is noteworthy that the cementing agent must have a more negative reduction potential than the metal to be cemented for cementation to take place. In the particular case of copper cementation, iron has a lower reduction potential than copper as it is shown in Eqs. (2.8) and (2.9).

$$Cu^{2+} + 2\,e^{-} \rightarrow Cu \qquad (E^{0} = +0.34\ V) \qquad (2.8)$$

$$Fe^{2+} + 2\,e^{-} \rightarrow Fe \qquad (E^{0} = -0.44\ V) \qquad (2.9)$$

Thus, the standard reduction potential ($\Delta E°$) of the cementation reaction is positive (+0.78 V) and thus the standard free energy ($\Delta G°$) is negative ($\Delta G° = -nF\Delta E°$). This indicates that this process is favourable thermodynamically and, therefore, the cementation reaction of copper with iron is spontaneous. The cementation process ends

when the concentration of copper ions in solution is reduced to a certain value in which the electrode potential of copper is equal to the potential of the iron electrode. This moment occurs when the system reaches the equilibrium state.

Chapter 3

Objectives

3.1. General and specific objectives

The main goal of this thesis was the study, characterization and technology application of autotrophic microbial populations as an alternative to the current procedures to extract valuable elements from metal-containing materials and thus explore optimal conditions of operation and limitations of applicability.

For the achievement of this general goal it is essential to consider a number of specific objectives which are defined below:

- To determine the procedure to implement the bioleaching process to extract valuable metals from metal-containing materials.

- To study the bases of the process by determining the effectiveness of the bioleaching process to recover copper from low-grade mineral ores in batch conditions.

- To characterize the potential toxicity of different bioleached metals to the microorganisms involved in the process by microrespirometric technique.

- To elucidate and to assess the evolution of the biological activity in response to operational parameters during the bioleaching process by means of microrespirometries and fluorometric measurements.

- To apply and to adapt the biorecovery process in the field of electronic waste to recover copper from printed circuit boards (PCB) in batch conditions.

- To extend the methodology for extracting metals from PCB to a continuous stirred-tank reactor as well as to a column reactor in order to determine the capacity of bioleaching under these conditions for its application on an industrial scale.

- To propose a new methodology based on the use of a leaching column to analyse key operational parameters to optimize the steps of the process reducing time consumption and keeping high recovery efficiencies.

Chapter 4

General materials and methods

4.1. Experimental equipment

4.1.1. Microbial consortium cultivation

A mixed microbial consortium was initially used in the experiments (specifically in Chapter 5), which was obtained from a lab-scale gas-phase biotrickling filter (López et al. 2016). The biomass was cultivated in a fermenter (Figure 4.1) (*Minifors, Infors HT, Switzerland*). The microorganisms were cultivated under aerobic conditions using 3.5 g/L of sodium sulphide as substrate. Moreover, the dissolved oxygen (*Oxyferm FDA 225, Hamilton, Switzerland*), the pH (*405-DPAS-SC-K8S, Mettler Toledo, Switzerland*) and the temperature (*Pt100, Infors, Switzerland*) were controlled inside the tank. All these parameters were monitored and their data acquired using a specific software (*Iris 6, EMPA, Switzerland*). The pH was maintained at pH 7 by the addition of HCl 0.1 M or NaOH 0.1 M, the temperature was controlled at 30 ºC and the dissolved oxygen (DO) was checked to ensure it was not below to 7 ppm. Moreover, 70 rpm were fixed for the tank stirring. The mineral medium used for their growth are detailed in Table 4.1.

Figure 4.1. Fermenter used for the cultivation of the mixed microbial consortium.

The mixed consortium was previously characterized by Maestre et al. (2010) by cloning and sequencing 16S rRNA fragments. The most abundant species identified were *Thiothrix spp*, *Sulfurimonas denitrificans*, *Halothiobacillus neapolitanus*, *Thiobacillus denitrificans* and *Thiomonas intermedia*. Nevertheless, the mixed consortia also contain iron-oxidizing microorganisms since the packing material (steel) inside the biotrickling filter presented an advanced oxidation state that could be produced by the presence of this bacteria. Taking into account that iron-oxidizing microorganisms are one of the most effective microorganisms for bioleaching processes (Valix 2017), the use of the consortia for this purpose was tested. In addition, the culture was also adapted to bioleaching conditions (acidic medium, for instance) to evaluate the improvement on metal recovery. For this purpose, 100 mL of the mixed consortia suspension (500 mg of

biomass/L) from the fermenter was inoculated in a 500 mL Erlenmeyer flask with 100 mL of mineral medium (its composition is detailed in Table 4.2). Then, 10 g of chalcopyrite powder within 2 and 3 mm of particle diameter were added and the flask was incubated at 30 ºC and 130 rpm using orbital shaking (*SI500, Stuart, United Kingdom*) for 25 days.

Table 4.1. Composition of the mineral medium used for the growth of the mixed microbial consortium.

Inorganic salt		Quantity (for 1 L)
NH_4Cl		1.000 g
$MgSO_4 \cdot 7\ H_2O$		0.200 g
KH_2PO_4		0.152 g
$CaCl_2 \cdot 2\ H_2O$		0.032 g
Trace solution (10 mL)	Distilled H_2O	1.000 L
	HCl 37%	11.270 mL
	$FeCl_2 \cdot 3\ H_2O$	2.500 g
	H_3BO_3	0.100 g
	$MnCl_2 \cdot 4\ H_2O$	0.170 g
	$CaCl_2 \cdot 6\ H_2O$	0.200 g

Table 4.2. Composition of the 6K mineral medium used for the growth of *Acidithiobacillus ferrooxidans*.

Inorganic salt	Concentration (in g/L)
$Fe_2SO_4 \cdot 7\ H_2O$	30.000
$(NH_4)_2SO_4$	3.000
K_2HPO_4	0.500
$MgSO_4 \cdot 7\ H_2O$	0.500
KCl	0.100
$Ca(NO_3)_2 \cdot 4\ H_2O$	0.014

4.1.2. Pure *Acidithiobacillus ferrooxidans* cultivation

The bacterial strain *Acidithiobacillus ferrooxidans* (ATCC 23270) was also used in this thesis. It was kindly provided by the Department of Chemical Engineering from the University of País Basco (Spain). 10 mL of the original supplied sample was inoculated with 190 mL of mineral medium into a 500 mL Erlenmeyer flask. The mineral medium used was the 6K mineral medium (Table 4.2), according to the indications of the microorganisms' suppliers. It is noticed that iron (II) is the main energy source for the growth of *Ac. ferrooxidans (*6 g/L of iron(II) are used). After that, the flask was introduced

in an incubator at 30 ºC and 130 rpm (Figure 4.2). ORP was monitored and when it reached a value over 600 mV, it was considered that the iron (II) was almost oxidized (Diaz 2016).

Figure 4.2. Incubator used for the cultivation of *Acidithiobacillus ferrooxidans* in batch conditions.

4.1.3. *Acidithiobacillus ferrooxidans* cultivation in a discontinuous stirred tank reactor (DSTR)

To perform the experiment at larger scale and, thus, increase the volume and the concentration of the microorganisms, the strain *Acidithiobacillus ferrooxidans*, previously cultivated in an incubator, was used to inoculate a bioreactor. Therefore, their growth was conducted in 2 L jacketed discontinuous stirrer-tank reactor (DSTR) (Figure 4.3) (*VFOC.77/2, Vidrafoc, Spain*). In the inoculation, 600 mL of the inoculum and 1400 mL of 6K mineral medium was used (see the composition in Table 4.2). A thermostatic bath maintained the reactor at 30 ºC whereas a mechanical stirrer maintained an agitation at 200 rpm. Because the microorganisms are aerobic, the reactor was aerated at 0.2 L/min by compressed air. The pH was controlled at 1.75 by the dropwise addition of 3 N H_2SO_4 and the ORP was also measured to control when the reduced species (e.g. iron (II)) was completely bio-oxidized by the microorganisms. Hence, when the ORP exceed 600 mV the tank was fed with 200 mL of fresh 6K medium (every two days approximately). For that, 200 mL of the solution from the tank was removed, being replaced by 200 mL of fresh 6K mineral medium. After the medium addition, the pH was adjusted to pH 1.75 if necessary with 3 N H_2SO_4.

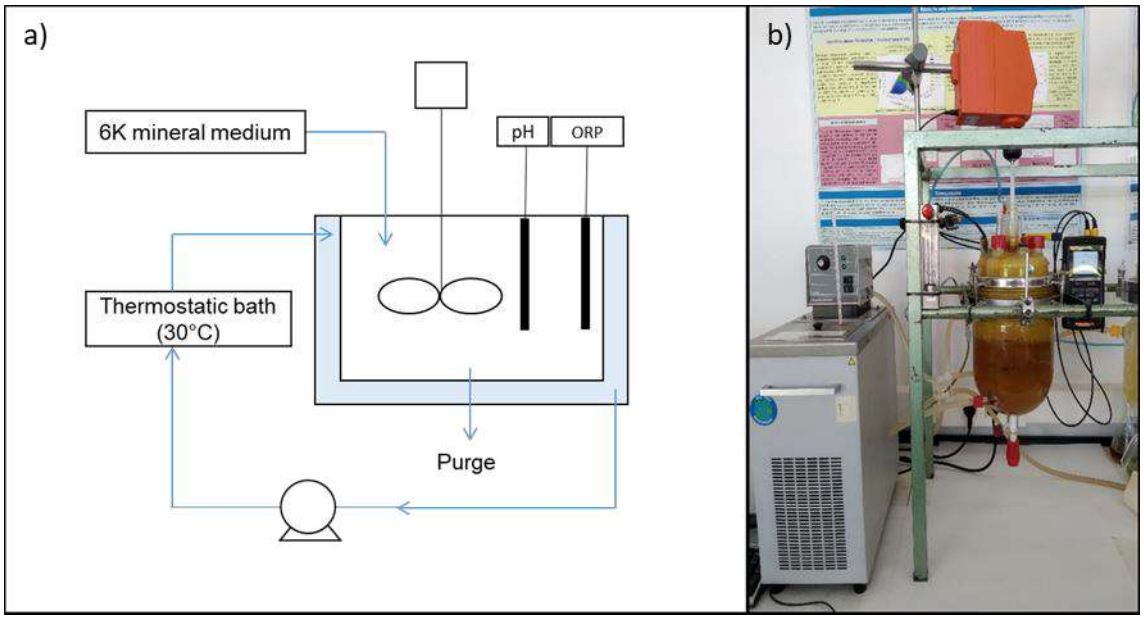

Figure 4.3. (a) Diagram and (b) picture of the discontinuous stirrer tank reactor in which the strain *Acidithiobacillus ferroxidans* were grown.

Although the biological sample was provided as a pure culture of *Ac. ferrooxidans*, a DNA extraction was performed in order to corroborate and to characterize the microorganisms that have been grown in the provided strain. For the DNA extraction, microorganisms withdrawn from the original inoculum were grown in a 4 L DSTR at the same conditions explained above and then the volume was centrifuged at 10000 rpm, finally obtaining 1 g of pellet. The extraction was carried out by the soil DNA isolation plus kit (in section 4.2.7 the protocol followed is described). The DNA metabarcoding analyses were carried out by AllGenetics & Biology SL. Results affirmed that all the sequences analysed were assigned to the genus *Acidithiobacillus spp*. Nevertheless, the analysis cannot confirmed that the strain provided for the experiments was a pure culture strain. It is noted that despite the continuous reinoculation of the culture during several months and their use in bioleaching experiments, the strain continues being a culture of *Acidithiobacillus spp.*, which indicates that this strain is quite difficult to be contaminated by any other species of microorganisms. This is beneficial in bioleaching processes since it avoids having significant variations of the microbial population initially inoculated for this purpose. It is noteworthy that actually, bioleaching process does not usually works with pure cultures but mostly one organism dominate the process. Moreover, *Acidithiobacillus spp*., as autotroph microorganisms, present the advantage over heterotrophs that contaminations are very hard as there is no organic in the system.

4.1.4. Microscopy

For the observation of the microorganisms used in this work, an optical and a Scanning Electron Microscope (SEM) were used.

The optical microscope (*BA310LED, Motic, Germany*) consists on a lens system that allows enlarging the image in order to visualize the microorganism present in a biological sample (Figure 4.4). The compound microscope has two system of lenses for greater magnification: the ocular and the objective lens. In particular, the four objectives lens of this microscope allow an increasing of x4, x10, x40, x100 although an increasing of x1000 was also possible with immersion oil.

Figure 4.4. (a) Optical microscope used for the visualization of the microorganisms during this work and (b) detail of the objectives used.

The scanning electron microscopy (*TS-1000, Hitachi, Japan*) uses electrons instead of light beam (protons) to create a high-resolution image, allowing a deep approach to the atomic world or organic materials (Figure 4.5). Since the wavelength of electrons are much smaller than photons, the electron microscopes have a higher resolution that optical ones and the resolution of a microscope is inversely proportional to the wavelength used.

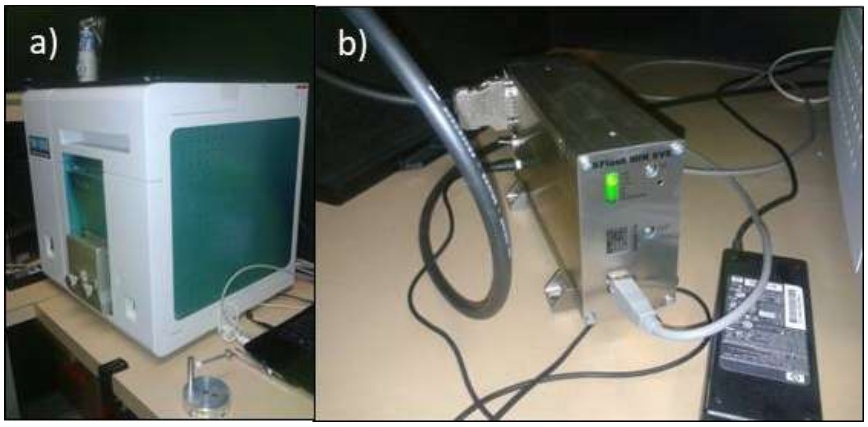

Figure 4.5. (a) The Scanning Electron Microscope (SEM) and (b) the Energy Dispersive X-Ray Spectroscopy analyser used in this work.

In the case of SEM observation, the sample must be previously dried by a dryer (*Conterm 150L, JP Selecta S.A., Spain*) in order to remove all moisture, since the vacuum is made inside the SEM microscope to create the image and moisture can cause interference on it.

4.1.5. Size reduction equipment

In some experimental designs, a reduction of the particle size of the waste was required. For the experiments in which chalcopyrite (Figure 4.6a) was tested for copper recovery in Chapter 5 of this thesis, a roller crusher (*Serie 24, Humboldt Wedag Española SA, Spain*) was used. After the reduction by a roller crusher, the particles were sieved to collect the particles between 2 and 3 mm of diameter (Figure 4.6b).

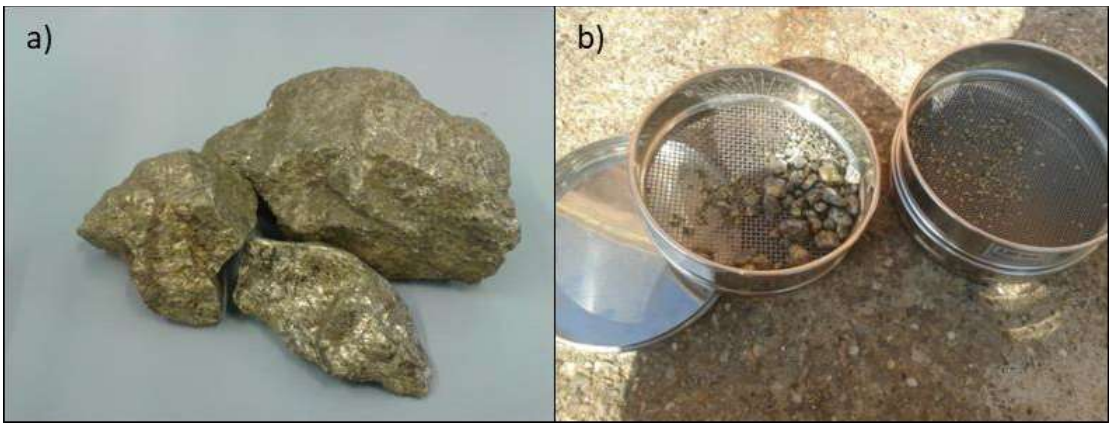

Figure 4.6. (a) Chalcopyrite used in the experiments before the particle reduction and (b) the sieves used for the selection of the desirable size.

During the research performed in this thesis (in particular from Chapter 6 to Chapter 9) end-of-life mobile phones have been used as a source for valuable metals (Figure 4.7a). They were provided by the company Electrorecycling S.A. (*El Pont de*

Vilomara i Rocafort, Spain). The printed circuit boards (PCBs) were manually removed from their structure (Figure 4.7b) and the main components as resistors, capacitors and chips were also separated. The size of the PCB was firstly reduced with a shears to 1 cm^2 particles and then, these particles were crushed with a grinder (Figure 4.8a) (*MF 10 basic, IKA, Germany*). The obtained particles were classified in different particle ranges: less than 0.2 mm of diameter, between 0.2 and 1.0 mm of diameter and higher than 1.0 mm of diameter with sieves of different granulometry, according to the desirable sizes (Figure 4.8b).

Both chalcopyrite and the different particle sizes of the PCB were digested and analysed to determine the copper content as well as to determine the concentration of other metals. The procedure followed for their digestion of samples is explained in detail in section 4.2.3.

Figure 4.7. (a) End-of-life mobile phones used in the experiments of electronic waste bioleaching and (b) the printed circuit boards removed from them.

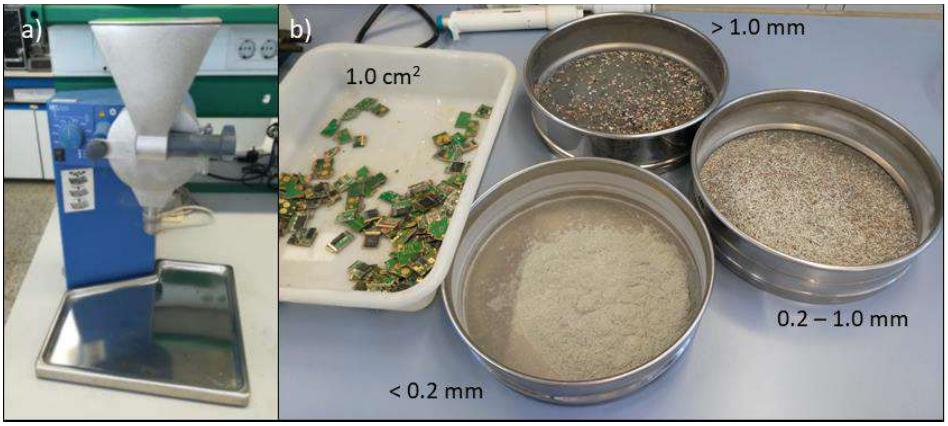

Figure 4.8. (a) Grinder used for particle size reduction of the electronic waste and (b) particles size obtained after their crushing.

4.2. Analytical techniques

4.2.1. Multimeter for pH and oxidation-reduction potential (ORP) measurements

A multimeter (*Multi 3620IDS, WTW, Germany*) was used to register and visualize the pH (*SenTix 980, WTW, Germany*) and the ORP (*Electrode SenTix ORP-T900, WTW, Germany*) in the leaching solutions as well as in the other liquid samples.

4.2.2. Dissolved oxygen monitoring system

The activity of the microorganisms was measured by microrespirometry, which consists on the measurement of dissolved oxygen in a small liquid sample after the addition of a pulse of substrate (less than 2 mL). In this sense, the evaluation of microorganisms' activity is possible through the oxygen consumption (final electron acceptor in aerobic bacteria). The system consists on an optical oxygen measuring device (*FireStingO2, PyroScience GmbH, Germany*) (Figure 4.9a) composed by a combined excitation and detection module, which is connected to a sensor spot by a fibre-optic cable (Figure 4.9b). The sensor spot, coated with an oxygen-sensitive fluorophore, is located opposite to the fibre-optic at both sides of the glass (Figure 4.9c). The operating principle is based on the red light excited (REDFLASH indicators), which show luminescence in the near infrared (NIR). The light emitted depends on the oxygen concentration, being higher when there is less oxygen concentration detected (Figure 4.9d). The optode signal was registered with a PC using the software Pyro Oxygen Logger v.3.213. An integrated temperature sensor from the optode system compensate automatically the temperature discrepancies (measurements are highly dependent). Even so, all the oxygen measurements were taken in a thermostat cabinet or thermostatic bath at 30 ºC to compensate temperature fluctuations. According to the manufacture's indications, the sensor spots were calibrated using air-saturated water (100% O_2) and 2% w/v sodium sulphite (0% O_2) as references.

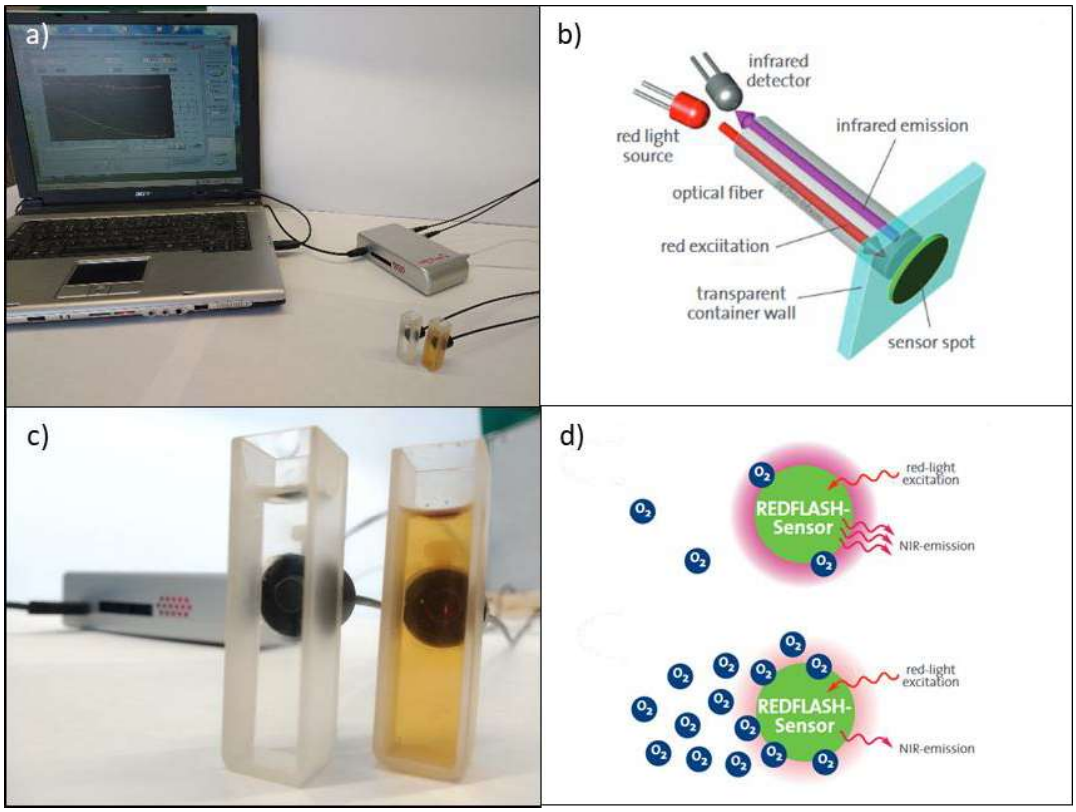

Figure 4.9. (a) Optical oxygen system used to perform microrespirometries in order to evaluate the activity of the microorganisms in a biological sample; (b) scheme of the oxygen sensor; (c) detail of the chambers of the system; and, (d) operating principle of the optode system ((b) and (d) were adapted from PyroScience 2018).

4.2.3. Waste digestion by microwave apparatus

In order to determine the content of metals in the PCB and in the chalcopyrite samples an acid digestion was performed. For this purpose, approximately 0.1 g of the solid sample and 10 mL of HNO_3:HCl (3:1) were introduced in the appropriate container (Figure 4.10a). Subsequently, samples were introduced in the microwave apparatus (4.10b) (*Microwave System, Millestone, Italy*) at 150 ºC during 15 min. Afterwards, the dissolution from the container was filtered at 0.45 µm to separate the liquid from the solid particles that could not be completely digested. Finally, an appropriate dilution was needed in order to analyse the metal's concentration by atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) since the linearity of copper measurements by these equipments are between 0 and 10 ppm. The repeatability of the analysis was determined making between 3 and 6 measurements.

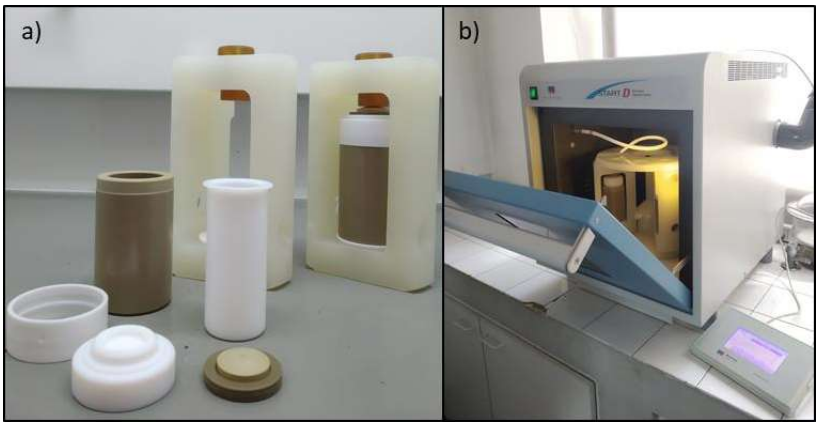

Figure 4.10. (a) Detail of the containers used for the microwave apparatus and (b) the microwave used in the experiments.

4.2.4. Metals determination by atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS)

Atomic absorption spectroscopy (Figure 4.11) is used to analyze metal elements that could be found in liquid samples. This technique is based on the radiation that can be absorbed at specific frequency when free atoms are generated in an atomizer. In particular, the amount of energy that is put in the flame is known and the remaining amount of energy on the other side can be measured by means of a detector. In this way, a calculation of how many of these transitions take place is possible, thus obtaining a signal which is proportional to the concentration of the element that is measured. During the experiments, an AAS (*Solar S2, ThermoFisher Scientific, United States*) was used to analyze copper and total iron concentration (when these two metals are found in high concentrations, ppm). On the contrary, when trace elements have to be analyzed, the ICP-MS (*7500CX, Agilent Technologies, United States*) was employed (ppb). ICP-MS, as its name suggests, uses an inductively coupled plasma to ionize the sample. It atomizes the sample, creating atomic and small polyatomic ions, which are then detected. In addition, the ICP-MS allows the analysis of metals and several non-metal elements whereas the AAS can only measure metal elements. Samples were filtered previous to dilution (through a compact 0.45 µm filter) in order to avoid possible obstructions in both the AAS and ICP-MS tubes.

4.2.5. Iron (II) and iron (III) determination by ultra-violet visible (UV-VIS) spectrophotometer

Since AAS can only measure total iron concentration, Ultra-violet visible (UV-Vis) spectrophotometer was carried out to discriminate iron (II) and iron (III) concentrations from measuring the intensity of a light that passes through the sample and comparing to

the light intensity without sample. The relation between both light intensities is called transmittance and it is related to the concentration of the analyte measured. Hence, in this work, the UV-Vis spectrophotometer (Figure 4.12) (*Lambda 25, PerkinElmer, United States*) was used to measure iron (II) and iron (III) concentrations from the colorimetric method with 1,10-phenantroline (Jeffery et al. 1989). Method consists on the reaction between the iron (II) and the *o*-phenantroline ($C_{12}H_8N_2$) which formed a red-orange compound (Eq. 4.1).

$$C_{12}H_8N_2 + \mathrm{Fe}^{2+} \rightarrow [(C_{12}\mathrm{H}_8N_2)Fe]^{2+} \qquad (4.1)$$

This compound only reacts with iron (II) ions, so if iron (III) want to be analyzed, the reduction of the iron (III) from the sample by its reaction with hydroxylamine is necessary (Eq. 4.2). In this way, the measurement of the iron (III) concentration is possible from the difference between the total iron and the iron (II) concentrations.

$$4\,Fe^{3+} + 2\,NH_2\mathrm{OH} \rightarrow 4\,Fe^{2+} + N_2O + 6\,H^+ + H_2O \qquad (4.2)$$

4.2.6. Microplate reader for total fluorescence measurements

A microplate reader (Figure 4.13) is used to measure the total fluorescence and thus estimate the cell number in a biological sample. This technique is based on a physical phenomenon in which certain substances (fluorophores) absorb energy in the form of electromagnetic radiation and then emits it at greater wavelength in a very short period of time. Specifically, when the fluorophore absorbs light, one of its electrons enters to an excited state (of higher energy) that is unstable, so when it returns to its basal state, the excess energy is released in the form of light, which is in a longer wavelength (less energy) in comparison to the excitement energy. These differences on the energy are detected by the microplate reader (*SpectraMax M2e, Molecular Devices, United States*), giving a signal which is proportional to the concentration of the fluorophore. The fluorophore used in the measurement was the PicoGreen®, which becomes intensely fluorescent upon binding nucleic acids. Hence, the signal obtain by the microplate reader is proportional to the amount of PicoGreen® detected and thus, to the biological material of the sample which is, in turn, related to the cell concentration. Therefore, after performing a correlation between the microplate reader signal and the cell number, the total fluorescence measurements allow to determine the cell concentration in a biological sample.

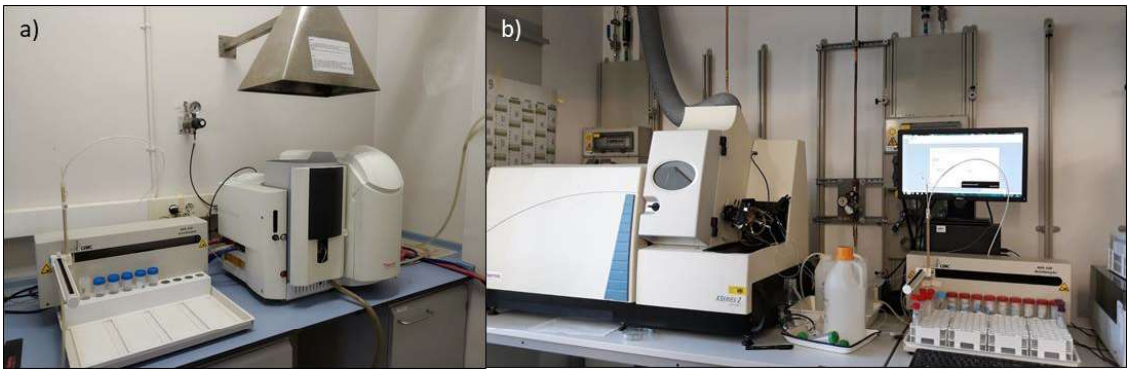

Figure 4.11. (a) Atomic absorption spectroscopy for the analysis of metals in a liquid sample and (b) inductively coupled plasma mass spectrometry for the measurement of low metals concentration.

Figure 4.12. UV-Vis spectrophotometry to analyse iron (II) and iron (III) in liquid samples.

Figure 4.13. Microplate reader for total fluorescence measurements to determine cell number concentration in a biological sample.

4.2.7. DNA extraction

The microorganisms *Acidithiobacillus ferrooxidans* was the inoculum used in almost all the experiments performed in this work. Although they were provided as a pure culture, the purity of the biological sample was tested by the analysis of the DNA present in the sample after 2 months of inoculation. In order to obtain the DNA to analyze, an extraction of them is necessary. There are different methodologies to extract the genetic material. In this case, the soil DNA isolation plus kit from Norgen Biotek (*product # 64000*) was selected. The kit provides the protocol to follow with the chemical reagents needed. The detailed explanation of the protocol could be found in the website of the company but a schematic flow chart of the protocol is shown in Figure 4.14.

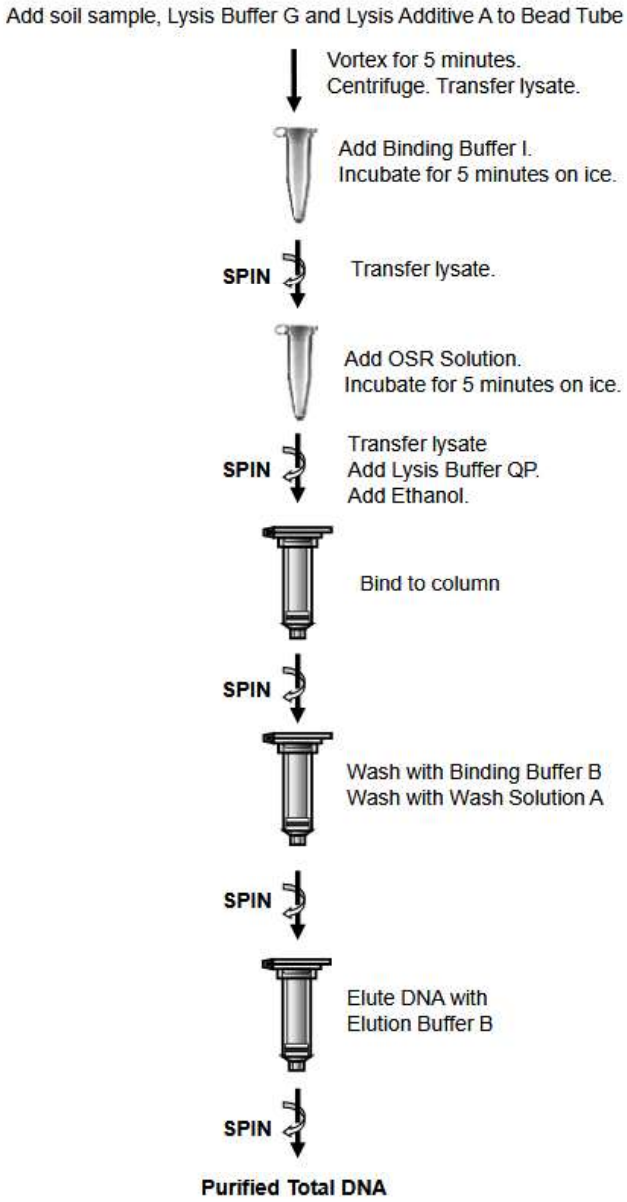

Figure 4.14. Flow chart of the protocol followed to extract the DNA by the soil DNA isolation plus kit (from Norgen Bioteck 2016)

Chapter 5

Development of a batch bioleaching process to recover copper from chalcopyrite using a mixed microbial consortium

The main motivation of this chapter was to gain knowledge about the possibility to use mixed cultures instead of pure cultures in bioleaching processes, initiating the experiments with a well-documented mineral, the chalcopyrite. In this way, the possibility to apply this technique in the electronic waste field will be evaluated. The first approach was the development of the process to recover copper biologically, beginning with the recovery from chalcopyrite to test the technique in the mining field. After testing the bioleaching performance, the knowledge acquired was used in the following chapters in order to develop an alternative methodology to recover metals in a more sustainable way than the traditional, studying what is the best strategy to achieve the highest extraction.

Abstract

In this chapter, bioleaching was applied to recover copper from chalcopyrite. Specifically, the bioleaching was studied for a long-term operation, investigating key parameters in the process, such as the role of the biomass (origin and adaption), the composition of the mineral medium, the buffer capacity and the influence of different ore grades and their potential associated alkalinity. A mixed microbial consortium obtained from a gas-phase biotrickling filter treating high loads of H_2S was used and showed significant copper extraction by biological leaching. Moreover, results revealed that the kinetics and efficiencies of copper extraction were determined by the mineral medium composition, the buffer capacity and the matrix and grade of the mineral used, allowing to set the limits of applicability due to the enhancement of ion precipitation at specific conditions. In addition, the influence of the buffer capacity of mineral medium on the global performance was underlined as critical as well as the chemical composition of the ore matrix where the mineral is contained. The study of this chapter constitutes an initial step in the bioleaching research, providing the methodologies, which can be used to extract metals from various metal containing materials by biotechnological process.

A modified version of this chapter has been submitted for publication as:

Benzal, E., Solé, M., Lao, C., Gamisans, X., Dorado, A.D., 2020. Influence of ore grade and mineral medium on chalcopyrite bioleaching with mixed microbial consortia. Environmental Progress & Sustainable Energy.

5.1. Introduction

Chalcopyrite ($CuFeS_2$) is the most abundant ore of copper. It is estimated that 80% of the copper reserves worldwide are formed by low-grade chalcopyrite deposits (Morin 2008). However, these ores are especially recalcitrant so that copper extraction by hydrometallurgical processes is complex and expensive, especially from low-grade ores (Tanne and Schippers 2019). For this reason, most of the studies focused on metals extraction by bioleaching consider low-grade ores in their experiments (Saitoh et al. 2017; Wang et al. 2014; Yaghobi Moghaddam et al. 2012; Yin et al. 2008).

Microorganisms involved in bioleaching have an important role in the process. In this sense, it is well known that the use of pure cultures of *Acidithiobacillus ferrooxidans* or *Acidithiobacillus thiooxidans* in bioleaching results in high extraction yields of copper from minerals such as chalcopyrite (Wang et al. 2014). However, mixed consortiums, containing these types of microorganisms can also be used for this purpose (Dorado et al. 2012; Qin et al. 2013; Sajjad et al. 2018; Zhang et al. 2008).

Bioleaching has been principally employed to extract metals such as copper, nickel, cobalt and zinc, among others (Martinez et al. 2015). Usually efforts have been placed on low-grade ores because biological leaching is more profitable than chemical leaching with these type of ores (Saitoh et al. 2017; Song et al. 2011). Nevertheless, some studies focused on biological leaching did not perform abiotic control experiments to consider the chemical processes that can also take place in the biological leaching (Dong et al. 2013b; Liang et al. 2013; Zhou et al. 2009). These chemical factors can supply information on what other factors are involved in metals extraction.

The most commonly used mineral medium for microbial growth in bioleaching, named 9K, was described for the first time by Silverman and Lundgren (1959). Afterwards, some authors slightly modified its composition. In particular, Fu et al. (2008) and Zhang et al. (2008), added K_2HPO_4 instead of KH_2PO_4 as well as $(NH_4)_2SO_4$ and $Ca(NO_3)_2$. Some authors modified the 9K composition by adding $(NH_4)_2SO_4$ and K_2HPO_4 instead of KH_2PO_4. Other authors modified the amount of iron, this is the case of Córdoba et al. (2008) or Fu et al. (2013) who added 22 g/L of ferrous sulphate instead of the 44,2 g/L in the 9K. These changes in the mineral medium composition affected copper recovery, despite there are no specific studies focused on this bioleaching aspect.

pH also plays an important role in bioleaching processes. Rohwerder et al. (2003) concluded that the bioleaching process only could take place at pH around 2 in order to avoid a significant abiotic oxidation of ferrous iron. However, Bosecker (1997) affirmed

that below pH 2, a considerable inhibition of the microorganisms occurs. For this reason, many authors agree that a maintained value of pH 2 along the whole bioleaching process leads to better metal recoveries (Dong et al. 2013b; Yin et al. 2008; Zhou et al. 2009). In order to keep acidic conditions, many authors add sulfuric acid to the medium along experiment (Khoshkhoo et al. 2014; Qin et al. 2013; Yaghobi Moghaddam et al. 2012; Zhang et al. 2008). Another way to maintain a constant pH value could be the use of appropriate buffer solutions in order to avoid the continuous acid addition.

The aim of the present chapter is to evaluate the effectiveness of copper bioleaching from chalcopyrite under different conditions using a mixed microbial consortium obtained from a gas-phase biotrickling filter operated at neutral pH and treating high loads of H_2S. Both biotic and abiotic experiments were carried out in parallel under the same conditions in order to distinguish between chemical and biological processes. Besides, the influence of two different mineral media on the process was also tested. Additionally, the influence of the buffer capacity of the mineral medium in contact with the mineral ore and the effect of the purity grade of the ore were analysed in terms of metal recovery. Finally, a pure culture of *Acidithiobacillus ferrooxidans* was used to bioleach the mineral at the best conditions observed, comparing the results to those obtained with the mixed microbial consortia.

5.2. Materials and methods

5.2.1. Mineral samples

The mineral used in this work was a chalcopyrite ore from La Negra's mine (*Querétaro, Mexico*). Hereinafter, it will be called high-grade chalcopyrite. It was analysed by atomic absorption spectrometry (AAS) after acid digestion. For this purpose, 0.15 g of ore (particle size below 63 µm) was digested with 10 mL of HNO_3:HCl (3:1) at 150 ºC for 15 minutes in a microwave apparatus (*Microwave System, Millestone, Italy*). The digestate was analysed with an atomic absorption spectrometer (*Solar S2, Thermo Scientific, United States*). The whole procedure was repeated 5 times to assess its repeatability. A second ore from the Misky deposit (*Arequipa, Perú*) was used to investigate the effect of the mineral range on the bioleaching process. Hereinafter, it will be called low-grade chalcopyrite. Moreover, X-ray diffraction analysis was performed with a Panalytical X'Pert PRO MPD X-ray diffractometer to determine a semi-quantitative mineral phase analysis of the samples by the Rietveld method (Rietveld 1988). The particle size used in the bioleaching experiments was between 2 and 3 mm, according

to the recommendations of Dorado et al. (2012). To obtain this size, the mineral was grinded with a hammer mill and sieved to the desired diameter range.

5.2.2. Microorganisms

A mixed microbial consortium obtained from a lab-scale gas-phase biotrickling filter operated at neutral pH and treating high loads of H_2S was used in this study. In particular, three pieces of packing material with biofilm attached were collected ans washed in 500 mL of mineral medium. Afterwards, the mineral medium with the suspended biomass was used to inoculate a sterilized reactor (2.8 L), which was operated as a continuous stirrer-tank reactor. This biomass was then culture in an Erlenmeyer flask at 30 ºC and 200 rpm before being used as an inoculum in bioleaching experiments. This was previously characterized by Maestre et al. (2010) by cloning and sequencing 16S rRNA fragments, identifying *Thiothrix spp*, *Sulfurimonas denitrificans*, *Halothiobacillus neapolitanus*, *Thiobacillus denitrificans* and *Thiomonas intermedia* as the most abundant species. In this work, the consortium was used with and without previous adaptation. To adapt the culture, an initial sample of the mixed microbial consortium was inoculated in a 500 mL Erlenmeyer flask which contained 100 mL of mineral medium and 10 g of chalcopyrite powder within 2-3 mm particle diameter. This flask was kept at 30 ºC and 130 rpm using orbital shaking for 25 days.

Additionally, a pure culture of *Acidithiobacillus ferroxidans* (ATCC 23270) was used to compare the process with two different cultures. It was kindly provided by the Department of Chemical Engineering from the University of País Vasco (Spain).

5.2.3. Mineral media

Two different mineral media were tested in this study. Medium 1 is the one used in the lab-scale gas-phase biotrickling filter from which the consortium was obtained. Its composition was: 1.000 g/L NH_4Cl, 0.200 g/L $MgSO_4 \cdot 7H_2O$, 0.152 g/L KH_2PO_4, 0.032 g/L $CaCl_2 \cdot 2H_2O$ and 10 mL of trace solution (11.27 mL/L HCl 37.5%, 2.500 g/L $FeCl_2 \cdot 4H_2O$, 0.100 g/L H_3BO_3, 0.170 g/L $MnCl_2 \cdot 4\ H_2O$ and 0.200 g/L $CaCl_2 \cdot 6H_2O$). The non-adjusted pH of these medium was 7. Medium 2 was a modified 9K medium, which is the most widely employed in literature-based copper bioleaching studies. The composition was: 3.000 g/L $(NH_4)_2SO_4$, 0.500 g/L KH_2PO_4, 0.500 g/L $MgSO_4 \cdot 7\ H_2O$, 0.100 g/L KCl and 0.014 g/L $Ca(NO_3)_2 \cdot 4\ H_2O$. The pH was adjusted with 3 N H_2SO_4 to pH 2. Regarding to the buffering agent influence study, buffer solutions HCl/KCl (0.10 M/0.09 M) or Na_2HPO_4/KH_2PO_4 (0.065 M/0.025 M) were prepared before the salts of mineral medium were added. Finally, the pH was adjusted with either 37% HCl or 85% H_3PO_4 to pH 2.

5.2.4. Bioleaching experiments

Bioleaching experiments were performed in 500 mL Erlenmeyer flasks containing 100 mL of mineral medium, 10 g of chalcopyrite ore (sieved between 2 and 3 mm diameter) and 100 mL of inoculum (500 mg/L bacteria density). Abiotic experiments were carried out at the same conditions without inoculum. The flasks were kept at 30 ºC and shaken at 120 rpm, and samples were taken for pH measuring and to analyze copper concentration along time.

5.3. Results and discussion

5.3.1. Ores composition

Semi-quantitative mineral phase analysis of the high-grade sample performed with the X-ray diffractometer showed a relation of chalcopyrite 68.0%, calcite 12%, sphalerite ferrous 5.0%, pyrite 5.0%, troilite 4.0%, pyrrhotite 3.0% and quartz 3.0%. The mineral composition of the low-grade chalcopyrite was quartz 98.0%, chalcopyrite 1.2% and malachite 0.5%, with minor content of other sulphite minerals. Hence, in terms of copper concentration, this was 26.4% for the high-grade chalcopyrite whereas it was 0.62% for the low-grade one.

5.3.2. Influence of the mineral medium on copper recovery

The bioleaching process was studied using two different mineral media (medium 1 and medium 2) with the high-grade chalcopyrite sample. Values of copper recovery and pH along time are plotted in Fig. 5.1. Results revealed that copper release was detected after 30 days of experimentation in the biotic sample with medium 2, being negligible in the rest of samples. This means that despite medium 1 was ideal for the growth of the consortium in the biotrickling filter (from where it was obtained) when it comes to bioleaching, medium 2 was more appropriate. In particular, the main difference between the composition of medium 1 and medium 2 is the amount of sulphate ions (0.078 g/L of SO_4^{2-} and 6.055 g/L, respectively).

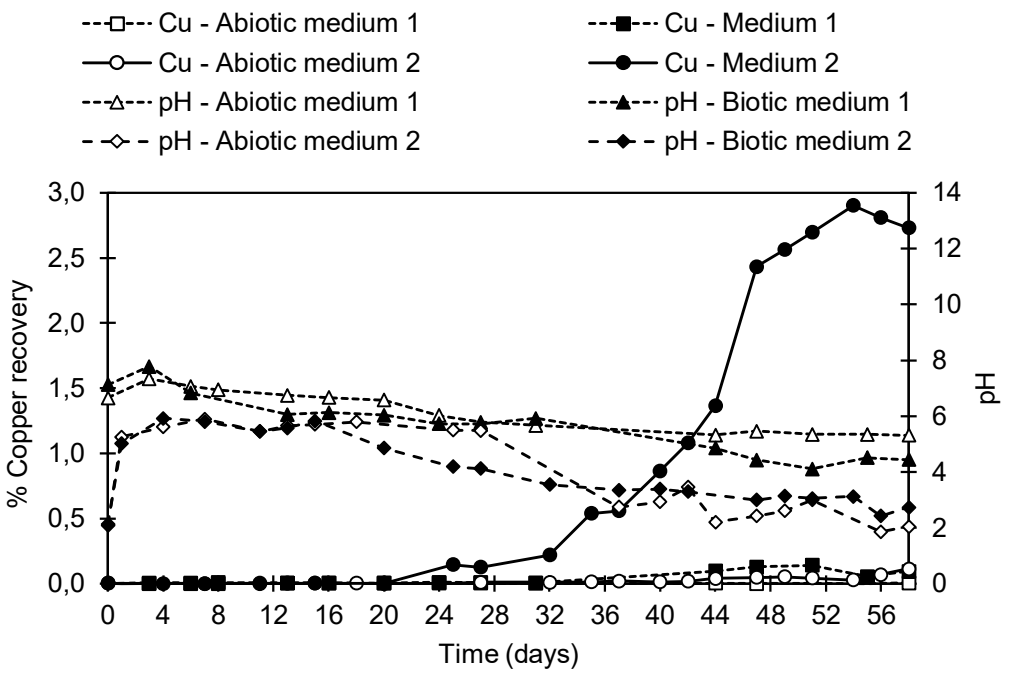

Figure 5.1. Copper recovery and pH evolution along operating time in the study of the mineral medium influence.

It is also worth noticing that, although medium 2 was adjusted to pH 2 at the start-up of the process, the pH increases up to 5 after 24 hours of contact with the ore in all biotic and abiotic experiments. This means that those differences observed in copper extraction between medium 1 and 2, cannot be attributed to the initial pH of mineral media, but to the medium composition itself. This alkalisation was also described by Rodríguez et al. (2003), who attributed it to the protonic attack onto chalcopyrite. However, this is not probably the cause of basification since it takes place very quickly during the first five days, long before the copper release began, in both, biotic and abiotic samples. In this work, it is assumed that the pH increase is likely due to the solubilisation of some components originally contained in the mineral matrix such as calcite. According to McGeouch et al. (2012), calcite can be dissolved by the action of protons, resulting in alkalisation of the mineral medium (Eqs. 5.1 and 5.2):

$$CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \quad (5.1)$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \rightarrow CO_2 + H_2O \quad (5.2)$$

Conversely, it can be also observed that protons concentration increases after 30 days in biotic samples. This acidification could be associated to the oxidation of sulphide by the sulphur-oxidizing microorganisms in mixed microbial consortium (Sand et al. 2001). Regarding abiotic samples, pH values keep quite constant corroborating that

acidification observed in biotic samples is due to microorganisms' activity. Although pH values decreased in both biotic samples, copper bioleaching is far higher in medium 2 than in medium 1, which confirms that this behaviour is related to the composition of the medium. As commented above, medium 2 contains 75 times more sulphate ion concentration than medium 1. According to Tuovinen and Kelly (1973), sulphate is required by some microorganisms as a sulphur source for biosynthesis, but also for several other enzymatic functions. Thus, it seems that sulphate is a key parameter in the microorganisms' development and probably is the main reason for the differences in the recoveries of copper obtained from the different media.

To ensure a proper operation over the entire bioleaching process, suspended biomass and acidic conditions have to be maintained. However, originally, in the biotrickling filter from which the biomass was obtained, the biomass was attached on a packed support and under neutral pH. These differences between biotrickling and bioleaching conditions could result in a poor efficiency of bioleaching process. Therefore, a study with a previous adapted biomass used was carried out (section 5.2.2). The evolution of pH and copper concentration in the bioleaching media from the adapted and the non-adapted cultures are shown in Figure 5.2.

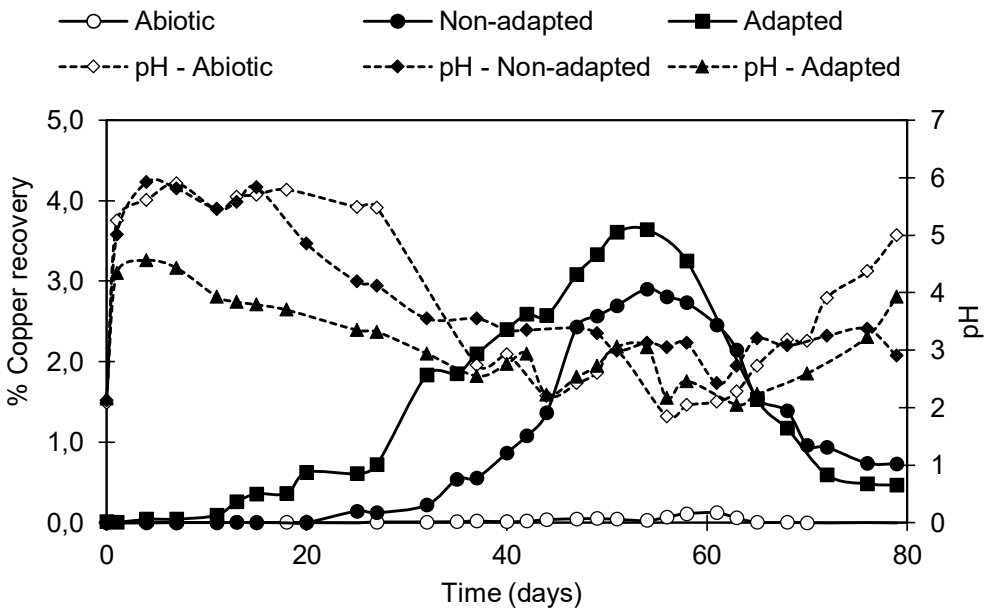

Figure 5.2. Copper recovery and pH evolution along operating time in the study of the effect of adapted and non-adapted microorganisms during chalcopyrite bioleaching.

As can be observed, the adapted culture improves both kinetics and efficiency of copper extraction respect to the non-adapted one. Regarding kinetics, with the non-

adapted biomass, copper bioleaching was detected after 25 days, whereas with the adapted biomass it began in 10 days (halving the start-up). On the other hand, when the adapted culture was used, the bioleaching effectiveness increased achieving a copper recovery of 3.64% in front of the 2.9% obtained with the non-adapted one. Nevertheless, it is noteworthy that the kinetic of copper recovery was different. The non-adapted culture began to recover copper after 30 days with a leaching rate of 11.66 mg/(L·day) whereas the adapted culture began before, but in this case, two velocities were observed. During the first 30 days, the copper leaching rate was 3.63 mg/(L·day), whereas from this time until 55 days the leaching rate was three times higher, achieving a rate of 12.13 mg/(L·day). Regarding pH, this parameter increased at the beginning of the experiment in both, adapted and non-adapted microorganisms. However, during the first 30 days, there is a greater pH decline in the case of the adapted microorganisms, which indicates higher activity of the sulphur-oxidizing microorganism since the protons concentration in the medium increases by the sulphur oxidation (Eq. 5.3) (Pathak et al. 2019).

$$S + H_2O + 1.5\,O_2 \leftrightarrow H_2SO_4 \qquad (5.3)$$

Moreover, as can be seen in Figure 5.2, after 60 days of experimentation, depletion on copper concentration occurred in all biotic samples. It is not usual to found articles that perform bioleaching experiments for such long time to observe this behaviour, or in case to carried out for long periods of time did not remark this observation (Cancho et al. 2007; Thurston, Mandernack, and Shanks 2010; Wang et al. 2014; Zhang et al. 2008; Zhao et al. 2013). The decrease of copper concentration coincided with an increase in pH values, which in turn can be related to a decrease on biological activity since biological activity produces protons to the medium.

This suggest that reduction of copper concentration, at this time, could be likely due to copper ions precipitation through the formation of poor soluble species, given place to copper (II) hydroxide. Nevertheless, at the pH achieved at this time almost all the copper is found in its soluble form according to the copper speciation diagram (Figure 5.3). Hence, the copper depletion could not be associated to its precipitation. It is noticed that when the decrease of copper concentration was detected, formation of an orange-brown precipitate was observed. It is though that the precipitate could be jarosite ($KFe_3^{+3}(OH)_6(SO_4)_2$), which formation is favoured by the presence of potassium and ammonium cations brought by the mineral medium (Guezennec et al. 2015). Nevertheless, schwertmannite ($Fe_{16}^{3+}O_{16}(OH)_{12}(SO_4)_2$) can be also formed in bioleaching environments (Liao et al. 2009). According to these authors, extremely high SO_4^{2-} content (>8000 mg/L) and low pH in the system favored the existence of

schwertmannite, that are the conditions of the present experiment. It is well known that in chalcopyrite bioleaching process, the formation of iron precipitates is pH dependent (Daoud and Karamanev 2006). According to these authors, the most jarosite precipitation was observed after 46 hours at pH 2.96, which is the pH measured when the copper decreasing was observed (Figure 5.2). Accordingly, it was though that the reason why copper concentration decreased was related to the formation of jarosite or/and schwertmannite in the experiment since it has been reported that the formation of these minerals may lock some of the extracted copper, producing a decrease in copper concentration (Zhou et al. 2009).

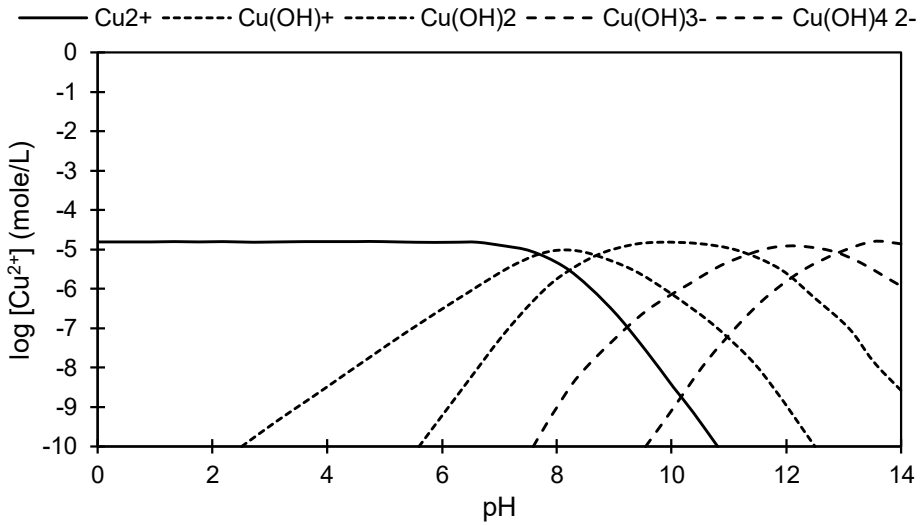

Figure 5.3. Theoretical copper speciation for hydroxo complexes. Adapted from Cuppett, Duncan, and Dietrich (2006).

Copper precipitation has not been described before in chalcopyrite bioleaching studies found in the literature. Many bioleaching studies in batch conditions were performed during less than 60 days (Klink et al. 2016; Peng et al. 2016; Shabani et al. 2019; Vilcáez, Yamada, and Inoue 2009) and the number of studies that experimented more days are scarce in the literature. The main interest of long-term experiments is to observe the behaviour of the system when not all copper has been extracted in a short time period. In this sense, few long-term chalcopyrite bioleaching studies have been performed (Cancho et al. 2007; Thurston et al. 2010; Wang et al. 2014; Zhang et al. 2008; Zhao et al. 2013) but they did not report a reduction in copper concentration. However, the conditions under which the experiments were performed in these studies differ from those of the present study (e.g. the pH maintained at very acidic values along all the duration of the experiments which reduces the formation of jarosite). Hence, based

on the results obtained it is noticed how important is to avoid the conditions that favours mineral precipitation as jarosite or schwertmannite in order to maintain the copper in solution. One of the main ways to reduce the formation of jarosite is to keep acid pH in the leaching solution either by the constant acid addition or by buffering the media used in the leaching experiment to avoid pH changes.

5.3.3. Effect of buffering the bioleaching media

Most of microorganisms used in metals recovery by bioleaching grow at very acidic pH (below 2.5) (Rohwerder et al. 2003). However, the optimum pH value for bioleaching processes depends on the type of microorganisms (Plumb, Muddle, and Franzmann 2008). On the other hand, the release of gangue components from the ore can also affect the pH of the medium and, thus, have some influence on the efficiency in the bioleaching processes as seen in Figure 5.2. Despite the initial pH of the media was adjusted to pH 2, this value increased quickly during the first stage of the bioleaching experiments (Figure 5.2). This is associated to the protons consumption by the ore components solubilisation. This alkalisation has been observed in numerous studies and, generally, the maintenance of pH along the bioleaching process is accomplished by periodic addition of sulphuric acid (Akcil, Ciftci, and Deveci 2007; Cancho et al. 2007; Zhang et al. 2008; Zhao et al. 2015).

In order to avoid the constant acid addition, which raises the cost and makes the recovery process less sustainable, two different buffer solutions HCl/KCl (chloride buffer) and Na_2HPO_4/H_3PO_4 (phosphate buffer) were tested during chemical leaching of low-grade chalcopyrite. Both buffer solutions are usually used in biological and chemical processes to keep pH at values of 2 (Ashour, Chehna, and Bayram 2006; Jiao et al. 2008; Léonil and Mollé 1991; Sarafra-Yazdi and Es'haghi 2006). However, in case that buffer solutions allow maintaining pH at constant acid value during the leaching experiment, it should be study the effect of Cl^- and PO_3^{4-} to the biological activity of the strain used in biological tests since these salts have been reported to be toxic depending on the concentration and the strain used (Huynh et al. 2019). Evolution of pH over time in buffered media is showed in Figure 5.4.

Results revealed that while the phosphate buffer kept well the pH around 2, chloride buffer was not able to maintain the pH of the media at pH 2 when the ore was present in the bioleaching media. Nevertheless, although the phosphate buffer maintained the pH, the formation of a precipitate was observed which could be due to the reaction between phosphate anions and metallic cations present in the mineral medium, such as iron and calcium, which produces iron (III) phosphate (Kps = $9.9 \cdot 10^{-16}$)

and calcium phosphate (Kps = $2.0 \cdot 10^{-29}$). This can seriously affect the bioleaching process because of the decrease in concentration of essentials ions which are necessary for the biological process. Hence, none of the two buffers tested resulted totally effective to be applied during the bioleaching process without producing negative effects. Thus, the use of concentrated reagents can be one of the most suitable alternatives in order to maintain acid pH, at the same time that the consume of acid can be reduced in the process.

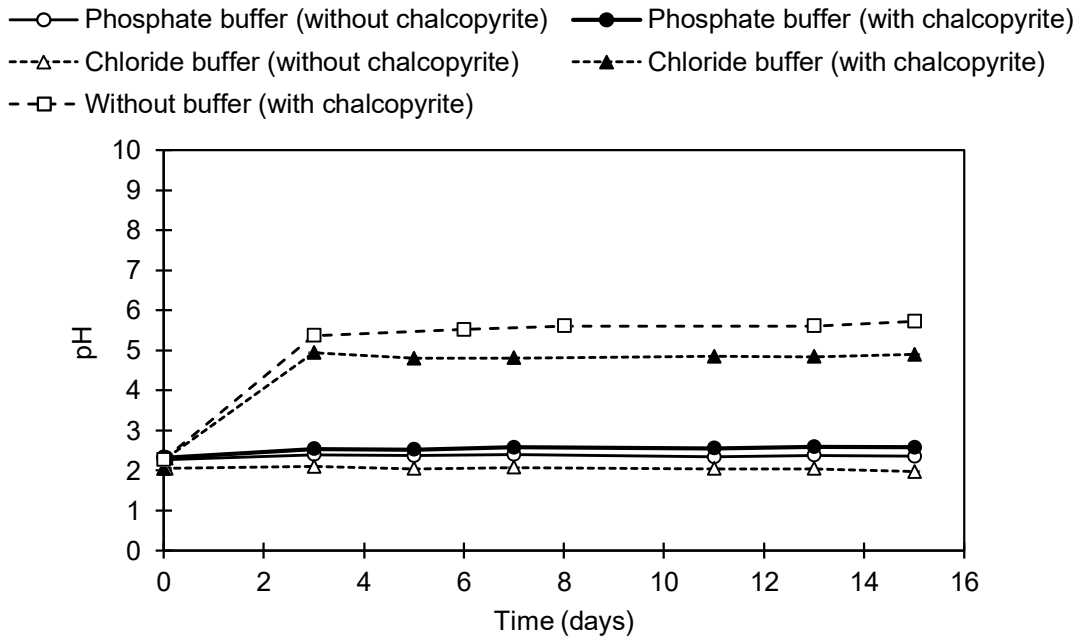

Figure 5.4. pH evolution along time during the study of mineral's medium buffer capacity. Non-filled symbols represent samples without mineral and filled symbols represent samples with mineral.

5.3.4. Effect of the ore grade on the bioleaching process

Bioleaching process can be applied to copper ores of different grades and compositions. This fact might influence the process because some components contained in the ore would be able to react with the mineral medium. For this reason, two different copper ores, which have different percentage of copper and mineral matrix, were investigated. Experiments were performed with the medium which presented higher recovery (medium 2), which composition is described in section 5.2.3.

Copper concentration and pH evolution along time are shown in Figure 5.5. Results revealed that copper extraction occurred from both minerals. As can be seen, after 18 days, the amount of copper extracted was higher from high-grade chalcopyrite (47 mg/L) than from low-grade chalcopyrite (12 mg/L). It is noteworthy that the amount

of copper extracted from low-grade chalcopyrite was the same in biotic and abiotic samples. This suggest that copper obtained from the low-grade ore was leached chemically without the intervention of microorganisms. The inactivity of the biomass in this case could be caused by some components present in the mineral matrix capable of inhibit the metabolism of the microorganisms. Therefore, it confirms that the mineral composition is important and not all copper ores are suitable to be bioleached. On the contrary, there was a noticeable difference between biotic and abiotic samples from the high-grade chalcopyrite. Copper obtained from biotic sample was over 50 times greater than the copper obtained from the abiotic experiment.

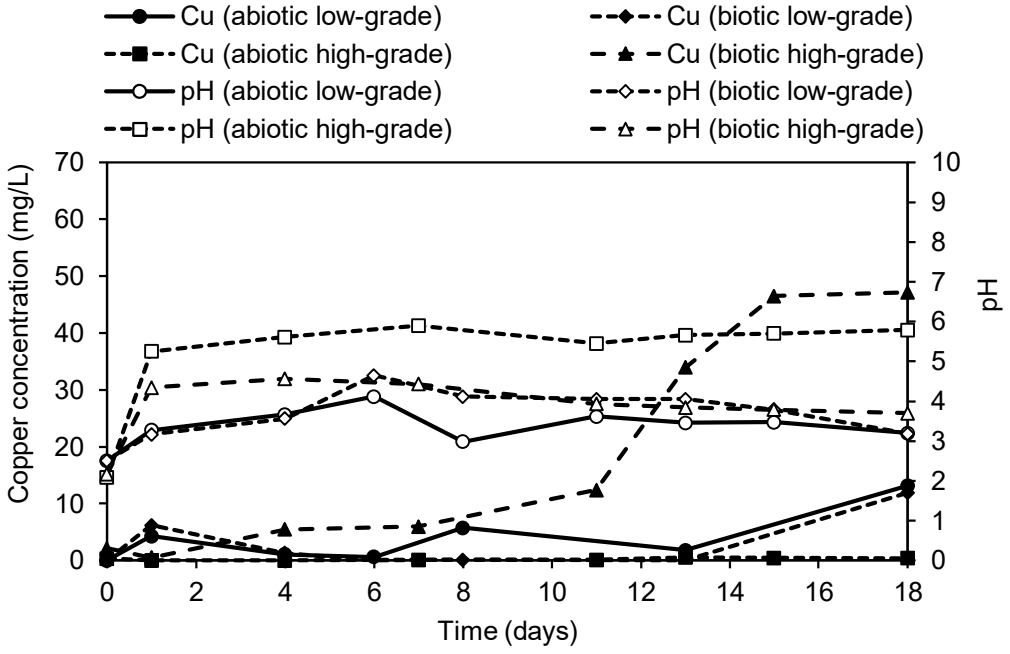

Figure 5.5. Copper concentration (filled symbols) and pH evolution (non-filled symbols) along time on bioleaching of two different copper ores.

In terms of copper extraction, the amount of copper obtained with the high-grade chalcopyrite represents an extraction of 0.4% in weight. These results are in agreement with those obtained by Dong et al. (2013b), who also obtained less than 10% of copper from two samples of chalcopyrite with similar composition of the mineral used in the present work (24% Cu, 27% Fe and 30% S) during the same experimental time. The low copper recoveries achieved are related to the pH of the mineral medium, since pH over 2 do not favour the process, despite this pH value is used in many of bioleaching studies (Dong et al. 2013b; Fu et al. 2008, 2013; Saitoh et al. 2017).

Copper concentration obtained with the high-grade chalcopyrite is 5 times greater than that obtained with the low-grade chalcopyrite. However, in terms of copper recovery,

1.9% of copper from the low-grade ore was obtained whereas only 0.4% was obtained from the high-grade sample. These results might be related to the high quartz content in the low-grade chalcopyrite (98%). Dong et al. (2013c) demonstrated that copper bioleaching from chalcopyrite was improved by the addition of quartz in the bioleaching medium. According to these authors, the presence of fine particles of quartz could reduce the formation of a passivating jarosite layer on the mineral that negatively affects copper extraction.

It is noteworthy that pH values have a similar behaviour in all cases. After the first 24 hours, the pH increases substantially and, then, the values were almost constant until the end of the experiment. The initial alkalisation was more pronounced in the high-grade chalcopyrite sample probably due to the matrix composition of the mineral, since one matrix component is calcite, which alkalinizes the medium (see Eq. 5.1 and 5.2). Despite the higher pH increasing, the amount of copper obtained was also higher in the high-grade chalcopyrite. This means that the efficiency of copper extraction can be increased when a pH control is performed, even if the pH used is not low.

5.3.5. Effect of the inoculum characteristics during bioleaching

Although some copper was recovered by bioleaching in the previous experiments, the percentage of copper recovered was quite low. Since the mixed culture used did not contained, one of the microorganism most used in bioleaching processes (Rawlings 2002), the next step was to consider the use of a pure culture. For this reason, the bioleaching of chalcopyrite with a pure culture of *Acidithiobacillus ferrooxidans* was compared to the bioleaching process with the microbial consortium of this work. As it is shown in Figure 5.6, the use of a pure culture of *Acidithiobacillus ferrooxidans* increased the efficiency of copper recovery since it recovered 30% of copper in 13 days whereas the mixed microbial consortium spent more time. For the latter, only 3% recovery was achieved after 50 days approximately (see Figure 5.2). Therefore, there was an important improvement when the specific strain was used.

Some authors affirmed that mixed cultures obtained better recoveries in copper bioleaching, however, they defined mixed culture for cultures composed by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, basically (Fu et al. 2008; Qiu et al. 2005). In this work, the mixed culture was originally composed by *Thiothrix spp*, *Sulfurimonas denitrificans*, *Halothiobacillus neapolitanus*, *Thiobacillus denitrificans* and *Thiomonas intermedia* as the most abundant species, as stated previously. These microorganisms allowed to bioleach copper, especially when they have been previously

adapted. Nevertheless, the results were not as good as it was expected due to the pure culture allowed higher copper recoveries in less time than the mixed culture.

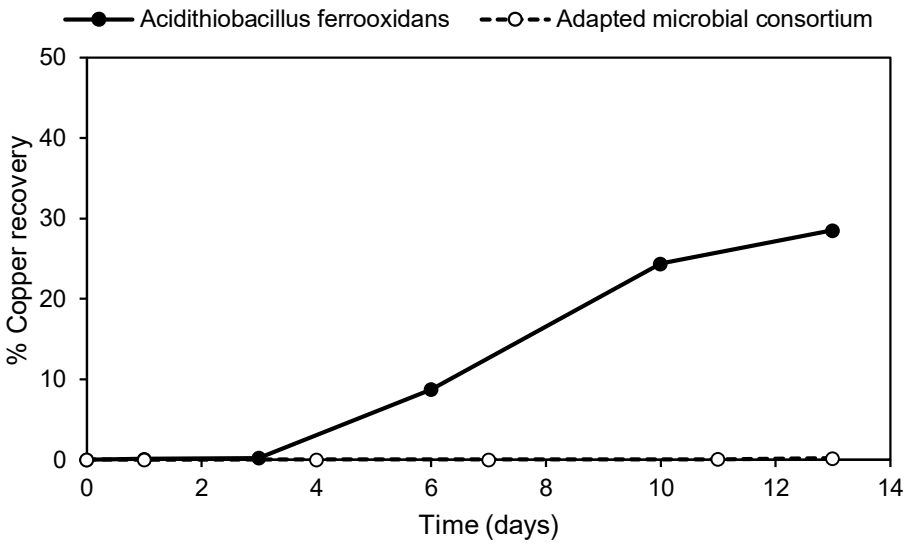

Figure 5.6. Copper evolution along time when pure and mixed culture were used during the bioleaching process.

5.4. Conclusions

Bioleaching experiments performed with a mixed microbial consortium showed that it was possible to recover copper from chalcopyrite ore, but the rate achieved was quite low. Nevertheless, acclimation of the mixed culture to bioleaching conditions resulted in a copper recovery rate 40% higher than that of non-acclimated biomass. After 60 days of experimentation, depletion of copper concentration occurred in all biotic samples coinciding with increase in pH values. This reduction of copper concentration is likely related to the formation of jarosite or schwertmannite that could lock some of the extracted copper. Moreover, the use of two different buffer solutions to maintain the pH at low values showed that both were unsuitable, since HCl/KCl buffer did not keep pH = 2, whereas the Na_2HPO_4/H_3PO_4 buffer maintained pH around 2, but caused the precipitation of some species needed for the bioleaching process such as iron or calcium. The type of ore where the metal is contained also affected the efficiency of retrieved copper by bioleaching. The amount of copper extracted from the high-grade chalcopyrite in biotic tests was nearly 50 times greater than under abiotic conditions. In contrast, the amount of copper extracted from the low-grade chalcopyrite was the same in both, biotic and abiotic tests. This was likely caused by the composition of the mineral matrix. In this

sense, this study confirmed that the presence of quartz in the mineral ore is beneficial for the copper extraction process by avoiding possible surface passivation. Regarding the culture used, notably better recovery of copper was achieved when a pure culture of *Acidithiobacillus ferrooxidans* was used. In particular, nearly 8 times the copper was recovered with the pure culture as compared to using the mixed microbial consortium obtained from a desulfurization biotrickling filter. Besides, the pure culture reached the maximum recovery in 13 days, whereas the mixed culture needed 54 days.

Finally, the procedure developed in this chapter to bioleach metals was successfully applied, which established the foundations for the recovery of copper from metal containing material. Clearly, a pure culture of *Acidithiobacillus ferrooxidans* was necessary to improve the efficiency of the process. For this reason, all of the experiments of the following chapters were performed with pure cultures of *Acidithiobacillus ferrooxidans*.

Chapter 6

Adaptation of the mineral bioleaching process for metal recovery from e-waste

The motivation of this chapter was to gain knowledge about the mechanisms of the bioleaching process using the procedure developed in the previous chapter and to apply it in the field of the electronic waste. The first approach was to evaluate the differences betweenf copper bioleaching from chalcopyrite and from electronic waste. Then, the bioleaching from e-waste was optimized by studying different parameters such as pH control and PCB dosage that could affect the efficiency of the process. In addition, the bioleaching process was also performed in two steps, in order to evaluate the benefit of separating the two main processes that take place in bioleaching to avoid possible toxic effects: the bio-oxidation and the leaching itself.

Abstract

In this chapter, bioleaching was applied to recover copper from electronic waste. In particular, the e-waste used came from PCBs of mobile phones. The process developed in the previous chapter with ores was applied to electronic scrap, analysing the differences between both cases. This chapter was focused on the suitability of applying the technology to the metal recovery from e-waste. In particular, the waste dosage and the pH control during the process were studied to understand some of the key parameters that could affect the efficiency of the process. New methodology based on the separation of the main processes that take place during the bioleaching was applied in order to increase the amount of copper recovered and to minimize the possible toxic effects of the bioleached metals. Thus, the bioleaching process was performed in two different steps, one for the biological oxidation of iron and the second for the leaching reaction to obtain copper from the scrap. Results demonstrated that more copper was bioleached within 13 days when e-waste (48.3%) was used instead of mineral ores (28.5%). This implied that it is more interesting to put efforts into the use of e-waste due to the huge amount and availability of raw materials. Moreover, when the process took place in two-step the recovery rate increased, achieving 90% of copper recovery in just 24 hours at the best conditions found, whereas it took more than 20 days to obtain similar results in only one step.

A modified version of part of this chapter has been published as:

Benzal, E., Solé, M., Lao, C., Gamisans, X., Dorado, A.D., 2020. Elemental copper recovery from e-wastes mediated with a two-step bioleaching process. Waste and Biomass Valorization, 11, 5457-5465.

6.1. Introduction

Bioleaching process has been studied for many years in the mining field (Dong et al. 2013a; Olson, Brierley, and Brierley 2003; Rohwerder et al. 2003), especially when low-grade ores have to be treated due to the low cost of bioprocesses (Dorado et al. 2012). The technique has proven to be effective in this field, so its use has been extended for the treatment of other materials (Klink et al. 2016; Qu and Lian 2013). The main trend is the application of bioleaching in the field of the electronic wastes because of the increasing generation of these products as in Chapter 2 was stated. Nevertheless, the structures and the compositions of the ores and e-waste are very different. On the one hand, a typical composition of PCB is 40% of metals that could include more than 10 different metals, 30% of ceramics (SiO_2 or Al_2O_3, among others) and 30% of plastics (Khaliq et al. 2014), although the composition varies depending on the age and the type of the discarded item (Chen et al. 2018a; Robinson 2009). On the other hand, ores such as chalcopyrite are mostly composed by $CuFeS_2$, depending on its purity. In the case of low purity ores, normally the matrix is formed by other mineral phases instead of plastics and the amount of metals is very low compared to the amount of metals that can be found in PCBs (Valix 2017). Moreover, in e-waste copper is found as a metallic state in a simpler structure in relation to the structure of the chalcopyrite, in which copper is found inside a complex crystalline structure (Khaliq et al. 2014; Tao and Dongwei 2014).

As a consequence of the complex composition of the e-waste (Baldé et al. 2015; Das and Ting 2017; Fornalczyk et al. 2013; Priya and Hait 2018), the amount of processed material is an important factor to take into account in bioleaching. In the case of e-waste bioleaching in one step, some authors have focused on this parameter (Adhapure et al. 2013; Zhu et al. 2011) as well as the authors who studied the bioleaching in two steps (Yang et al. 2014). All of them concluded that the best results are obtained when the concentration of e-waste treated is lower than 15 g/L. Yang et al. (2014) affirmed that concentrations above 15 g/L of e-waste contain alkaline substances that might lead to inhibitory effects on bacterial growth. In addition, Zhu et al. (2011) also attributed the toxicity of metals ions when powder dosage is beyond the inhibitory limitation of the bacteria in solution. Nevertheless, Brandl, Bosshard, and Wegmann (2001) observed higher recoveries when 5 and 10 g/L of e-waste were treated instead of 50 or 100 g/L. Therefore, clear results have not been already reported regarding the best waste dosage in bioleaching studies. Furthermore, none of these studies have taken into account the possible chemical leaching, because the authors have considered that the whole copper extraction was only produced by the biological activity.

Moreover, another important factor influencing the leaching process is the medium pH as stated previously. The microorganisms involved in bioleaching typically grow in very acidic conditions (pH 1.5 – 3.0), so maintaining acid pH during the entire experimentation period would be important to ensure a proper biological performance. Despite this relevance in e-waste bioleaching, only few authors studied the pH influence on the process by performing experiments at different pH adjustments (Hong and Valix 2014; Shah et al. 2015; Xiang et al. 2010), but they did not compare the process carried out under uncontrolled pH.

So far, two different bioleaching methodologies (one-step and two-step) have been suggested by researchers based on the kind of biomass exposure to the waste (Baniasadi et al. 2019). In the one-step method, the e-waste is added immediately to the culture medium, so bacterial growth takes place in the presence of e-waste. Conversely, in the two-step method the e-waste is added after the microorganism reaches its logarithmic growth phase. To date, most of the bioleaching studies has been focused on the recovery of metals in one step (Bas, Deveci, and Yazici 2013; Ilyas et al. 2007; Liang et al. 2013) but it has been reported that the bacteria could be affected by the toxic compounds which could be released during the e-waste treatment (Isildar et al. 2016; Xia et al. 2017; Zhu et al. 2011). For this reason, Brandl, Bosshard, and Wegmann (2001) suggested to develop the bioleaching process in two steps to avoid the toxicity and to improve the efficiency of the technique. In this regard, these authors grew the microorganisms in the absence of electronic scrap and, then, the scrap was added to the culture to do the leaching process (Shah et al. 2015; Yang et al. 2014). Shah et al. (2014) concluded that biologically obtained iron (III) allows to leach higher concentration of waste by two-step method when a dominant culture of *Leptospirillum ferriphilum* was used in 15 days. In addition, another study concluded that the two steps methodology allows to treat higher amount of e-waste in the process (Shah et al. 2015). However, most of the two steps bioleaching experiments reported in the literature took from 3 to 15 days (Brandl et al. 2001; Shah et al. 2015; Shah et al. 2014; Yang et al. 2014). This means quite long time for an economically viable application, especially to scale-up the technology as an alternative to conventional processes.

The aim of the work presented in this chapter was to evaluate the suitability of applying the bioleaching to extract metals from e-waste using a pure culture of *Acidithiobacillus ferroxidans*. In this regard, the PCB dosage and the pH control in one-step bioleaching have been studied. Moreover, the process in two-step was performed, testing the improvement of using two different techniques to separate the biomass from the bio-oxidation step.

6.2. Materials and methods

6.2.1. Mineral sample

The mineral used in these experiments was a chalcopyrite sample from La Negra's mine (*Querétaro, México*). It is the same sample used in the previous chapter, so the mineral characteristics are explained in section 5.2.1. The particle size used was between 0.2 and 1.0 mm of diameter, which was selected based on comparative purposes with the result of the e-waste pretreatment. To obtain this size, the mineral was grinded with a hammer mill and sieved to the desired diameter range.

6.2.2. Electronic scrap

The PCBs used in this chapter and in the following chapters come from end-of-life mobile phones. The PCB was removed manually from the phone structure and the main electronic components such as resistors, capacitors and chips, among others, were also separated manually. The particle size was reduced firstly with a shears, and then strips were crushed, collecting the particles between 0.2 and 1.0 mm of diameter through a sieve. According to Wang et al. (2009), metals solubilisation increased when decreasing the sieve fraction due to the superficial area increase, demonstrating that particles lower than 1.0 mm obtained higher metals extraction than particles over this size.

6.2.3. Microorganisms and mineral medium

The bacterial strain *Acidithiobacillus ferroxidans* (ATCC 23270) was used. It was kindly provided by the Department of Chemical Engineering from the University of País Vasco (Spain). The mineral medium used in the experiments, named 6K, was prepared as follows: $(NH_4)_2SO_4$ 3.00; K_2HPO_4 0.50; $MgSO_4 \cdot 7\ H_2O$ 0.50; KCl 0.10; $Ca(NO_3)_2 \cdot 4\ H_2O$ 0.014 grams were dissolved in 900 mL of distillate water. The pH was adjusted with 3 N H_2SO_4 to 1.75. Then, 30 grams of $FeSO_4 \cdot 7\ H_2O$ were dissolved in 100 mL of distillate water and the pH was also adjusted with 3 N H_2SO_4 to 1.75. After that, both solutions were mixed and the pH was readjusted again to 1.75 if necessary.

6.2.4. Bioleaching experiments

For the first experiments, the same methodology than the previous chapter was used. Hence, bioleaching experiments were performed in 500 mL baffled Erlenmeyer flasks containing 180 mL of mineral medium, 20 mL of the inoculum and different concentrations of either mineral or PCB (2.5 – 10.0 g/L). For the PCB bioleaching experiments, abiotic assays were carried out at the same conditions without inoculum.

The flasks were kept at 30 ºC and shaken by orbital agitation at 130 rpm in an incubator (*SI500, Stuart, United Kingdom*), measuring the pH and the ORP periodically. For iron and/or copper analysis samples were taken, which were filtered before their analysis.

Moreover, in this chapter the two-step bioleaching methodology was used. The first step consists in the biological oxidation of iron (II), which was carried out in 500 mL baffled Erlenmeyer flasks. It was initially inoculated with 30% of fresh culture using the medium described in the above section, until a total volume of 350 mL. As in the previous methodology, the flask was stirred by orbital agitation at 130 rpm and kept at 30 ºC in an incubator. In addition, the pH and the ORP were measured periodically. When all the iron (II) was oxidized to iron (III) by the microorganisms, it was considered that the first step finished and, then, the next step consisting on putting in contact the solution containing this bio-generated iron (III) with the e-waste was performed. For this purpose, 350 mL of the bio-generated iron solution was transferred to 500 mL baffled Erlenmeyer flasks and the PCB dosage (7.5 or 15 g/L) was added. In this work, when the effect of biomass separation between bio-oxidation and leaching steps was studied, the separation was performed by filtration and sedimentation, so in these cases, only the bio-generated iron (III) without biomass were transferred to the flasks for the leaching step. The leaching flasks were incubated at 30 ºC, stirring them by orbital agitation at 130 rpm. Since the microorganisms were not used during the second step, abiotic experiments were carried out only during the first step. Samples taken every 2-3 hours, whenever possible during the two steps, were filtered before being analyzed to determine the iron and copper concentrations.

6.3. Results and discussion

6.3.1. Chalcopyrite and PCB metal composition

On one hand, the analysis of the chalcopyrite used in this chapter, as the ore used in the previous chapter, reveal that the average content of Cu, Fe, S, Si and O in g/kg was 265, 272, 308, 14 and 73, respectively. On the other hand, the analysis of the PCB used in this chapter showed that the average content of Cu, Ni, Fe, Ag, Au, Al, Pd, In, Sn, Pb, Co and Mn in g/kg was 390.38, 11.51, 1.95, 0.19, 0.80, 1.33, 0.15, 0.12, 28.92, 16.16, 0.14 and 0.58, respectively (Figure 6.1). From the data obtained, Cu was found as the major component, being the total metal content per kilogram in the PCB 452.23 g. This means that PCB from mobile phones could be a good source of metals to take advantage of them in comparison to the metals found in the ore used and

according to some other currently exploited ores (Cancho et al. 2007; Third et al. 2000; Zhao et al. 2013).

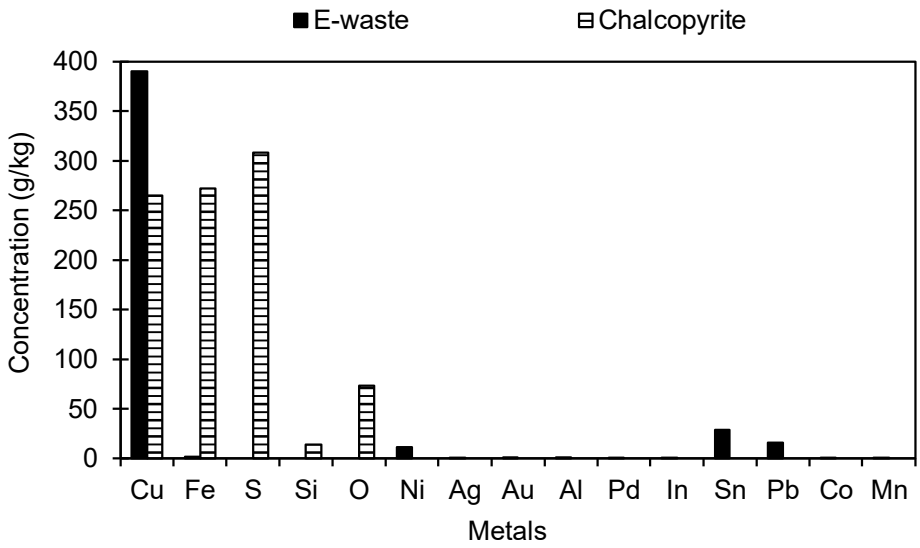

Figure 6.1. Metals composition and oxygen content of chalcopyrite and the e-waste used in the experiments.

According to Hagelüken and Corti (2010), the "mineralogy" of e-waste is much different comparing to of the natural ores from metals refining. This is because the e-waste contains up to 60 different elements that are closely interlinked with complex assemblies and sub-assemblies which physical and chemical properties are also much different. In addition, these authors also affirmed that the metals contained in the e-waste are often crosslinked to organic compounds which could be toxic for the microorganisms, for instance. Therefore, the composition of e-waste results much more complex than the natural mines, which makes recycling metals processes from the electronic scrap more complex. For this reason, e-waste recycling and management is not simple and straightforward (Lu and Xu 2016; Ylä-Mella et al. 2014), so their recycling and management requires special attention. Nevertheless, although the complexity of e-waste may seem a limitation for recycling, biorecovery of metals has been effectively applied in this type of waste (Annamalai and Gurumurthy 2019). Hence, e-waste results a good source of metals for their recovery, since a lot of metals could be found in them.

6.3.2. Comparative study between mineral bioleaching and e-waste bioleaching

Based on previous studies (Bosecker 1997; Dorado et al. 2012), bioleaching of e-waste was investigated at the same operational conditions than chalcopyrite bioleaching. In this case, one-step bioleaching was performed. Thus, a solid-liquid ratio of 10 g/L with a particle size below 1.0 mm of diameter, initial pH=2, 30 ºC of temperature, 120 rpm stirring and the same strain of *Acidithiobacillus ferrooxidans* were selected. Results of both processes are shown in Figure 6.2.

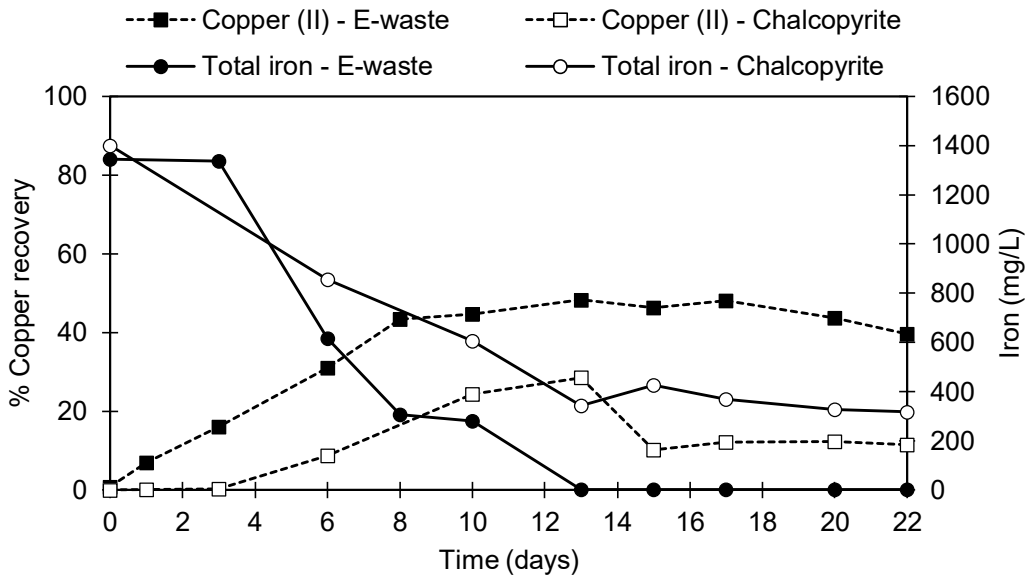

Figure 6.2. Comparison between bioleaching of e-waste and bioleaching of chalcopyrite.

Higher copper recovery from electronic waste than from chalcopyrite was observed. In particular, 48.3% of copper has been recovered from the PCB whereas 28.5% has been recovered from the mineral in 13 days. This difference is mostly associated to the matrix that contains the copper because of the differences on their structures, as mentioned previously. Moreover, Figure 6.2 also shows that the kinetics of copper extraction in e-waste is higher than in the case of the ore, in which the recovering started 3 days later. This behaviour is also related to the structure of the material bioleached and the accessibility of microorganisms to copper. For instance, the machining of the electronic devices varies the copper distribution inside the e-waste, making it more inaccessible in comparison to the natural copper resource. Nevertheless, once the process has started, the trend is the same in both cases during the first 13 days. After this period, a copper depletion occurred when the ore was bioleached. As it was suggested in Chapter 5, reduction of copper could be likely due to the formation of

jarosite or/and schwertmannite, which may locked some of the extracted copper (Zhou et al. 2009).

It is noticeable that iron concentration decreased during the experiment in both cases, although in the case of the e-waste all the iron precipitated in 13 days. This was related to pH changes (Fig. 6.3) since the pH gradually increased, rising pH values over 4.5 after 13 days of experimentation, which produced iron hydroxide precipitation. This alkalisation was caused by the e-waste itself, since it has been reported to be alkaline in nature (Arshadi et al. 2016; Işıldar et al. 2019). In the case of chalcopyrite bioleaching, the pH remained quite constant at pH 2 at the conditions tested and, as a consequence, not all the iron precipitated during the experiment. Although in Chapter 5 the pH increased during chalcopyrite bioleaching, the differences are associated to the changes in working conditions such as the use of a different culture, the particle size used or the amount of iron present in the culture medium.

The iron precipitation observed also explained why no more copper was recovered after 13 days of experimentation in the case of the e-waste, since the leaching reaction was limited by the lacking iron, so no more copper could be extracted at this conditions. Similar limitation was observed in the case of chalcopyrite bioleaching, but taking into account that there was soluble iron in the leaching solution during all the experiment, this could not be the cause. It is suggested that the limitation in this case could be produced by the passivation of the ore, which difficult its dissolution and, thus, its metal recovery (Zhao et al. 2019).

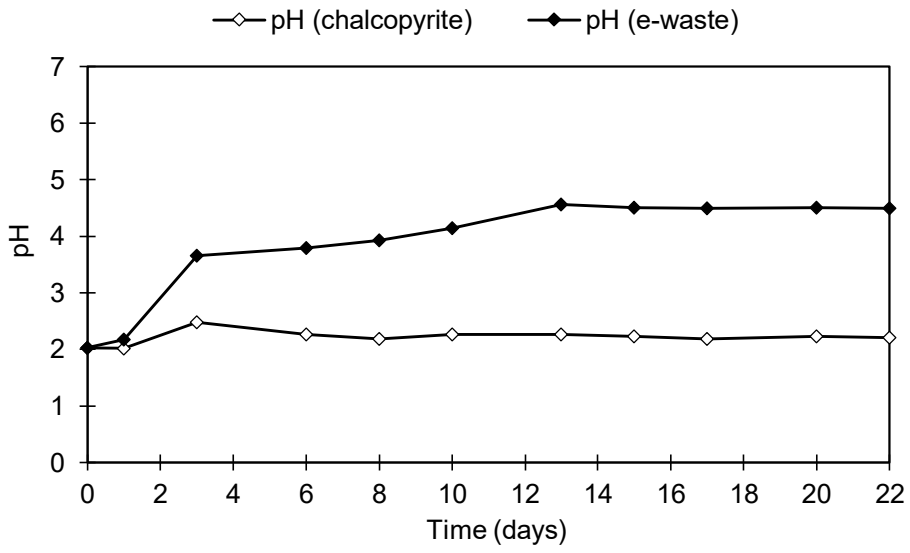

Figure 6.3. Evolution of pH in chalcopyrite and e-waste bioleaching.

6.3.3. Effect of e-waste concentration on bioleaching process

The influence of e-waste dosage in copper bioleaching has been investigated in previous works and diverse results were obtained without a global agreement about the optimal PCB dosage (Adhapure et al. 2013; Xiang et al. 2010; Yang et al. 2014; Zhu et al. 2011). Xiang et al. (2010) and Adhapure et al. (2013) studied the effect of e-waste dosage on PCBs bioleaching by a mixed bacteria consortium. They experimented with electronic waste concentrations from 10 to 50 g/L, both concluding that the maximum leaching for copper was obtained at 10 g/L in one-step bioleaching. They observed that copper extraction decreases with the increase of waste concentration in the range of 10 and 50 g/L of PCB and they attributed this behaviour to the toxicity of the high concentration of copper extracted. Yang et al. (2014) obtained the maximum copper extraction at a waste dosage of 15 g/L, whereas no bioleaching was observed at concentrations of 25 and 35 g/L. According to these authors, metallic or plastic components in waste might lead to an inhibitory effect on biological process.

Taking into account the results observed when 10 g/L of e-waste was used in this work, a set of biotic experiments were carried out at lower waste concentrations (5.0 and 2.5 g/L). Control test without biomass were also tested for all the three dosages. Results of the copper recovery along time are plotted in Figure 6.4.

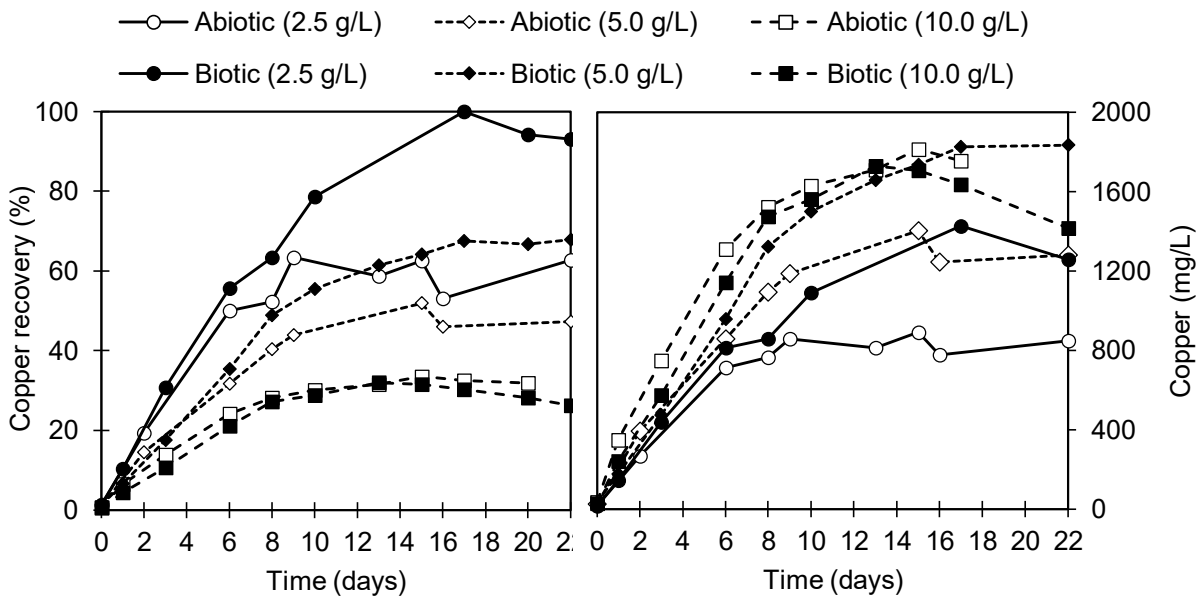

Figure 6.4. Copper recovery along time at different electronic waste concentrations performed in one step bioleaching process with biomass (black dots) and without it (white dots).

As can be seen, the less amount of e-waste, the more recovery of copper was obtained with respect to the amount of total metal content. In particular, recovery close to 100% was achieved using microorganisms in the tests with 2.5 g/L of PCB. In this case, the bioleached copper was nearly twice the amount of copper retrieved by chemical leaching. When 5.0 g/L of PCB were tested, the same behaviour was observed, but differences between the abiotic and biotic samples were less pronounced. For that, in this study, e-waste concentrations that allowed maximum copper bio-extraction were lower than those found in the literature during similar periods of time (Adhapure et al. 2013; Xiang et al. 2010). The fact that the differences between biotic and abiotic assays were more pronounced at higher e-waste concentrations means that biological activity was significantly affected by the PCB dosage. This behaviour can be attributed to the fact that the higher the residue amount used, the higher concentration of inhibitory compounds. For this reason, the effect of these compounds on the biological activity will be studied in Chapter 8. Nevertheless, the extraction of copper, in terms of copper concentration along time, revealed that more copper was recovered at higher e-waste concentrations although the differences between biotic and abiotic assays were the same than those observed in terms of copper recovery. This behaviour could be also related to reagent shortage since the velocity of the biological oxidation of iron (II) is slower than the velocity of the copper oxidation (Nemati et al. 1998; Yazici and Deveci 2014).

Regarding to pH, and as can be observed in Figure 6.5, although the bioleaching media were initially adjusted to pH 2, this parameter increased in the three tests along the first 6 days of experimentation. It is noteworthy that the more amount of PCB was added, the more alkalization of the media was observed. It confirms that the alkalization observed in many studies (Adhapure et al. 2013; Brandl et al. 2001; Ilyas et al. 2013; Wang et al. 2009) is due to the consume of protons by reaction with the waste and it seems not be related to biological activity of iron (II) oxidation (there was no significant differences between biotic and abiotic experiments). In spite of this similar behaviour during the first days, after 10 days of experimentation the pH decreased to pH 2 in the tests with 2.5 g/L of e-waste (where the maximum recovery was achieved), whereas the pH scant increased to pH 4.5 in the tests with 10.0 g/L. In case of the tests with 5.0 g/L of PCB, they remained quite constant among pH 3 – 4. These pH changes could be related to the heterogeneity of the PCB sample (Villares et al. 2016). As a consequence, alkalizing compounds could be found in higher concentrations when higher scrap amounts were treated. For instance, this could explain why pH increased in tests with 10.0 g/L but decreased with 2.5 g/L after 12 days. Wang et al. (2009) also observed a

depletion on pH when 8.0 g/L of PCB were treated. They assumed that this acidification is produced by the hydrolysis reaction of iron (III), producing protons as shown in Eq. (6.1), which results in pH decrease.

$$Fe^{3+} + 3\,H_2O \rightarrow Fe(OH)_3 + 3\,H^+ \qquad (6.1)$$

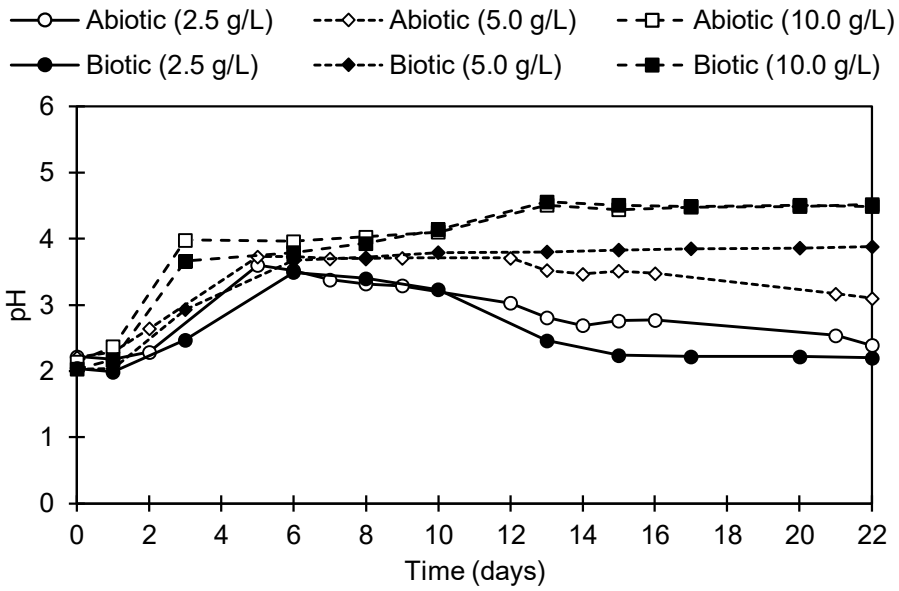

Figure 6.5. Evolution of pH along time at different PCB dosages without pH control in one-step bioleaching.

This alkalization could considerably reduce the biological activity since it is well established that *Acidithiobacillus ferrooxidans* growth and development take place at pH below 2.5 (Meruane and Vargas 2003). These authors related the inhibition of the biological activity to the formation of a ferric oxide layer on bacteria, hindering the protons diffusion. For this reason, we investigated the bioleaching process under controlled pH adjusting pH values over bioleaching process by addition of 3 N H_2SO_4 to keep pH between 2 and 2.5. Figure 6.6 illustrates how the control of pH can affect the operation by comparing experiments with and without pH control.

As can be observed, in both dosages tested (2.5 and 5.0 g/L of PCB, respectively) copper recovery was faster when the pH was adjusted; in particular, the improvement was more noticeable during the first 10 days of experimentation. This fact means that, for example, when 2.5 g/L of PCB were treated, 80% of copper recovery was reached 4 days before when the pH was controlled and up to 7 days before to achieve 65% of extraction when 5.0 g/L were treated. Hence, the bioleaching process were faster under pH control. However, after this period, no significant improvement was observed when the pH was adjusted, independently of the dosage treated. Despite the results obtained

in the second period, a pH control at acidic values improved the kinetic of the process, as it was also demonstrated by other authors (Yang et al. 2009; Zhu et al. 2011).

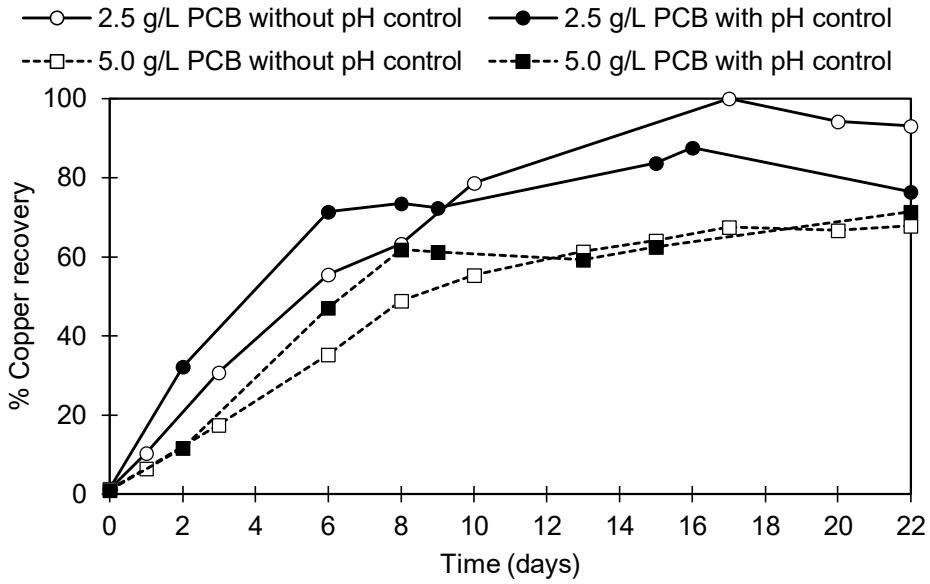

Figure 6.6. Copper recovery along time in biotic samples with 2.5 and 5.0 g/L of PCB with and without pH control.

It is noteworthy to point out that these experiments were performed using one-step process. In this sense, the microorganisms could be affected by the toxicity of the bioleached metals or the kinetics of the leaching process could be limited by the slow kinetic of the biooxidation. In this sense, two-steps bioleaching might avoid these limitations.

6.3.4. Development a two-step bioleaching process: bio-oxidation of iron (II)

Figure 6.4 allowed to corroborate that the e-waste could negatively affect the biological activity, depending on the e-waste dosage used. In order to avoid its negative effect, a novel methodology has been developed. It is based on the separation of the two main processes involved in the bioleaching global process which are the iron biological oxidation and the chemical leaching from scrap. In this way, it is possible to see the effect of each process separately and, therefore, assess whether the effectiveness of the overall process improves. Depending on the procedure, the two-step process could be performed by temporal-driven or spatial-driven steps. In temporal-driven procedure the two processes were carried out in the same flask but sequentially since the biomass was not separated, so after the iron oxidation the scrap is directly added. On the contrary, in spatial-driven procedure the two processes take place in two different flasks due to the

separation of the biomass makes it necessary to transfer the bio-generated iron from one container to another one.

In both procedures, the goal of the first step was to obtain the iron (III) concentration necessary in the second step to extract copper from the PCB waste. This iron is obtained from the biological oxidation of the iron (II) (Eq. (6.2).

$$4\,Fe^{2+} + 4\,H^{+} + O_2 \xrightarrow{Biological\ activity} 4\,Fe^{3+} + 2\,H_2O \qquad (6.2)$$

An effective transformation of iron (III) is absolutely necessary to assure a proper bioleaching process. Since iron may be found in different speciation forms depending on the pH media (Figure 6.7), it is important to maintain the properly conditions since the speciation form of iron is crucial for its solubility and bioavailability by microorganisms (Hogle et al. 2014). Hence, maintaining the pH solution as acidic as possible is important in order to achieve higher iron (III) availability.

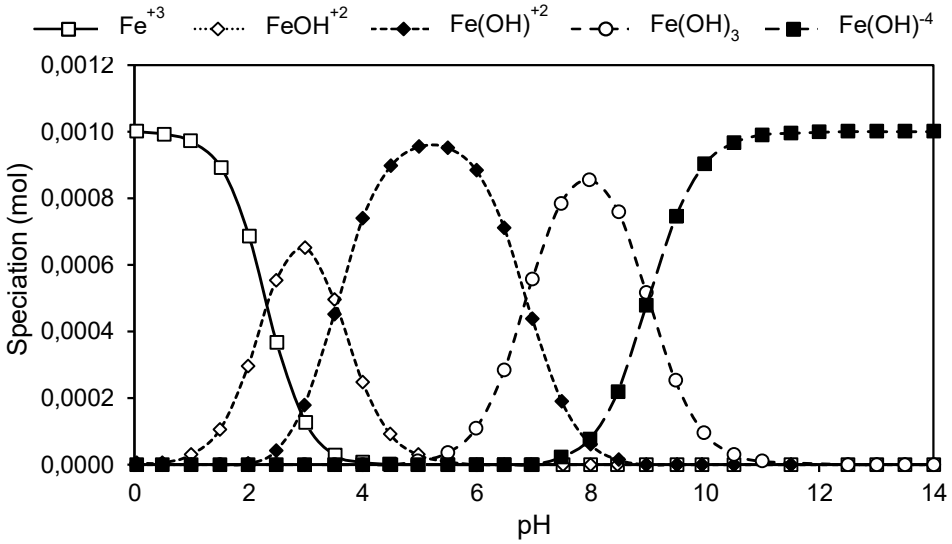

Figure 6.7. Diagram of the speciation of iron in front of pH.

The microorganisms employed in this research grow in very acidic conditions (pH 1.5 – 3.0), therefore, it would be very important to maintain acid pH over the whole experiment in order to assure a proper biological performance and to prevent iron precipitation. The effect of pH control during biological iron oxidation could be appreciated in Figure 6.8. Although the oxidation of iron was observed in both experiments, it is noticed that the total iron concentration remained constant only with pH adjustment, thus, avoiding iron precipitation. Otherwise, the total iron was reduced from 6000 mg/L to near 4000 mg/L in 45 hours without pH control. Hence, 28.4% of the

available iron (III) from the solution was lost due to its precipitation when the pH was not adjusted, resulting in a reduction of the reagent needed for the leaching stage. As stated before, this result remarks the importance of pH adjustment during the experiment to facilitate its solubility and bioavailability by microorganisms.

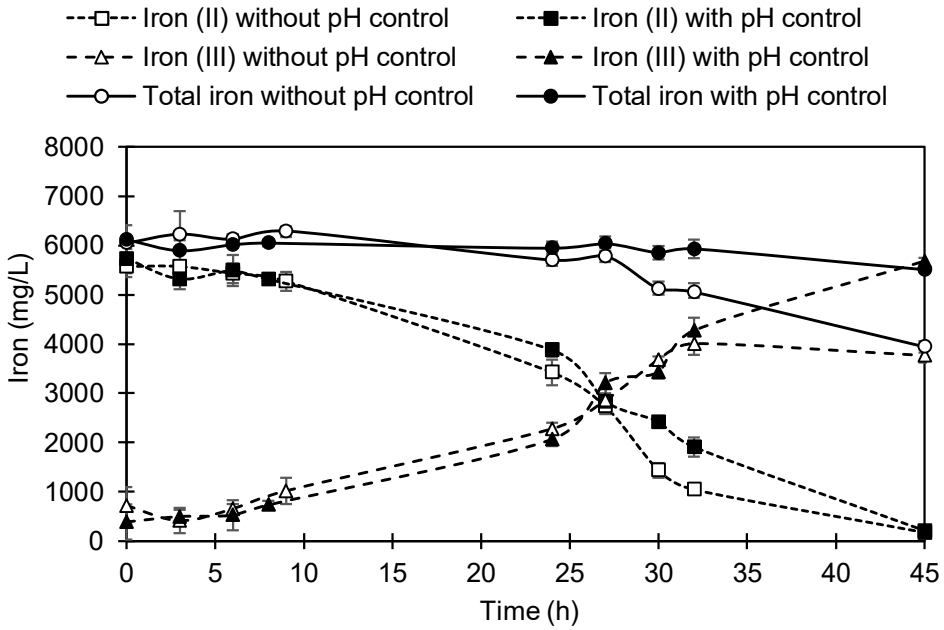

Figure 6.8. Evolution of iron concentration during the bio-oxidation with pH adjustment in the two-step bioleaching.

In spite of the results observed, abiotic control was necessary to validate the effective microbiological action and to discriminate the possible chemical oxidation of iron that may occur at the conditions tested. The biotic and abiotic comparison is showed in Figure 6.9. It can be seen that 96% of the iron (II) has been oxidized to iron (III) in 45 hours in presence of microorganisms whereas in the abiotic test the iron (II) remains constant and around 6300 mg/L. This corroborates that iron is only oxidized by the microorganisms at the experimental conditions used in 45 hours. These results are in agree with Xiang et al. (2010) who also observed that the chemical oxidation of iron (II) was not produced at pH 1.5 even after 7 days.

Regarding to the initial iron (II) concentration in the medium, Pina et al. (2010) reported that the higher the initial iron concentration, the higher the iron oxidation rate. Therefore, this study suggests a non-zero order kinetic. According to Pina et al. (2010), obtained similar effect was observed when the initial iron concentration was below 10 g/L whereas at higher iron concentrations the oxidation rate did not improve. Although these authors carried out their experiments with *Sulfobacillus thermosulfidooxidans*, the results obtained were in agreement with Gómez et al. (1996) who performed similar

experiments using a pure culture of *Acidithiobacillus ferroxidans* (the microorganism used in the present thesis). On the contrary, a recent study of Hubau et al. (2018) stated that the bio-oxidation rate obtained with an influent concentration of 6 g/L of ferrous iron was higher than the rate obtained with an influent of 9 g/L of iron (II), although they observed substantial variations in these rates at the same conditions. Because of that, and taking into account that there are some studies using initial concentration of 9 g/L of iron (II) (Fomchenko and Muravyov 2017; Mražíková et al. 2013; Nie et al. 2014; Willner and Fornalczyk 2013), a comparison of the evolution of iron concentration along time was performed with 6 and 9 g/L of iron (II) (Figure 6.10).

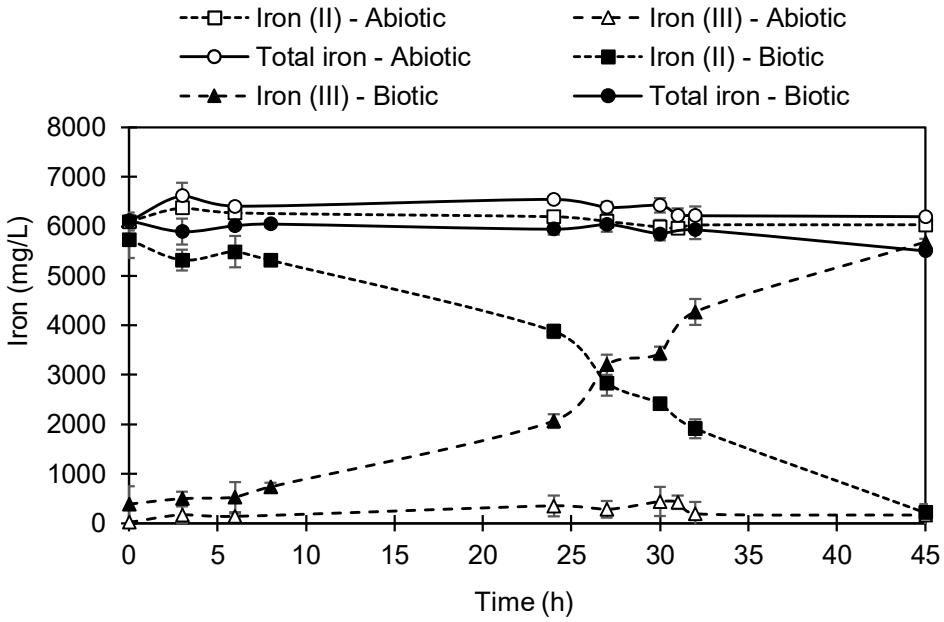

Figure 6.9. Evolution of iron concentration along time during biotic and abiotic iron oxidation under pH control.

Figure 6.10 shows that the complete iron (II) oxidation was achieved in 30 hours when the medium contained 6 g/L, whereas the medium with 9 g/L needed 48 hours to be completely oxidized. However, in both cases the iron oxidation ratio was very similar (152 mg/Lh for the medium with 6 g/L of iron (II) and 158 mg/Lh for the 9 g/L one). Moreover, the iron (II) concentrations at the beginning of the experiments were lower than the theoretical concentrations, especially, the medium with 9 g/L of iron. This might be caused by iron precipitation. In this regard, although the oxidation rate was very similar in both cases, the use of the medium with 6 g/L of iron (II) will reduce the amount of precipitates accumulated during the bio-oxidation. Therefore, from the results obtained, a concentration of 6 g/L of iron (II) was used for next experiments. Moreover, 6 g/L of iron is the stoichiometric amount needed to recover all the copper from the scrap,

taking into account that maximum values of 3.4 g/L of copper is usually found after the e-waste bioleaching process.

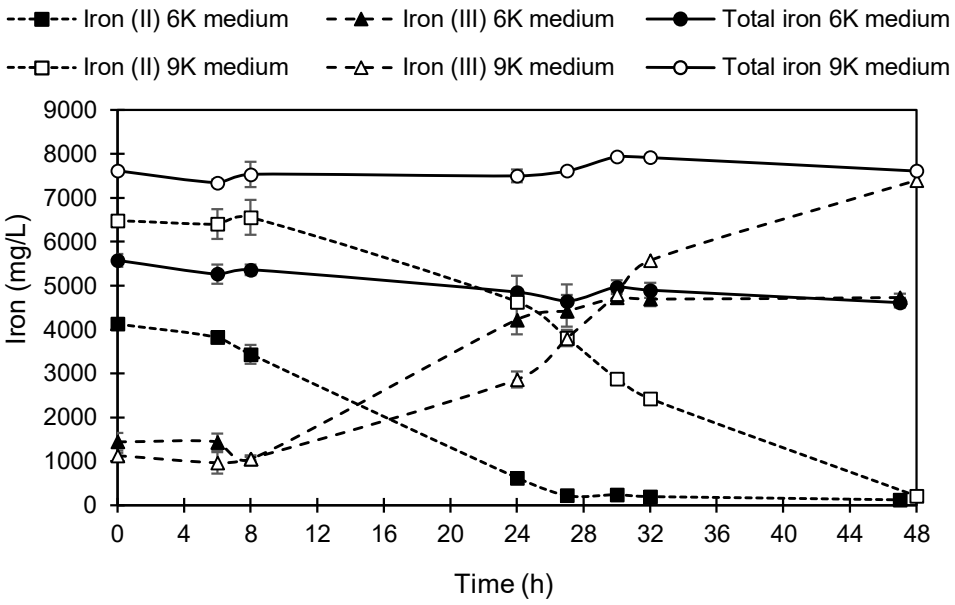

Figure 6.10. Evolution of iron (II), iron (III) and total iron concentration along time during the growth of *Acidithiobacillus ferroxidans* using 6 and 9 g/L of iron (II) in the mineral media.

6.3.5. Development a two-step bioleaching process: e-waste leaching

As explained at the beginning of the previous section, the methodology developed to achieve higher copper recovery rates consists on the biological oxidation of iron (II) (step one) and the leaching of PCB (step two). After testing the appropriate conditions for the biological action, the second step of the bioleaching was carried out.

Since better results were obtained when the bio-oxidation of iron took place under pH adjustment, the same effect on the leaching phase was studied. For this, two sets of experiments were done, one with pH adjustment (pH 1.7 – 1.8) during the PCB leaching and the other without it.

In Figure 6.11 shows that the main significant changes occurred during the first hours. In particular, iron (III) was consumed during the first 6 hours of experimentation whereas 37% of copper was recovered in this period. Then, the iron (III) concentration remained almost constant despite the total iron concentration as well as the iron (II) concentration decreased by 50%. The decreasing was associated to iron precipitation, which was also observed by Xiang et al. (2010) during their bioleaching experiments. They attribute this decrease to iron (III) precipitation as jarosite or other ferric hydroxides.

In the present experiment, at constant iron (III) concentration in the solution, it is assumed that iron (II) precipitates just after it has been previously oxidized. This behaviour is associated to the pH increase observed, since it raised up to pH 2.5 and remained constant at this value although the pH was adjusted initially at pH 1.75.

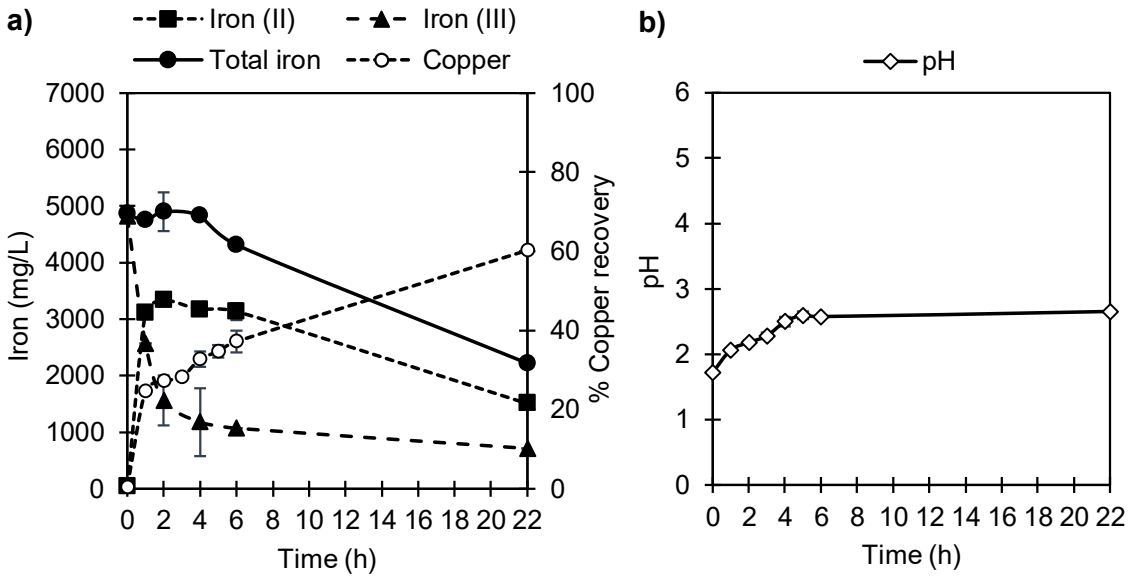

Figure 6.11. (a) Copper and iron evolution and (b) pH evolution over time during the leaching step of PCB when the pH is not controlled in the two-step bioleaching process.

The initial pH increase in the first hours has been associated to the e-waste nature itself, considering that the electronic scrap has been reported to be alkaline in nature (Brandl et al. 2001; Xiang et al. 2010). However, there are no previous study focusing on which component of e-waste produced the alkalinity observed. In addition, Xiang et al. (2010) also related the pH increase to the consumption of protons by other metals or metal oxides contained in e-waste (Eqs. 6.3 and 6.4).

$$M + H^+ \rightarrow M^{n+} + H_2 \quad (6.3)$$

$$MO + H^+ \rightarrow M^{n+} + H_2O \quad (6.4)$$

It is noteworthy to point out that despite not having a decrease on iron (III) concentration after 6 hours, copper recovery increased up to 60%. According to Torres and Lapidus (2015) this response is associated to the presence of oxygen in the acidic medium. They affirmed that the continuous stirring of the leaching solution improved the oxygen gas incorporation and its dissolution in the liquid, which produced the oxidation of metallic copper (Eq. 6.5).

$$2\,Cu^0 + 4\,H^+ + O_2 \rightarrow 2\,Cu^{2+} + 2\,H_2O \quad (6.5)$$

This copper solubilisation by the action of oxygen was also described by Bas, Deveci, and Yazici (2013) who carried out their experiments at pH 1.7 and 35 ºC. Taking into account that the conditions in the present work were pH 1.75 and 30 ºC, it could be assumed that the oxygen is the responsible of the copper recovery observed in Figure 6.11 during the last hours of the experiment. Nevertheless, if it occurred, the protons consumption should cause a pH increase, which was not observed. Moreover, the kinetics of this reaction is very slow in comparison to the iron oxidation since Bas et al. (2013) only recovered 18% of copper by the action of oxygen in acidic media after 90 experimental hours, i.e. much more experimental time than in this work. However, this hypothesis was not completely rejected since other metals could be leached and many other chemical reactions could take place, modifying and also compensating pH changes in the solution.

As it was observed in the experiment without pH control, in the case of pH adjustment, the main changes were obtained during the first three experimental hours (Figure 6.12). Iron (III) was consumed quickly in the first hour (68% of the initial iron (III)), and then, it was consumed slowly to achieve their complete reduction after 22 hours. In consequence, the iron (II) concentration increased along time. In contrast to the previous experiment, the total iron concentration remained almost constant during the entire experimentation period when the pH was adjusted, so it was possible to avoid its precipitation in the solution. In relation to metal extraction, higher amount of copper was retrieved under pH control, reaching 55% of copper recovery in 6 hours and 70% in 22 hours.

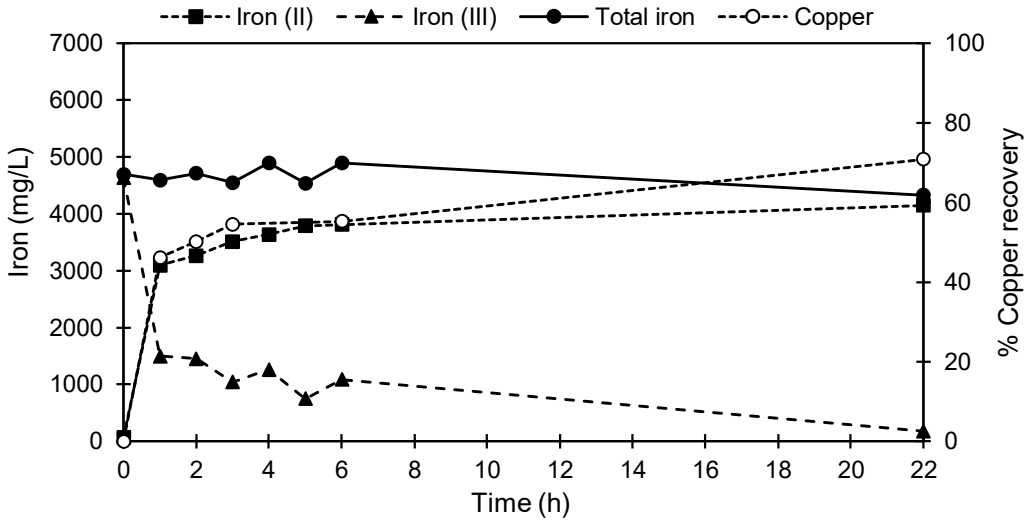

Figure 6.12. Copper and iron evolution along time during the PCB leaching step when the pH is controlled in the two-step bioleaching process.

Therefore, a pH control implies an improvement of the process since it results in superior copper recovery performance and in less iron precipitation. This improves the global process performance since if the total iron concentration remains constant in the solution, the microorganisms could oxidize it to regenerate the iron (II) again and to start the cyclic loop.

6.3.6. Development a two-step bioleaching process: biomass separation

A part from copper, some other metals could be also leached from the scrap, affecting the microorganisms activity due to the toxic composition of the e-waste (Akcil et al. 2015; Villares et al. 2016). In this regard, two different techniques were tested in order to separate the biomass from the soluble iron (III). Among the different processes for solid-liquid separation, filtration and sedimentation are usually found in literature (Holdich and Butt 1997). While filtration is a very effective separation process, sedimentation is characterized to be simpler, economic and easier to operate autonomously besides having lower maintenance costs in comparison to filtration processes. Therefore, the leaching of copper from PCB after the removal of the biomass by filtration and sedimentation were comparatively assessed after the bio-oxidation stage in two-step bioleaching.

For the filtration method, the bio-generated iron (III) was obtained after the filtration of the solution through a 0.22 µm membrane filter. The clarification of the media was determined by optical density (OD) at 500 nm (Barron and Luecking 1990) before and after the filtration, revealing that the OD was 1.0307 and 0.1560, respectively. The iron concentration and copper recovery along time of the filtered solution during the leaching stage are shown in Figure 6.13. A fast extraction was observed at first, obtaining up to 50% of copper from PCB in 6 hours. This behaviour could be attributed to the quickly extraction from the most available sites on the waste particles. Then, a slower extraction occurred achieving a total copper recovery of 71% in 25 hours. Regarding iron concentration, iron (III) strongly decreased during the first 6 hours, which is directly associated to their reaction with copper. After 25 hours practically all the iron was reduced to iron (II).

As mentioned before, the filtration for biomass removal was compared to the biomass removal by sedimentation. Hence, leaching of copper from PCB after the sedimentation was performed. As in the filtering case, the clarification of the media was monitoring by determining the optical density (OD) at 500 nm (Barron and Luecking 1990) at different times. In Figure 6.14, results showed the OD dropped sharply from 1.00 to 0.17 in two hours. After this period, the OD remained practically unchanged until

24 hours. It means that an important part of the biomass was deposited in just 2 hours. Hence, for practical considerations, the supernatant after two sedimentation hours was taken for leaching tests. In this sense, the solution was transferred to a beaker and after 2 hours of settling, the solution was separated from the biomass by decantation.

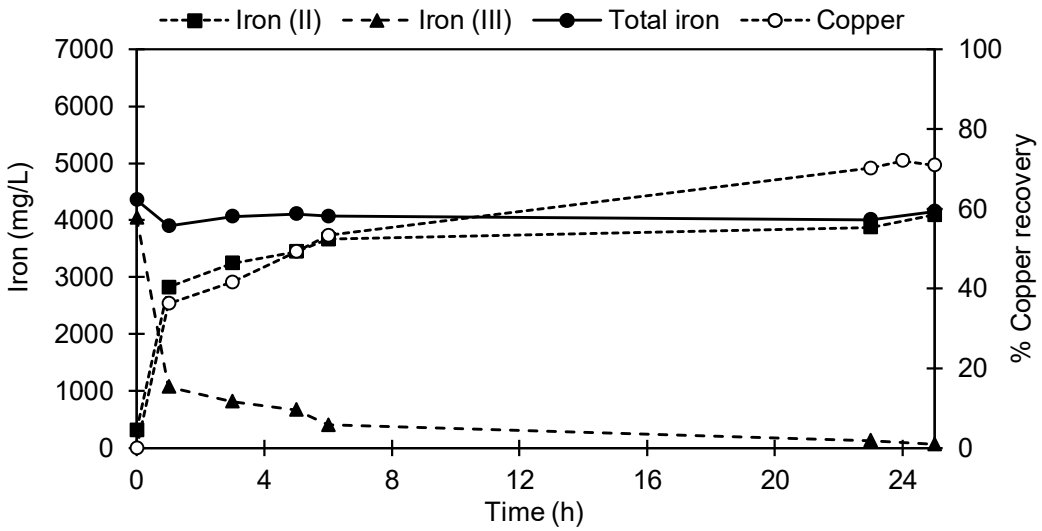

Figure 6.13. Concentration of iron and copper recovery during the bioleaching after filtration.

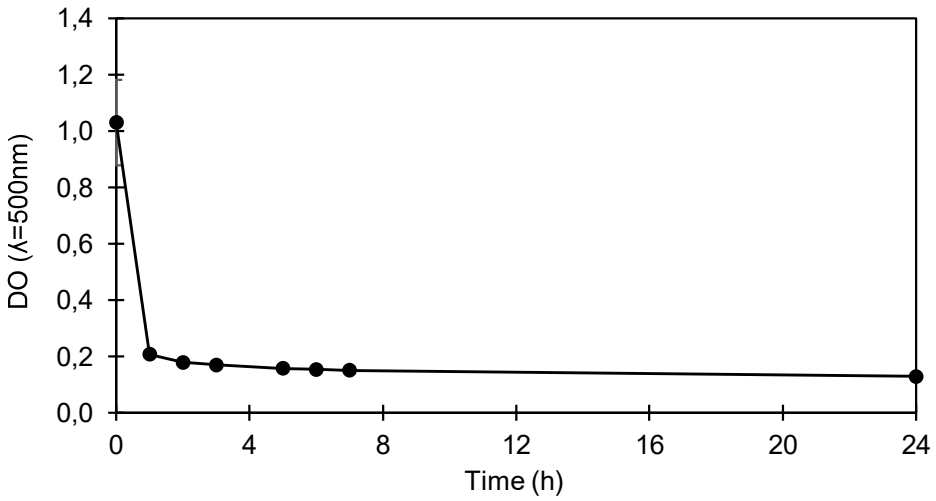

Figure 6.14. Evolution of optical density during the sedimentation of the biomass.

In Figure 6.15 copper recovery and iron evolution along time during the leaching of e-waste after the sedimentation of the bio-oxidation solution from the first step is shown. Iron (III) ions were consumed during the first 6 hours due to its reaction to the metallic copper from the PCB. In consequence, iron (II) concentration increased and

copper recovered was up to 55%. After this period, the copper recovery increased again achieving 90% after 25 hours although the iron (III) concentration increased. This is associated to the presence of microorganisms in the sample that have been grown, consuming iron (II) to produce iron (III). Although almost all biomass was deposited, a few amount of microorganisms could remain in solution (Figure 6.14). Therefore, the presence of microorganisms could appear as a result of the remaining microorganisms that could be in the supernatant after the sedimentation, which have been proliferated during the experimental time. Moreover, the total iron concentration was slightly reduced despite adjusting the pH throughout the experimental period at pH 1.75. Nevertheless, the decrease was not as significant as the iron precipitation observed when the pH was not controlled, as it was demonstrated in the previous experiments.

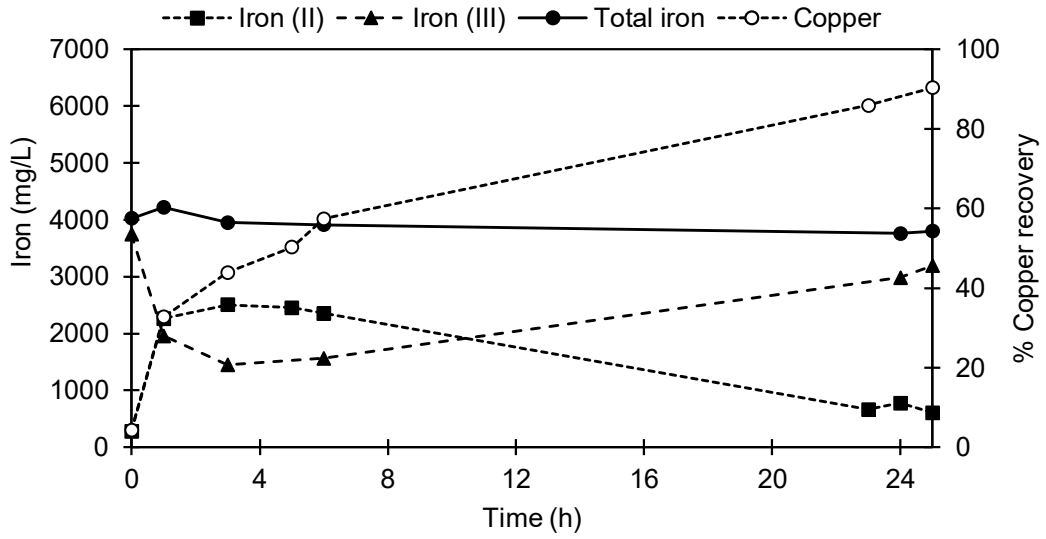

Figure 6.15. Concentration of iron and copper recovery during the bioleaching after sedimentation.

In order to facilitate the comparison between the no separation of the biomass and the use of filtration or sedimentation to separate it, the results of copper recovery in the three cases are depicted in Figure 6.16. Copper recovery was the same when the biomass was separated completely by filtration in comparison to the copper recovered when the biomass was not separated. However, the sedimentation process used for biomass removing increased the copper extraction up to 19%. It means that high concentrations of biomass or a complete removal of it did not improve the copper extraction but, on the contrary as it could be though, low concentrations of biomass did increase the efficiency of the process. Hence, low biomass concentrations allows to reoxidize the iron (II), making more iron (III) available for continuing metal extraction, however, high biomass concentrations are not possible to survive by the affection of the

possible toxic bioleached metals. In this sense, the separation of the biomass by sedimentation improved the process at the same time that allows their use to re-oxidize the iron (II) again separated from the PCB. Moreover, the simplicity of the sedimentation allows to be an easier methodology to adapt the bioleaching process performance to an industrial scale.

These results are in agreement with Yang et al. (2014) who reported better results when the samples were not filtered before the leaching of PCB in the two-step process. In their case, they obtained 70% more copper recovery when they did not filtrate the sample after the bio-oxidation, attributing the improvement to the regeneration of the iron (III) leaching agent by the microorganisms. Although the enhancement observed in the present work was not so high between the techniques used, it is worth noting that the 90% of copper extraction after sedimentation was obtained in just 25 hours, whereas Yang et al. (2014) spent 48 hours to achieve the same result. The difference is associated to the operational conditions, especially the pH, which was controlled under pH 2.25 by these authors whereas it was adjusted under pH 1.80 in the present study.

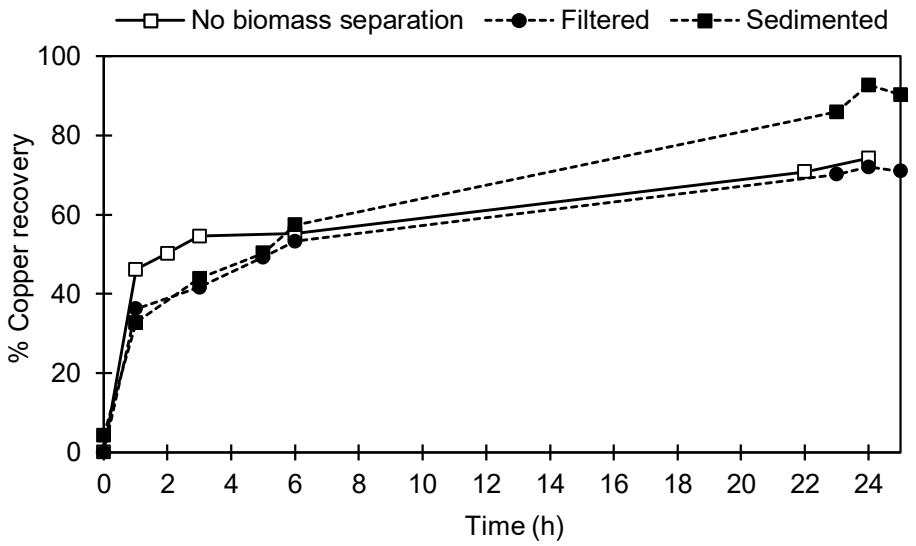

Figure 6.16. Comparison of the copper recovery along time when the biomass was not separated and when it was separated by filtration and sedimentation.

In the light of these results, although the difference on copper recovery was not so high between the techniques tested, in all cases the experimental time was clearly reduced in comparison to analogous experiments described in the literature, despite the use of a similar pH to this work (Shah et al. 2014; Xiang et al. 2010).

6.4. Conclusions

Remarkable differences appeared when the e-waste and the chalcopyrite were bioleached under the same operational conditions. The main difference was the amount of copper extracted, since copper obtained from the e-waste was 70% higher than the amount obtained from the mineral sample. This could be related to the role of the redox potential in the process but also to the matrix in which copper is placed, because the e-waste and the ore have quite different structures, affecting the accessibility and bioavailability of the copper to be retrieved.

Regarding to chemical and biological leaching at 2.5, 5.0 and 10.0 g/L e-waste concentrations, an effect of the PCB dosage to the process was observed. In particular, differences between biotic and abiotic tests were more pronounced when less e-waste concentration was treated. This means that e-waste results to be toxic for the microorganisms when the amount of e-waste is too great because of its metal composition. Even so, the improvement observed when *Acidithiobacillus ferroxidans* are involved was noticeable, obtaining up to 30% more copper recovery when 2.5 g/L of e-waste were treated biologically instead of chemically. An improvement of copper bioleaching was observed during the first 10 days of experimentation when controlling the pH in biotic samples of 2.5 and 5.0 g/L of PCB. This was attributed to overcoming the limitation of the alkalization process (caused by PCB decomposition) thanks to the pH adjustment. However, after this period, there was not a significant improvement on copper recovery between samples with adjusted pH and without it.

The bioleaching performance in two steps obtained better results than those obtained in one step, since the time required to extract copper was reduced from 22 days to just 2 days (one for each step). However, although the pH control in one-step bioleaching did not improve the process significantly, when the two-step methodology was used, the pH control was needed to maintain the total iron concentration in both steps. Regarding to the bio-oxidation step, the implication of the microorganisms to oxidize the iron (II) efficiently was verified, since a chemical oxidation was not observed during the same period of time at the conditions tested (pH 1.7, 30 ºC and 130 rpm). In addition, the 6K medium resulted as efficient as 9K medium. However, 6K medium was selected for further experiments since 9K usage led to higher amount of iron (III) precipitation.

In two-steps bioleaching, both filtration and sedimentation allowed to separate the biomass from the iron (III) solution. Though the biomass was not separated completely when sedimentation was used, copper recovery was higher (90%) than that

obtained after filtration (70%). Nevertheless, sedimentation process is more simple and cheaper. This led to the conclusion that sedimentation would be a better option for process scale-up purposes taking into account that not all biomass is effectively separated from the first step.

Chapter 7

Development of a bioleaching process in a continuous stirred-tank reactor (CSTR) to recover metals from e-waste

The next step in the bioleaching research performed in this thesis was to operate the developed process studied in the previous chapters at higher scale than laboratory using a system that allows an improvement on monitoring of key parameters. To that aim, in this chapter the biotechnological process to recover metals was carried out using a CSTR. Firstly, Acidithiobacillus ferrooxidans was cultivated in a bioreactor, monitoring its growth by fluorescence measurements. After the growth, cells were used in the bioleaching of PCBs. In this way, the process was tested in a bigger scale, so at this scale the process conditions were modified accordingly. Additionally, and to improve the monitoring of the process, in this chapter a methodology based on microrespirometric techniques was developed to monitor in a simple way the biological activity in a non-invasive manner that allows corrective decisions to be made in a short time. The work presented in this chapter has been carried out in collaboration with the research group Environmental Microbiology from Technische Universität Bergakademie Freiberg in Freiberg (Germany) during a research stay in this university in September 2017.

Abstract

Following with the metal extraction from e-waste by bioleaching, the process was focused on both, increasing the scale and improving the monitoring of the system according to the parameters previously set. In this regard, bioleaching was tested in a two-bioreactor system. In order to obtain enough biomass concentration for the extraction process, *Acidithiobacillus ferrooxidans* was previously grown in the bioreactor. During the growth, the oxygen consumption of the culture was monitored along time by an microrespirometric technique using an optode. Simultaneously, biomass growth was followed by fluorometric measurements to estimate the cell number, thus correlating it to the oxygen consumption of the biomass. These techniques were implemented in the two-bioreactor system in which the bioleaching of e-waste took place. After leaching, all the metals in the solution were analysed in order to evaluate the potential of the technology to recover valuable metals at the conditions tested. More than 10 valuable metals, such as tin, silver or gold, were extracted during the biological leaching. Control experiments without microorganisms were also carried out. Results demonstrated that more than 56% of copper was released biologically from the PCB by the two-bioreactor system. Although similar results were obtained in the abiotic mode (52%), more acid was consumed to maintain the pH at 1.75 in the abiotic case. The results demonstrate that the

microrespirometric technique emerged as an efficient tool to monitor the activity of the microorganisms during the bioleaching process as well as during their growth in the bioreactor overcoming limitations of other monitoring techniques as those influenced by formation of precipitates. Moreover, this technique allows to minimize the sampling effect on the system due to the small volume necessary for the measurements.

A modified version of this chapter is being prepared for publication as:

Benzal, E., Giebner, F., Gamisans, X., Schlömann, M., Dorado, A.D., 2021. Biorecovery of metals from printed circuit boards in a stirred-tank reactor.

7.1. Introduction

Bioleaching has been largely studied in batch mode at laboratory scale as it has been detailed in previous chapters. Hence, after testing and verifying the potential of the technology, the process needs to be adapted to a new scale to work in similar conditions than those used at industrial level to assess if the potential as an alternative to the current industrial techniques used. The most common configurations for metal's recovery by bioleaching are stirrer-tank bioreactors (Couillard and Mercier 1991; Ilyas and Lee 2014; Jujun et al. 2015; Rivera-Santillán et al. 2013), heaps (Ghorbani et al. 2015; Pradhan et al. 2008) and column bioleaching (Benzal et al. 2020a; Chen et al. 2015; Ilyas et al. 2010; Qiu et al. 2011). These systems are devoted to bioleach higher volumes with an accurate control of the process conditions as temperature, stirring rate, pH, oxidation-reduction potential or dissolved oxygen concentration. Moreover, although the studies focused on the bioleaching performed in a bioreactor like CSTR are studied for metals extraction from ores, in the e-waste field this set-up is scarce in the literature (Ilyas and Lee 2014). For instance, Jujun et al. (2015) recovered copper and gold from e-waste by a CSTR using *Pseudomonas chlororaphis*, whereas Ilyas and Lee (2014) also recovered copper from e-waste using *Sulphobacillus thermosulfidooxidans* and Tipre and Dave (2004) using a consortium of acidophilic chemolitotrophic auto- and hetero- trophic iron and sulfur oxidizers. In particular, Jujun et al (2015) found that the stirring had the major influence in the process, followed by the pH and the temperature, obtaining 88% of copper and 76.6% of gold in 5 days at the best conditions tested (pH 7, 22.5 ºC and 80 r/min). In the case of Ilyas and Lee (2014), they recovered copper (95%), aluminum (91%), zinc (96%) and nickel (94%) in 15 days. Tipre and Dave (2004) concluded that semi-continuous process obtain better results than batch one and, moreover, they suggest that continuous process can further improve the leaching rate although they cannot perform the continuous process to validate it due to the lack of a facility.

Cell counting and biomass quantification become a challenge in bioleaching processes because of the formation of precipitates, which difficult the most convenient classical approaches of turbidimetry and colorimetric protein quantification (Redmile-Gordon et al. 2013). In contrast, methods as fluorescence staining, microscopic documentation, computational image analysis and qPCR depict an important effort, besides its economic cost (Giebner et al. 2015). On the other hand, total fluorescence measurement appears to be an affordable technique for cell quantification during the biomass growth as well as their evolution along e-waste bioleaching (see section 4.2.6 from Chapter 4 for more detail of the fluorescence measurements process).

Moreover, the methodology frequently used to determine metabolic cell activity in bioleaching process is based on the measurement of the ferrous iron oxidation rates (Lambert et al. 2015; Nemati et al. 1998; Pina et al. 2010; Third et al. 2000) as an indirect measure following the product of the reaction. For this purpose, *o*-phenantroline and ferrozine are the commonly applied colorimetric methods (Braunschweig et al. 2012). However, as stated above, colorimetric measurements could be affected by the precipitates formed during bioleaching process and moreover, it has to take into account that not only microorganisms can oxidize iron (II) but also other chemical reactions can concert iron (II). Giebner et al. (2015) suggested that another obvious approach is to quantify the consumption of the electron acceptor oxygen since the instant consumption of this electron acceptor can be only attributed to microorganisms activity. They affirmed that the application of a Clark electrode could be the easiest and cheapest technique for respirometry control but, taking into account that Atkinson and Smith (1973) observed that this kind of electrodes consume oxygen due to its operation, Giebner et al. (2016) adapted an optode-based technique of respirometry control. This technique was adapted to measure oxygen consumption of *Acidithiobacillus ferroxidans* and, thus, to determine its metabolic activity.

The aim of the present chapter was to scale up the bioleaching process to recover metals from PCBs, performing the process in a more controlled bioreactor system to assess the potential for an industrial application. In this sense, the first approach was to grow the *Acidithiobacillus ferroxidans* in a bioreactor, conducting total fluorescence measurements to estimate the cell number and monitoring microbial activity by respirometric measurements by means of an optical oxygen sensor (optode-based system). Then, the bioleaching of e-waste was performed in two-bioreactor system comparing results with an abiotic control system. During the process, total fluorescence measurements were done as well as respirometric assays in order to monitor the activity of the microorganisms. Abiotic controls were carried out to discriminate contribution of biological in comparison to chemical conversion.

7.2. Materials and methods

7.2.1. Electronic scrap

The PCBs used were obtained from end-of-life mobile phones, as stated in the previous chapter. It is noted that all the phones used for the experiment were of the same model (Nokia 3510) in order to reduce the variability of the sample and, therefore, its

heterogeneity. After removing the PCB from the phone structure, the main electronic components (resistors, capacitors and chips, among others) were manually separated. Then, the particle size was reduced by shears, selecting the particles of approximately 2.0 cm^2.

7.2.2. Microorganisms and mineral medium

The bacterial strain *Acidithiobacillus ferroxidans* (ATCC 23270) was used. It was kindly provided by the Department of Chemical Engineering from the University of País Vasco (Spain). The mineral medium used in the experiments (named 6K) was prepared as follows: $(NH_4)_2SO_4$ 3.00; K_2HPO_4 0.50; $MgSO_4 \cdot 7\ H_2O$ 0.50; KCl 0.10; $Ca(NO_3)_2 \cdot 4\ H_2O$ 0.014 grams were dissolved in 900 mL of distilled water. The pH was adjusted with 3 N H_2SO_4 to pH 1.75. Then, 30 grams of $FeSO_4 \cdot 7\ H_2O$ were dissolved in 100 mL of distilled water and the pH was also adjusted with 3 N H_2SO_4 to pH 1.75. After that, both solutions were mixed and the pH was readjusted to pH 1.75 when necessary.

7.2.3. Cell number estimation by fluorometric measurements

The microplate reader (*SpectraMax M2e, Molecular Devices, United States*) used for the measurement of the total fluorescence and, thus, for the estimation of the cell number, is explained in detail in section 4.2.6.

Prior to the measurement of total fluorescence to estimate the cell number, and following the instructions of Giebner et al. (2015), the samples were treated as explained below (Figure 7.1). Firstly, 2.0 mL of culture sample were transferred into an Eppendorf tube and centrifuged (15.000 *g*, 5 min). After discarding 1.0 mL of the supernatant, 0.1 mL of 0.5 M oxalic acid was added. The mixture was vortexed 15 seconds and incubated for 5 minutes at room temperature to dissolve the precipitates. After centrifugation (15.000 *g*, 5 min), 0.1 mL of the supernatant was discarded and 0.1 mL of oxalic acid was added again. The process of oxalic acid addition was repeated twice in order to dissolve all the precipitates in the sample. After the last centrifugation, 1.0 mL of the supernatant was removed. The remaining sample of 0.1 mL was diluted with 0.5 mL of sterile 0.9% NaCl solution. The mixture was also centrifuged again (15.000 *g*, 5 min) and 0.5 mL of clear supernatant was now removed. After repeating the last step twice, the remaining 0.1 mL cell-containing sample was re-suspended with 0.9 mL of 50 mM phosphate buffer and it was also vortexed to homogenise the mixture. Finally, 50 µL of PicoGreen© reagent was added to 0.25 mL of the pre-treated sample. The mixture was incubated for 1 hour at room temperature in the dark. The resulting fluorescence signal of the assay was measured as relative fluorescence units (RFU) in a black 96-well plate (0.3 mL well volume, *Greiner bio-one*) using a micro-plate reader (*SpectraMax M2e,*

Molecular Devices). Equipment settings selected were: excitation at 485 nm, emission at 525 nm, cut-off at 495 nm, 400 V PMT-gain and 10 flashes per read. All the samples were measured in triplicate.

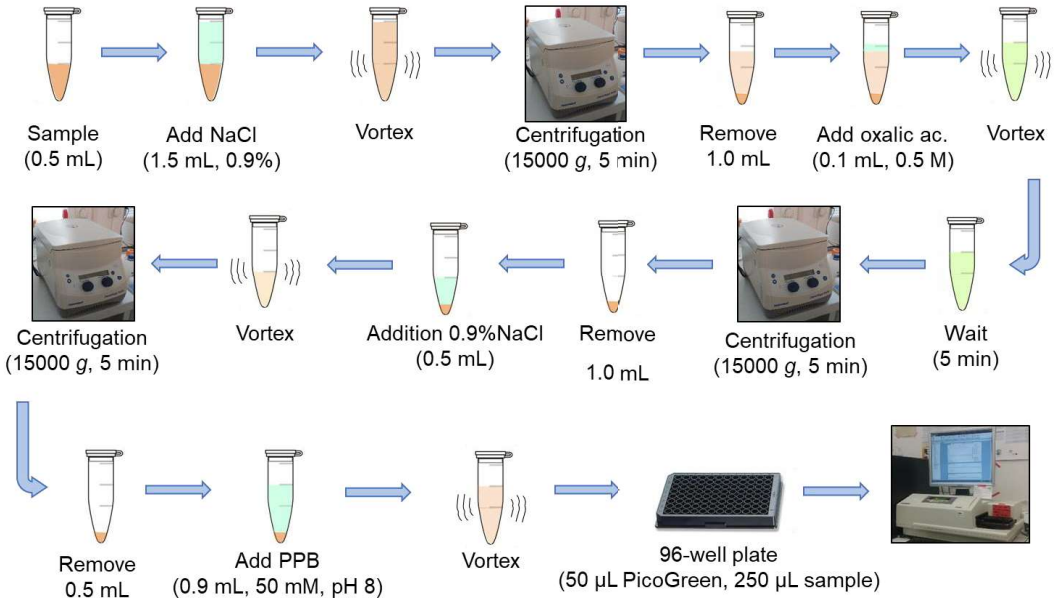

Figure 7.1. Diagram of the procedure followed to measure the total fluorescence in microbial samples. Abreviations: PPB (potassium phosphate buffer).

7.2.4. Experimental set-up

Bioleaching experiments were conducted in 1 L jacketed bioreactors (*Labfors 5, Infors HT, Switzerland*) equipped with baffles and a spiral propeller blade stirrer. Following the methodology used during the previous experiments in Chapter 6 in which the bioleaching was performed in a two-step process, the two steps of the bioleaching process were carried out separately. Hence, two bioreactors were used for the biotic experiment, as shown in Figure 7.2. The first one was used for the bio-oxidation of iron (II), whereas the second one was used for the leaching reaction. In this sense, bio-oxidized iron (III) was pumped to the leaching reactor while the leaching solution, including reduced iron (II), was pumped to the bio-oxidation tank continuously.

The bio-oxidation vessel was filled with 0.9 L of mineral medium and 0.1 L of inoculum, whereas the leaching tank was filled with 1.0 L of mineral medium. The pH was controlled at pH 1.75 by the dropwise addition of 3 N H_2SO_4 to the bio-oxidation tank, to keep the pH low for high Fe(III) solubility and still compatible with the growth of the microorganisms. Moreover, a thermostatic bath was used to maintain 30 ºC in both reactors, and they were stirred at 200 rpm (bio-oxidation reactor) or 300 rpm (leaching reactor), respectively. An increase in stirring in the leaching reactor was necessary in

order to avoid settling of the e-waste. Aeration was performed by injecting compressed air at 0.2 L/min in both vessels. When the solution turned red in the biotic tank and the ORP measurement was over 600 mV, the liquid from this tank was pumped to the other tank, considering that all iron (II) was biologically oxidized. In order to maintain a residence time of 24 hours in both bioreactors, the pumped flow was kept at 0.7 mL/min. A 16-days leaching test was performed with 15 g/L of PCB. An abiotic control was also carried out by using the mineral medium with iron (II) but without the presence of the microorganisms. In this case, only the leaching tank was used under the same conditions. Samples were taken daily and they were diluted before being analyzed to determine iron and copper concentrations. In addition, pH and ORP were also measured every day, whenever possible.

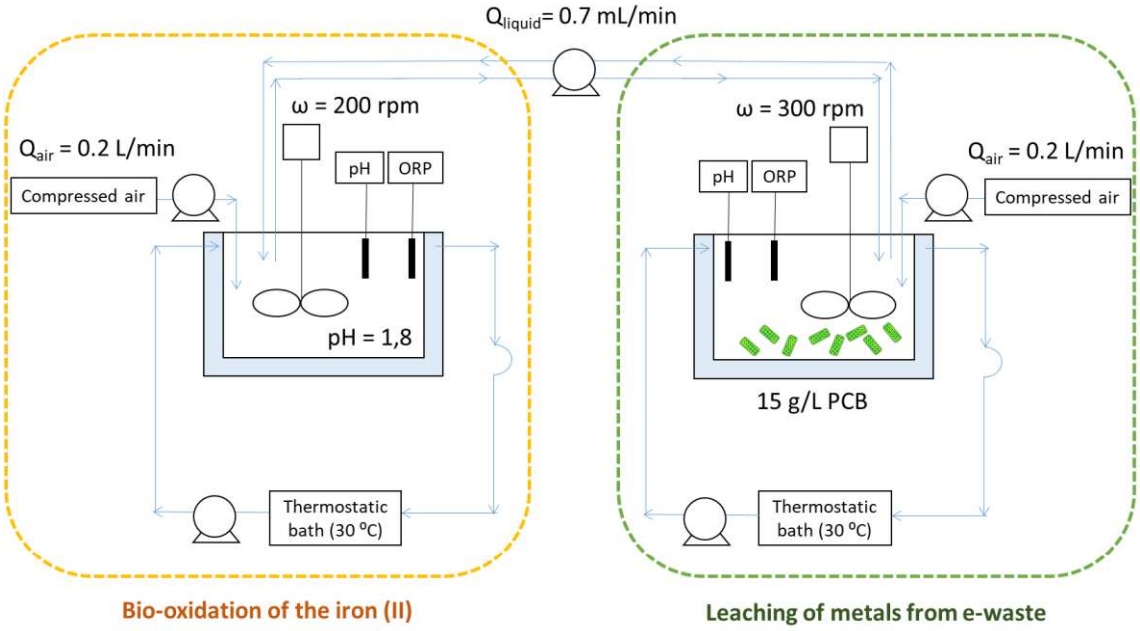

Figure 7.2. Scheme of the experimental set-up used for the biotic experiment during the bioleaching of PCB in stirred-tank reactors.

7.2.5. Optical system for oxygen measurements

The optical system (*FireStingO2, PyroScience GmbH, Germany*) used for the measurement of biological activity is explained in detail in section 4.2.2.

For a standard respirometric assay, the procedure explained by Giebner et al. (2015) was followed (Figure 7.3). For an assay, 2 mL of the biological sample was centrifuged (5000 rpm, 10 min). Then, the supernatant was removed and the pellet was re-suspended in the same volume of modified mineral medium, without ferrous ion (0K

medium). After the sample had been mixed by a vortex, it was transferred to a test tube. Afterwards, 2 mL of pre-tempered 6K mineral medium (pulse substrate) was added and the sample was mixed again. Just before the addition of 6K, the oxygen sensor was also introduced into the tube test to record the signal for 30 minutes. To express the respiration rates, the linear sections of the recorded oxygen decrease were used, defining the respiration rate in milligrams of oxygen consumed per litre in one hour. Respirometric measurements were made in triplicate.

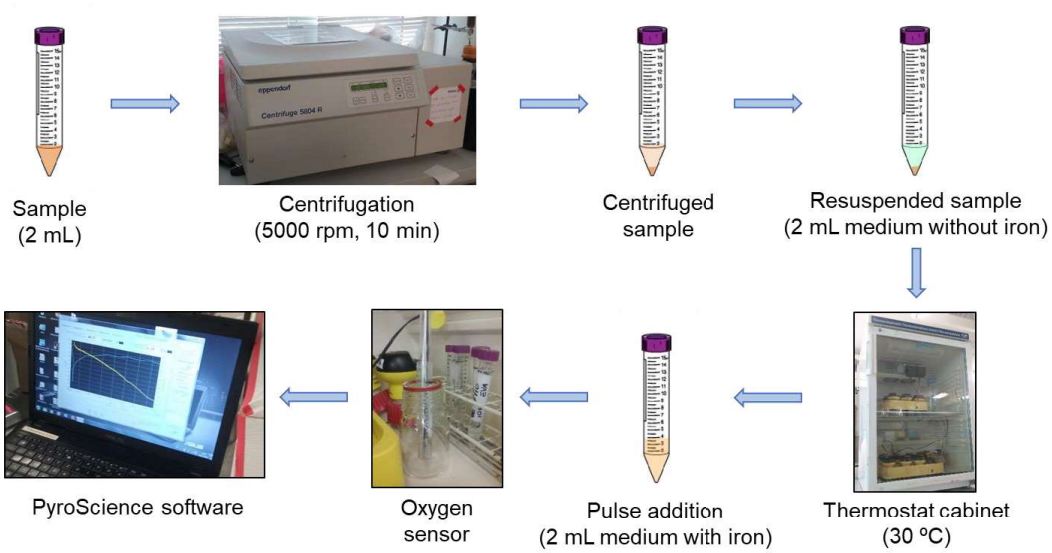

Figure 7.3. Diagram of the procedure followed to measure the biological activity with the optode.

7.3. Results and discussion

7.3.1. PCB metal composition

The analysis of the PCB used in this chapter exposed that the average content of Cu, Ni, Fe, Ag, Au, Al, Pd, In, Sn, Pb, Co and Mn in g/kg was 390.38, 11.51, 1.95, 0.19, 0.80, 1.33, 0.15, 0.12, 28.92, 16.16, 0.14 and 0.58, respectively. From the data obtained, Cu was found as the major component and the total metal content per kilogram in the PCB was 452.23 g, which means that PCB of mobile phones could be a good source of metals comparing with composition of metals found in the ore chalcopyrite and corresponding with composition of other ores reported (Cancho et al. 2007; Third et al. 2000; Zhao et al. 2013).

7.3.2. Growth of *Acidithiobacillus ferrooxidans* in the bioreactor

Acidithiobacillus ferrooxidans was cultivated in a bioreactor system in order to proliferate their growth. For that, 200 mL of fresh culture was added to the bioreactor,

which was filled with 6K mineral medium until 2 L. The pH was maintained at 1.8 by the dropwise addition of 3 N H_2SO_4. The solution was stirred at 200 rpm and aerated with compressed air at 0.2 L/min. During the growth process, the cell number was measured by fluorometric measurements while the oxygen consumption was determined by microrespirometries. For the conversion from the fluorescence signal intensity (RFU) to cell density in cell/mL, the correlation developed by Giebner et al. (2015) was used.

It could be seen in Figure 7.4 that the cell density remained almost constant during the whole experiment. Even so, the concentration on the first day was $8.4 \cdot 10^5$ cell/mL and after 48 hours it increased to $1.7 \cdot 10^6$ cell/mL. After this time, the cell density slightly increased until reaching $7.0 \cdot 10^6$ cell/mL in 11 days. Although the increase on cell concentration was significantly high, the oxygen consumption in this period was more noticeable. Regarding the oxygen consumed on the first day by the microorganisms, iron (II) oxidation from the pulse substrate was very low (0.13 mg/Lh), probably corresponding to the endogenous consumption. Nevertheless, on day 4, the consumption raised to 1.81 mg/Lh of oxygen, which indicated that the cells increased their activity, reaching the maximum consumption on day 10 (5.94 mg/Lh).

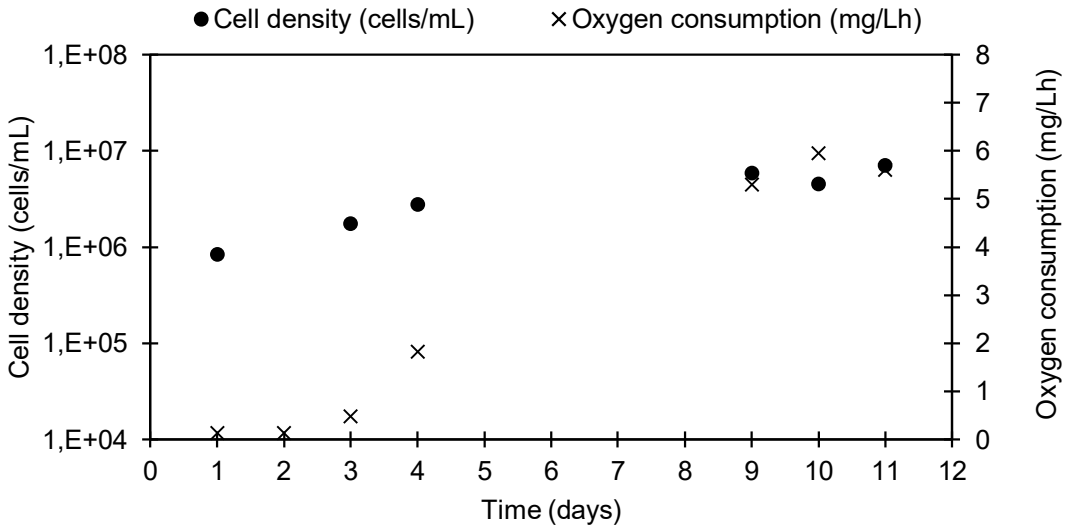

Figure 7.4. Cell density and oxygen consumption during the growth of *Acidithiobacillus ferrooxidans* in the bioreactor.

A correlation between the cell concentration and the corresponding oxygen consumption was stablished (Figure 7.5). The best regression was obtained through a lineal trend line with a gradient of $1 \cdot 10^6$ respect the oxygen consumption (in mg/Lh). The variation coefficient (R^2) of the potential correlation obtained was 0.9807.

$$Cell\ densisty\ [cells/mL] = 1 \cdot 10^6 \cdot Oxygen\ consumption\ [mg/Lh]\ + 9.55 \cdot 10^5$$

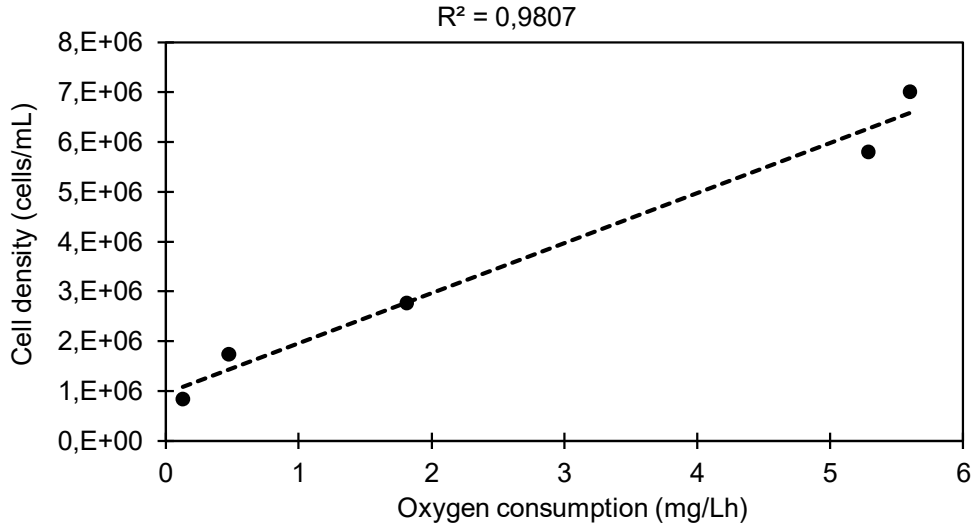

Figure 7.5. Correlation between the cell number of *Acidithiobacillus ferrooxidans* and their oxygen consumption after each pulse of substrate of iron (II).

7.3.3. Bio-recovery of metals with the two-bioreactor system

The bioleaching of copper from printed circuit boards was performed in a system consisting in two bioreactors, as mentioned in section 7.2.4. In Figure 7.6 the total iron concentration and the copper recovery along time are presented.

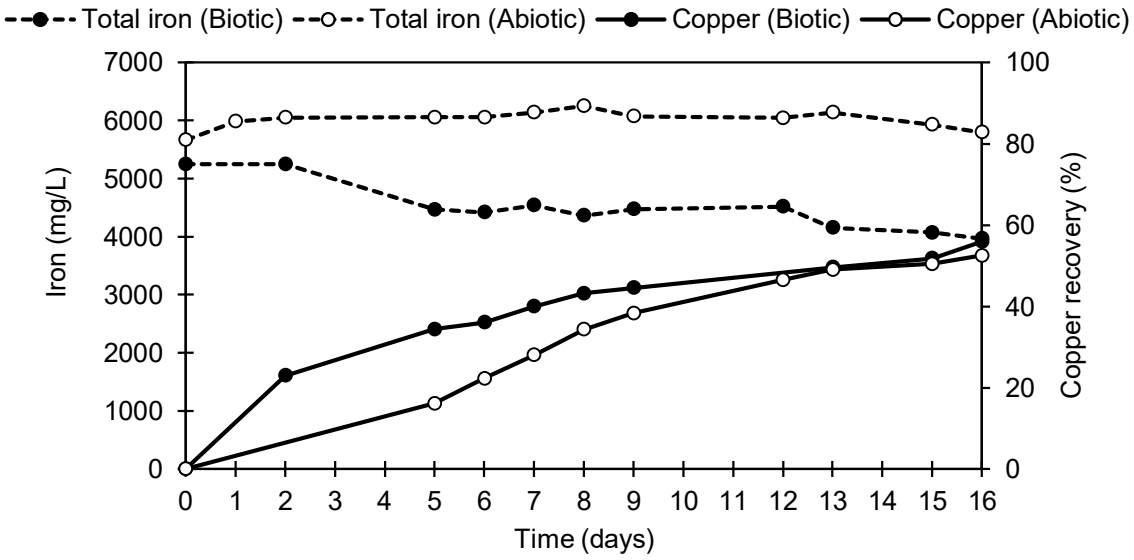

Figure 7.6. Evolution of total iron concentration and copper recovery during the bioleaching performed in the bioreactor.

As can be observed, in the lab-scale batch reactor 56% of copper from the e-waste was successfully oxidized in the biological experiment. Nevertheless, 52% of copper was also obtained in the abiotic experiment. Despite obtaining similar recoveries in 16 days of operation, it was noticed that the behavior was slightly different, since the biotic sample began the recovery earlier than the abiotic one. In particular, in the first 5 days the velocity of copper extraction in the biotic assay doubled the one of the abiotic experiment, but in the following 3 days just the opposite happened, achieving similar recoveries in 16 days. Therefore, the results indicate that extending the duration of the experiment does not improve the biological metal recovery. Regarding total iron concentration, the differences between biotic and abiotic experiments were more pronounced. While the iron concentration remained constant in the chemical assay, the metal concentration decreased almost 1000 mg/L in the biological one. It means that iron precipitated when biomass was used to oxidize the iron (II), although the pH was maintained in the bioreactor at 1.75. This was associated to fact that pH was not adjusted in the leaching tank. Though the leaching tank communicated to the other tank, the pH increased slightly (see Figure 7.7b). However, the increase was enough to precipitate the iron by the formation of iron oxyhydroxides (Baniasadi et al. 2019).

These differences in pH values are presented in Figure 7.7 for both the abiotic and the biotic experiment. As mentioned before, during the biotic experiment the pH was maintained at 1.75 in the bio-oxidation reactor whereas the pH in the leaching reactor was not controlled. Nevertheless, in the biotic experiment both reactors were communicated and the liquid was moved continuously from one tank to the other. In contrast, in the abiotic experiment the leaching reactor was pH-controlled, since the bio-oxidation reactor was not used in this case.

Figure 7.7a shows the pH evolution in the leaching tank as well as the cumulative acid volume added to maintain the pH during the abiotic experiment. As expected, in this case the pH was always controlled at pH 1.75, so no fluctuations on pH could be seen in this tank after the adjustment. Nevertheless, a total amount of 17.5 mL of 3 N H_2SO_4 for the 2 L reactor was required to maintain the solution at pH 1.75.

As observed in Figure 7.7b, the pH in the bio-oxidation reactor before the acid addition increased up to pH 2, especially in the first 7 days in which the acid addition was necessary to maintain the pH at 1.75. After this time, no more acid addition was needed, since the pH did practically not increase. Regarding the leaching tank, though the pH was not adjusted, the fluctuations in pH values was not as large as expected. During the whole experiment the pH variation in this reactor was between 1.82 and 2.02, although the pH was very close to pH 2 most days. For this reason, this fact was associated to

the iron precipitation observed in Figure 7.6, since, as mentioned before, at this pH iron (III) (jarosite or schwertmannite) could begin to precipitate (Baniasadi et al. 2019; Valix 2017).

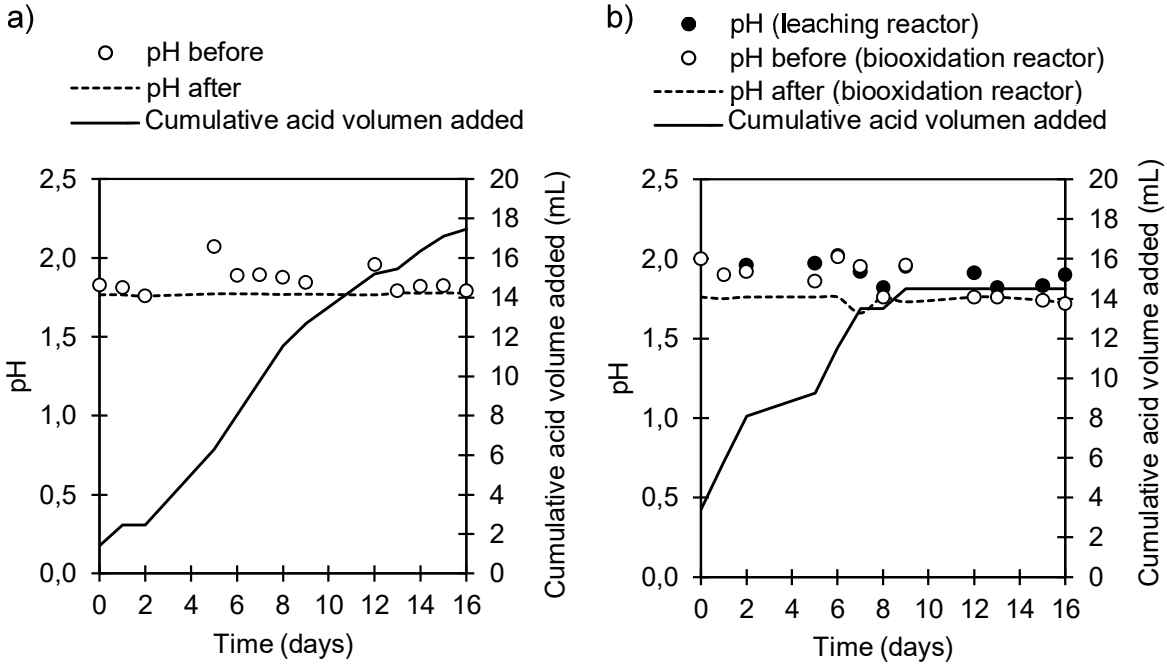

Figure 7.7. Evolution of pH along time and cumulative acid addition in the (a) chemical leaching and (b) biological leaching of PCB carried out in reactor. In the legend, "pH before" indicates the pH values before the pH adjustment whereas "pH after" indicates the pH values after the adjustment, which is after the acid addition. The pH adjustment was performed with 3 N H_2SO_4.

It is noteworthy that in the abiotic experiment a higher addition of acid was necessary to maintain the pH in comparis on to the biotic one. In particular, 14.5 mL of 3 N H_2SO_4 was required in the biotic assay, whereas 17.5 mL were needed for the abiotic one, which correspond to approximately 21.8 and 26.2 mmol of H_2SO_4, respectively. Moreover, the acid addition in the abiotic assay was needed during the whole experiment, whereas in the biotic it was needed one only during the first days. It means that the biological activity brings about that the pH of the solution does not drastically increase so the consumption of acid required to maintain pH 1.75 in the leaching solution was reduced. This is related to the biological activity of *Acidithiobacillus ferrooxidans*. Though the bio-oxidation of iron (II) consumes protons of the medium which alkalinizes the solution, the population of bacteria also increases with the oxidation of iron (II) to iron (III), thus accumulating iron (III). When this fact occurs, hydrolysis reactions of iron (III) could also occur which tend to decrease the pH, for example by formation of ammoniojarosite (Eqs. 7.1) (Wang et al. 2009).

$$Fe(OH)_3 + \frac{4}{3}SO_4^{2-} + Fe^{3+} + H_2O + \frac{2}{3}NH^{4+} \leftrightarrow \frac{2}{3}(NH)_4Fe_3(SO_4)_2(OH)_6 + H^+ \quad (7.1)$$

During the experiment the ORP was also measured, which results are shown in Figure 7.8. Results from the abiotic test indicated that iron (II) was not completely oxidized during the experiment since at ORP between 350 and 400 mV the percentage of iron (II) oxidized is less than 10% (Ballor, Nesbitt, and Luecking 2006). According to these authors, iron (II) was completely oxidized at ORP above 500 mV. Therefore, iron was completely oxidized in the bio-oxidation reactor during the biotic experiment as well as in the leaching reactor after 2 days of experimentation, assuming that the oxidation of iron in the leaching tank was produced during the biotic assay due to the continuous transfer of the liquid between reactors. For this reason, the ORP values in the two reactors used during the biological assay were quite similar since they were not isolated from each other. Nevertheless, these results indicated that in the biological leaching the iron (III) was the responsible of the copper solubilisation but not in the chemical leaching, although similar metal recovery was obtained after 16 days of experimentation (see Figure 7.6). Even so, it is noteworthy that the initial velocity of copper dissolution was higher in the biotic experiment, despite probably not maintaining the optimal conditions for the microorganisms' growth. As explained in the previous chapter, the leaching of copper in abiotic experiment was produced by the dissolved oxygen present in the liquid at very low pH since the reactor was aerated with 0.2 mL/min of oxygen to maintain the same conditions than the biotic experiment and the pH was controlled at 1.75. This behaviour was described by Torres and Lapidus (2015) and Bas et al. (2013). These authors affirmed that the oxygen produces the oxidation of metallic copper at acidic conditions (Eq. 7.3). In addition, Bas et al. (2013) demonstrates that this proton attack to copper occur after 90 hours and it represents 18 % of total copper extraction in their assays.

$$2\,Cu^0 + 4\,H^+ + O_2 \rightarrow 2\,Cu^{2+} + 2\,H_2O \quad (7.3)$$

In the case of the biotic experiment performed in the two-bioreactors system, the recovery of other metals a part of copper was also evaluated (Table 7.1). Despite other specific strains are required for the extraction of different metals than copper (e.g. cyanogenic bacteria for gold recovery), the possibility of other metals mobilization by *Acidithiobacillus ferrooxidans* was assessed (Awasthi and Li 2017; Baniasadi et al. 2019). In this sense, 40 different metals were analysed despite only 11 of them were found in the leaching solution after 16 days of experimentation. Results on additional metals releasing are shown in Table 7.1.

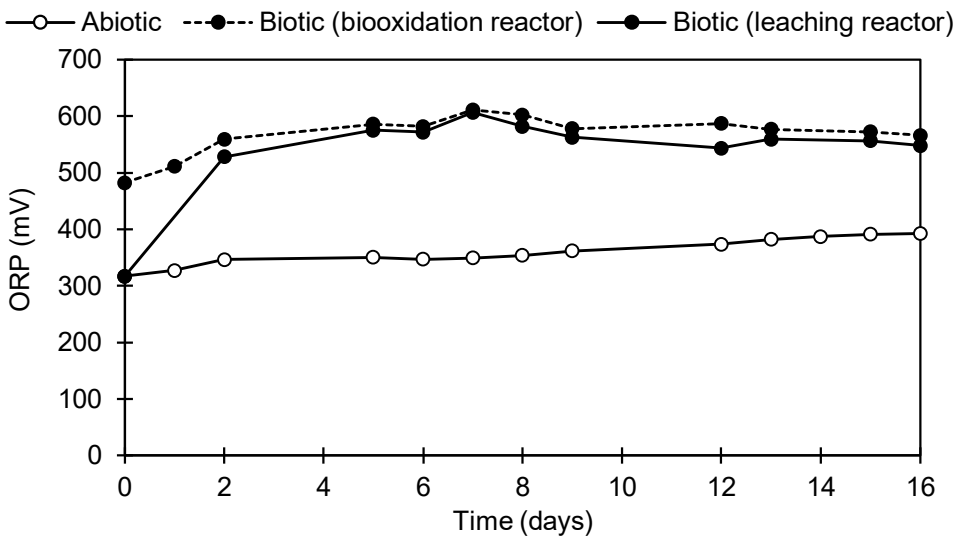

Figure 7.8. Oxidation-reduction potential along time in chemical and biological leaching performed in reactors.

Table 7.1. Metals bioleached after 16 days in the two-bioreactors system.

Metal	Concentration (in ppb)
Ag	1840
Al	1550
Au	129
Co	546
Dy	3.6
In	173
Mn	2170
Ni	27400
Os	15.8
Pd	43.2
Sn	166000

Although the concentrations obtained were in general not so high, in some cases, the presence of these metals allow to affirm that other metals a part of copper could be recovered by *Acidithiobacillus ferrooxidans* at the conditions tested. In particular, tin concentration was the highest one (166000 ppb) followed by nickel (27400 ppb). On the contrary, trace amount of dysprosium (3.6 ppb), osmium (15.8 ppb) and palladium (43.2 ppb) were observed. It is noteworthy that 129 ppb of gold were recovered although this metal was usually retrieved by the activity of cyanogenic bacteria (Isildar et al. 2016;

Yuan et al. 2018). The extraction of these metals are related to their redox potential, since they could be oxidized by the iron in accordance to their standard redox potential reported (Eq. 7.4 – 7.15) (Vanýsek 2005).

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (E^o = +0.77\ V) \quad (7.4)$$

$$Cu^{2+} + 2\,e^- \rightarrow Cu^0 \quad (E^o = +0.43\ V) \quad (7.5)$$

$$Ag^+ + e^- \rightarrow Ag^0 \quad (E^o = +0.80\ V) \quad (7.6)$$

$$Al^{3+} + 3\,e^- \rightarrow Ag^0 \quad (E^o = -1.67\ V) \quad (7.7)$$

$$In^{2+} + 2\,e^- \rightarrow In^0 \quad (E^o = -0.34\ V) \quad (7.8)$$

$$Mn^{2+} + 2\,e^- \rightarrow Mn^0 \quad (E^o = -1.03\ V) \quad (7.9)$$

$$Ni^{2+} + 2\,e^- \rightarrow Ni^0 \quad (E^o = -0.24\ V) \quad (7.10)$$

$$Sn^{2+} + 2\,e^- \rightarrow Sn^0 \quad (E^o = -0.14\ V) \quad (7.11)$$

$$Au^{3+} + 3\,e^- \rightarrow Au^0 \quad (E^o = +1.69\ V) \quad (7.12)$$

$$Co^{2+} + 2\,e^- \rightarrow Co^0 \quad (E^o = -0.28\ V) \quad (7.13)$$

$$Pd^{2+} + 2\,e^- \rightarrow Pd^0 \quad (E^o = +0.92\ V) \quad (7.14)$$

$$Dy^{2+} + 2\,e^- \rightarrow Dy^0 \quad (E^o = -2.20\ V) \quad (7.15)$$

Nevertheless, there is an exception in the case of the precious metals analysed (gold, silver and palladium). Since the redox potential of the precious metals is higher than the redox potential of the iron, the extraction of these metals can not be produced by the action of iron. This means that their solubilisation had to be produced by some other metals or compounds from the leaching solution (Kaksonen et al. 2014).

Additionally, a mass balance was carried out for the experiment, by measuring the weight of the PCB used in the experiment before and after the bioleaching. In particular, the loss of weigh of the PCB used in the experiment was 3.62 grams after 16 days, since 15.34 grams of e-waste was initially added, while the weight of the PCB after 16 days was 11.72 grams. Hence, 23.6% of the total weigh was reduced due to the metal extraction from which 21.3% is due to copper extraction whereas the remaining 2.3% is due to the set of the other extracted metals. This fact was corroborated by the total sum of the metals recovered, taking into account the concentration of the extracted metals and the experimental volume used. In this sense, according to the experimental measurements, 3.56 g of metals were extracted, which was quite similar to the mass of

PCB lost in the process (3.62 g). To that, the mass balance was verified since the experimental loss of weight of the PCB corresponds to the total mass of metals extracted. In addition, the difference in appearance of the waste before and after the process were also visible (see Figure 7.9).

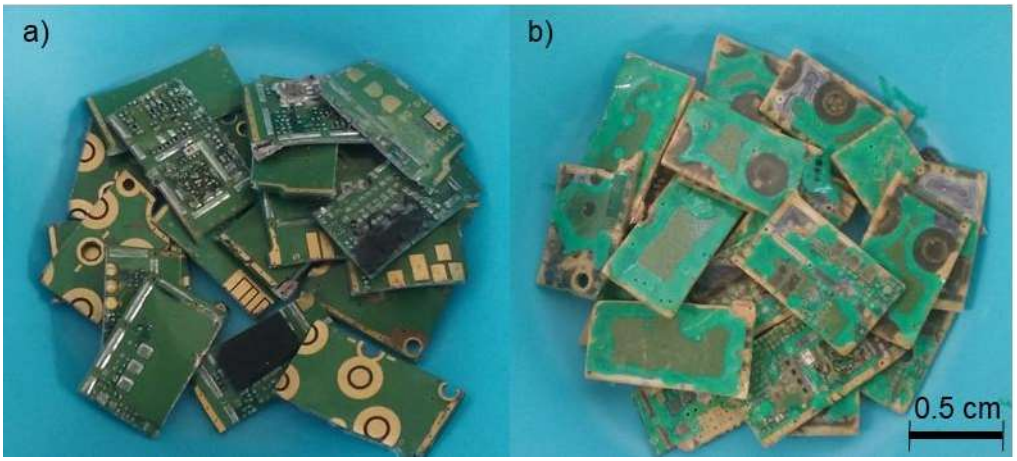

Figure 7.9. PCBs used for the bioleaching performed in bioreactor (a) before and (b) after the process.

7.3.4. Determination of microbial respiration rates with the optode system during the bioleaching process

Microrespirometric tests were performed periodically during the bioleaching experiments to evaluate the activity of the *Acidithiobacillus ferrooxidans* (Figure 7.10).

Figure 7.10 shows different respirometric tests performed after and during the bioreactor inoculation. As can be seen no oxygen was consumed in the abiotic test during the respirometric test. This fact corroborated that the oxygen consumed in biological samples was only produced by the action of the microorganisms, which consume oxygen to oxidize the iron (II) from the added pulse. Hence, chemical oxidation of the iron was avoided at these conditions, as Kim et al. (2008) also observed due to velocity of this process (much more lower than time of analysis). On day 0 the biomass was active, consuming 3.78 mg/Lh of oxygen. After 5 days, they increased their activity since an increased on the slope was observed (6.54 mg/Lh). It is noticed that two days later, the activity decreased and it decreased even more on day 13. Their inactivity could be related to the toxicity effect of bioleached metals, since at this time the amount of extracted metals could have reached inhibitory concentrations. In this regard, it has been reported that some metals such as nickel or silver, among others, could inactivate the cells, depending on the metals concentration (Cho et al. 2008; David et al. 2008). In this

sense, a complete study related to the toxic effect of leached metals (including copper) on the biological activity is needed (Chapter 8).

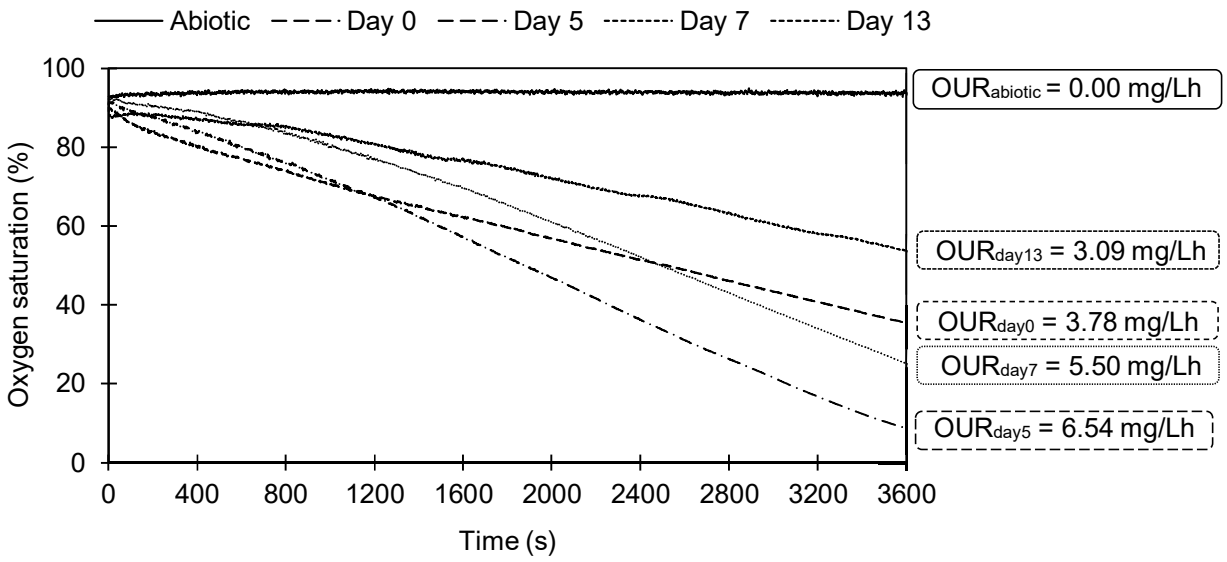

Figure 7.10. Oxygen consumption along time at different days during bioleaching of PCBs in the bioreactor, indicating the oxygen uptake rate (OUR) in each case.

After observing the oxygen consumption for some experimental days, the oxygen uptake rate for every sample taken during the bioleaching are presented in Figure 7.11 to observe the tendencies along the experiment. In addition, the cell density measured for each sample is also shown.

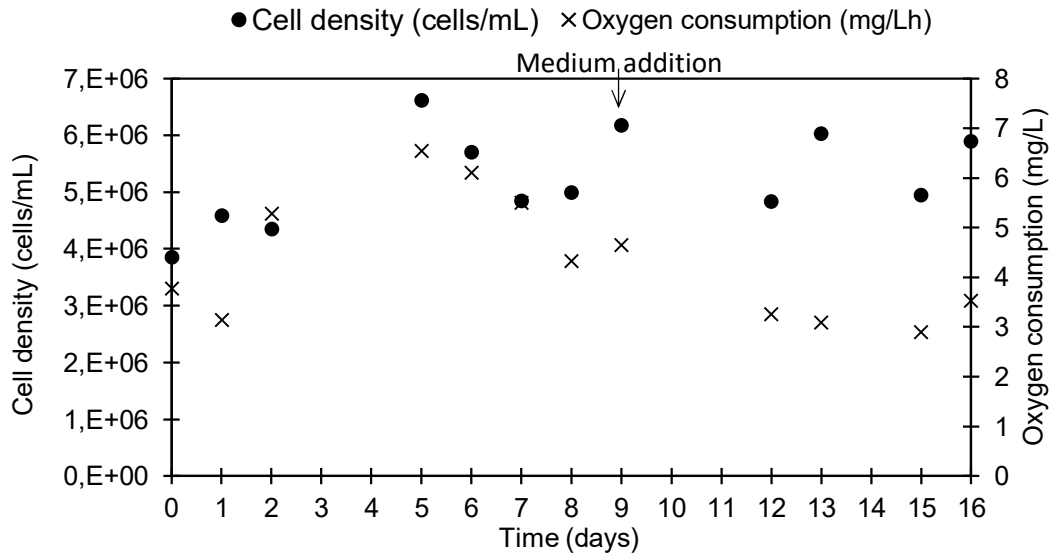

Figure 7.11. Evolution of cell density and oxygen consumption in bioleaching performed in two-bioreactors system.

According to Figure 7.11 cell density remained constant between $3.8 \cdot 10^6$ and $6.6 \cdot 10^6$ cell/mL during the experiment. This means that the specific growth rate was 0.0022 h^{-1} under these conditions, thus indicating that their growth was slow in comparison to reported values between 0.1 to 0.2 h^{-1} (Barron and Luecking 1990). The difference is associated to the conditions in which the microorganisms are found, since the reported values are measured during the growth of the biomass under optimal conditions, whereas the present value is measured during the bioleaching experiment. Hence, the bioleached metals as well as many other components that could be found in the solution affect the biomass activity and thus, its growth rate. This fact reinforced the need of a complete study related to the toxic effect of leached metals on the biological activity (Chapter 8).

Nevertheless, important changes were observed in the oxygen consumption evolution. In particular, it increased during the first 5 days, as Figure 7.11 show, indicating that the microorganisms were gaining activity although the number of cells did not reflect this increasing. After this period, the cell activity significantly decreased since the oxygen consumption dropped from 6.5 mg/Lh to 4.3 mg/Lh. At this time, after observing the depletion on cell's activity, fresh medium was added to renew part of the medium from the bioreactor. Hence, 200 mL of the bioreactor was removed and the same volume of fresh 6K medium was added instead. Although the activity slightly decreased, it remained almost constant in the following days. It was thought that the renovation of the medium helped to maintain the activity of the microorganisms, bringing them fresh nutrients. Nevertheless, it is noticed that despite adding fresh medium, the cell density did not vary. Therefore, although the oxygen consumption began to decrease after 9 days, the cell density remained almost constant. This behaviour is associated with the difficulty of accessing copper, which implies a lack of iron (II) production, which in turn, it would imply a lack of the main nutrient that provides energy to microorganisms to grow.

7.4. Conclusions

During the growth of *Acidithiobacillus ferrooxidans* performed in the bioreactor, an increase of their activity was observed by microrespirometry. Although the cell density increased slightly in comparison to the oxygen consumption, a correlation between these two parameters was possible by a linear regression (R^2= 0.9807).

Regarding the bioleaching process, the study concluded that copper was recovered in the process, but similar extractions were obtained during the biotic (56%) and abiotic (52%) experiments after 16 days of operation. However, in the biological

assay the extraction started earlier. In relation to iron concentration, this was maintained constant in the abiotic test, whereas it decreased in the biotic one. This fact was related to the pH behaviour, since in the biotic experiment the pH was only adjusted in the bio-oxidation tank, so that the pH in the leaching tank raised up to pH 2, which resulted in iron precipitation. However, the precipitation was not so noticeable, because both tanks were connected and the liquid was moved from one tank to the other, avoiding high increases of pH in the system. In the abiotic experiment, the iron did not precipitate due to the constant pH adjustment in the tank. It was noticed that more acid was needed to maintain pH 1.75 in the abiotic one, consuming 17.5 mL of 3 N H_2SO_4. In contrast, the biotic experiment consumed 14.5 mL of the same acid. The ORP revealed that the parameter remained constant over 500 mV, when biomass was used in the process, whereas it was maintained under 395 mV, when there was no biomass in the assay. This value increases when more oxidised species are in the solution. Hence, when biomass was used in the process, the ORP signal increased, since the iron (II) was biologically oxidised to iron (III) which increased the total concentration of oxidized species in the liquid.

From the experiment, it was also concluded that some other metals could be recovered from the PCB by bioleaching using *Acidithiobacillus ferrooxidans*. In particular, the metals recovered were (from the highest to the lowest concentration): tin, nickel, manganese, silver, aluminium, cobalt, indium, gold, palladium, and osmium, apart from copper. As a consequence of these recoveries, a loss of 23.6% of PCB initial weight was observed, which corresponds to the total mass of metals extracted.

During bioleaching in the two-bioreactor system, a microrespirometric technique allowed to observe that the activity increased for the first days, but it decreased after the fifth day. Avoiding the decrease was possible by replacing 200 mL of the medium with fresh one. Nevertheless, cell enumeration along the experiment revealed that the cell density remained almost constant in this period of time, despite the changes observed in oxygen consumption. Since the cell density was measured by means of fluorometric measurements, which does not distinguish between living and dead cells, the results do not only indicate a constant cell density. because of a compensation between living and dead cells, but an increase in the activity of the living cells. Finally, the results from this chapter allow to conclude that microrespirometry provides an efficient methodology to monitor biomass activity when biotechnological process take place avoiding limitations of traditional methods, where the presence of precipitates may affect the measurement.

Chapter 8

Resistance assessment of *Acidithiobacillus ferrooxidans* to heavy metals by means of toxicity assays through microrespirometric measurements

This chapter is focused on the possible negative effect on the activity of Acidithiobacillus ferrooxidans resulting from the complex composition of the e-waste. The presence of copper and of additionally leached heavy metals that could limit the biological process could affect the activity. Moreover, iron, which is the main energy source of these bacteria, could become inhibitory at high concentration. For this reason, the effect of concentration on the microorganisms' activity was evaluated to analyse the inhibition by the substrate iron(II) ($FeSO_4$). Finally, the evolution of the biomass activity along time when there is a lack of feeding was evaluated in order to check the robustness of the system. This allows to have a better control when the process takes place in continuous mode and there is an unexpected or maintenance shutdown.

Abstract

The e-waste has a complex composition, including potentially toxic metals, which could be retrieved during the bioleaching, as a consequence of the process mechanism, and which could accumulate in the solution. This fact could affect the biological activity of the microorganisms, which are among the key parameters in the process. For this reason, the toxic effect on the activity of *Acidithiobacillus ferrooxidans* of the three metals obtained in greater quantity during the previous bioleaching experiments (copper, nickel, and aluminium) was studied. Besides, the inhibition by iron substrate was also evaluated to know the maximum iron concentration that the microorganisms are able to tolerate. In addition, the evolution of microorganisms' activity without the addition of the mineral medium salts including iron was investigated in order to determine the limit time until the complete cell inactivation. In all cases, the activity was evaluated by microrespirometric measurements of the oxygen consumption after a substrate addition. Regarding toxicity tests, depending on the concentration and the time exposed, nickel, copper and aluminium affected the activity of the microorganisms. In particular, it was demonstrated that some metal concentrations were not completely innocuous for microorganisms when the contact with the solution was prolonged for several hours. It was found that, in general, aluminium was the most toxic metal studied, followed by copper, whereas nickel was the least toxic. With respect to iron inhibition, although low concentrations of iron helped to proliferate the microorganisms' growth, this substrate produced a total inactivation of the cells at a concentration over 0.75 M after 24 hours. Finally, some inactivation of the cells was also observed after 550 hours without iron addition, losing

82% of their initial activity at this time. This fact implies that the microorganisms are very resistant to long-time feeding shutdowns, which demonstrates the robustness of the system.

A modified version of part of this chapter has been published as:

Benzal, E., Cano, A., Solé, M., Lao, C., Gamisans, X., Dorado, A.D., 2020. Copper recovery from PCBs by *Acidithiobacillus ferrooxidans*: Toxicity of bioleached metals on biological activity. Waste and Biomass Valorization, 11, 5483-5492.

8.1. Introduction

In a common bioleaching operation, not only the metal of interest is extracted since many other metals could be also leached during the process, including potentially toxic metals. Moreover, their presence in the solution could affect the biological activity. In the case of *Acidithiobacillus ferrooxidans*, one of the most important microorganisms in bioleaching applications (Valdés et al. 2008), some studies have focused on their tolerances to different metals, evaluating its effect on the iron oxidation rate by measuring iron concentrations along time (Leduc et al. 1997). For this purpose, o-phenantroline and ferrozine are the commonly applied colorimetric methods (Braunschweig et al. 2012). However, the precipitates formed during the oxidation process could affect colorimetric measurements. According to Adhapure et al. (2014), in bioleaching processes the precipitate formation is common. This precipitation not only could affect the measurements of iron by colorimetric methodologies but it also affects the bioleaching recoveries due to the passivation of the waste (Zhu et al. 2011).

As it was suggested by Giebner et al. (2015), another obvious approach for iron measurements was to quantify the consumption of the electron acceptor (oxygen) since the microorganisms used in the present study, *Acidithiobacillus ferrooxidans*, consumed this molecule in their metabolism to grew and to obtain energy (Eq. 8.1).

$$4\,Fe^{2+} + 4\,H^{+} + O_2 \xrightarrow{Biological\ activity} 4\,Fe^{3+} + 2\,H_2O \qquad (8.1)$$

Hence, to know when the microorganisms are active is possible by measuring their oxygen consumption after the addition of a pulse of substrate. In this regard, an optode-based technique can be used to measure oxygen consumption of *Acidithiobacillus ferrooxidans* to determine their metabolic activity. The optode-based technique is characterized by the fact to use small sample volumes (1 or 2 mL) without losing sensitivity in the oxygen measurements, which avoid great impacts on the system due to sampling along time. In addition, this technology is also characterized to be a direct measurement, allowing to determine if there is oxygen consumption, and, therefore, biological activity in a sample at a real time. Hence, changes could be made before major problems could appear in the system due to the lacking activity.

As mentioned in the previous chapters, the e-waste has a complex metal composition in which it could be found more than 60 different elements (Hagelüken and Corti 2010). However, among these elements, some of them are valuable, some toxics or hazardous and some present both characteristics. Hence, some of these metals that are considered as valuable in the e-waste like silver are also used as biocides

(Khaydarov et al. 2014). Thus, although the valuable metals are interesting elements to recover from the e-waste, the use of biological techniques to recover them could be affected by its toxicity, hindering the biotechnological processes with these purposes. For this, the application of the optode system could result an effective technology to study the possible toxic effect of metals in biological solutions since it allows evaluating both the toxic concentration and the time exposure effect to them.

Iron is one of the main nutrients needed by *Acidithiobacillus ferrooxidans*, since these bacteria is one of the few microorganisms known which obtain their energy for bacterial growth by the oxidation of iron (II) to iron (III) (Zhan et al. 2019). It means that iron results essential for their metabolic processes, involving different enzymes and electron transfer proteins for this purpose (Valdés et al. 2008). In particular, the bacteria couples the energy derived from the oxidation to the production of the reducing agent NADPH and the synthesis of ATP for cellular materials construction (Nemati et al. 1998). Nevertheless, the higher the iron concentration, the more negative effect could have for their metabolism as it has been described that the inhibition of the microorganisms by the substrate could be occurred (Barron and Luecking 1990). Moreover, Valdés et al. (2008) affirmed that the abundance of soluble iron has the potential to pose sever oxidative stress to the *Acidithiobacillus ferrooxidans*, causing DNA and protein damage to them. So that, despite toxic metals could inactivate the microorganisms, their metabolism can be also affected and/or inhibited by substrate itself (Reed et al. 2010). According to these authors, many enzymes are inhibited by their own substrates, reaching a maximum value in their velocity curves and descending as the substrate concentration increases. Hence, when substrate inhibition is produced, there is a progressive decrease in activity at high substrate concentrations. This fact was corroborated by Barron and Luecking (1990), who reported that the substrate inhibition of *Acidithiobacillus ferrooxidans* was produced at iron (II) concentration over 3.0 g/L, despite the culture media generally used in bioleaching experiments is the 9K medium (which contains 9 g/L of iron (II)) (Pagnanelli et al. 2007). Nevertheless, Barron and Luecking (1990) also affirmed that 20.0 g/L of iron (II) caused the maximal inhibition at 30 ºC and pH 1.9. On the contrary, Okereket and Stevens (1991) demonstrated that the iron oxidation rate was almost constant from 2.0 to 6.3 g/L of iron (II) at 25 ºC and pH 2.4. Kelly and Jones (1978) reported that the substrate inhibition was produced at higher concentrations, above 39.0 g/L of iron (II), but in this case, they carried out the experiments at pH 1.6 and 30 ºC. Nemati et al. (1998) attribute these differences to accuracy of the ferrous iron measurements and the different operating conditions such as pH and temperature, among others. In the same way, Barron and Luecking (1990)

also affirmed that the results are largely dependent of the conditions in which the experiments take place. Nevertheless, as it has been abovementioned, Pagnanelli et al. (2007) reported that the culture media generally used for research studies exceed the amount of ferrous ion concentrations previously cited, as 9K medium, firstly described by Silverman and Lundgren (1959). So that, there is no general agreement on how the iron (II) concentration affects the microorganisms. Thus, the results focused on the substrate inhibition are confusing and a specific range has not been defined yet. Moreover, it is noteworthy that the authors focused on the study of substrate inhibition usually measuring just the iron concentration without taking into account the time exposure to it, although the bioleaching experiments are characterized to produce long exposures of metals with biomass.

The aim of the present chapter was focused on evaluating the potential toxicity of some metals and how they can affect the activity of *Acidithiobacillus ferrooxidans* by means of direct microrespirometric measurements as an improvement of previous methodologies overcoming their limitations. Thanks to this novel technology, the toxicity assays evaluated both the time exposed and the metal concentration of copper, nickel and aluminum, three of the main elements retrieved in the previous chapter during the e-waste bioleaching in bioreactor. Moreover, in this chapter the potential inhibition by iron substrate as well as the effect of long starvation over time on the microorganisms' activity was also evaluated. The latter effect was studied by means of cell enumeration, oxygen consumption and iron oxidation rate along time.

8.2. Materials and methods

8.2.1. Microorganisms and mineral medium

The same bacterial strain used in Chapters 6 and 7 was employed (*Acidithiobacillus ferroxidans*, ATCC 23270). Hence, the same 6K mineral medium was prepared, which contained (in g/L): $(NH_4)_2SO_4$ 3.00; K_2HPO_4 0.50; $MgSO_4 \cdot 7\ H_2O$ 0.50; KCl 0.10; $Ca(NO_3)_2 \cdot 4\ H_2O$ 0.014 grams. These salts were dissolved in 900 mL of distillate water and the pH was adjusted with 3 N H_2SO_4 to pH 1.75. Then, 30 grams of $FeSO_4 \cdot 7\ H_2O$ were dissolved in 100 mL of distillate water, also adjusting the pH with 3 N H_2SO_4 to pH 1.75. After that, both solutions were mixed and the pH was readjusted to pH 1.75 if it was necessary.

8.2.2. Toxicity tests development

Toxicity tests were evaluated with three different metals: copper, nickel and aluminium at different concentrations. The metals to evaluate were added as $NiSO_4 \cdot 7 H_2O$, $CuSO_4 \cdot 5 H_2O$ and $Al_2(SO_4)_3 \cdot 18 H_2O$ for nickel, copper and aluminium, respectively. For each metal, six different concentrations between 0.0005 and 1.5 M were also evaluated, which molarity depends on the metal studied. Nevertheless, the selection of the concentrations studied were based on the literature, in which it was found that the natural and the limit tolerances are defined for some metals for the *Acidithiobacillus ferrooxidans* strain (Magnin et al. 1998). For this, the concentrations of these tolerances were selected as well as the concentration found in previous leaching solutions and two more concentrations in order to evaluate the effect of different concentrations along time. Additionally, a concentration over the tolerance limit was also tested for each metal, which are 1.2, 1.5 and 0.5 M for copper, nickel and aluminium, respectively. Experiments were performed with two 500 mL baffled Erlenmeyer flasks for each metal and each concentration (36 flasks in total). One of the flasks was used as control without the metal addition whereas in the other the potential toxic metal concentration was added. The loss of activity was measured by respirometric assays with the optode system (described in 4.2.2 section). In particular, the relative loss of activity was calculated as a percentage between the sample with the toxic metal addition and its control (see Eq. 8.2). Samples for respirometric measurements were taken every two hours, whenever possible, during 48 hours.

$$\%\ Relative\ loss\ of\ activity = \frac{A_c - A_t}{A_c} \cdot 100 \qquad (8.2)$$

Where:

A_c = activity of the control sample (mg O_2/Lh)

A_t = activity of the sample with the toxic concentration (mg O_2/Lh)

8.2.3. Optical system for oxygen measurements

The optical system (*FireStingO2, PyroScience GmbH, Germany*) used for toxicity tests is explained in detail in section 4.2.2.

For respirometric measurements, two different methodologies were used depending on the presence or not of ferrous ion in the sample before the test. Hence, for the toxicity assays of heavy metals without ferrous ion in the solution, 2 mL of biological sample were taken and, after the addition of 2 mL of 6K medium, the mixture were vortexed. Then, the mixture was transferred to the cuvette of the optode system in which

the spot sensor had been previously incorporated (see Figure 8.1). On the contrary, the second methodology used (with ferrous ion in the solution), was carried out in the study of inhibition by iron substrate. In this case, the methodology was the same than the one used in Chapter 7. In this regard, 2 mL of the biological sample was centrifuged (5000 rpm, 10 min) and, after removing the supernatant, 2 mL of modified mineral medium, lacking ferrous ion (0K medium) was added in order to re-suspend the pellet. Then, 2 mL of 6K medium were added and the mixture, previously homogenate, were also transferred to the cuvette of the optode system. Once the 2 mL of 6K medium were added in both methodologies, the signal was recorded for 30 minutes. As in Chapter 7, to express the respiration activity, oxygen decrease rate was determined.

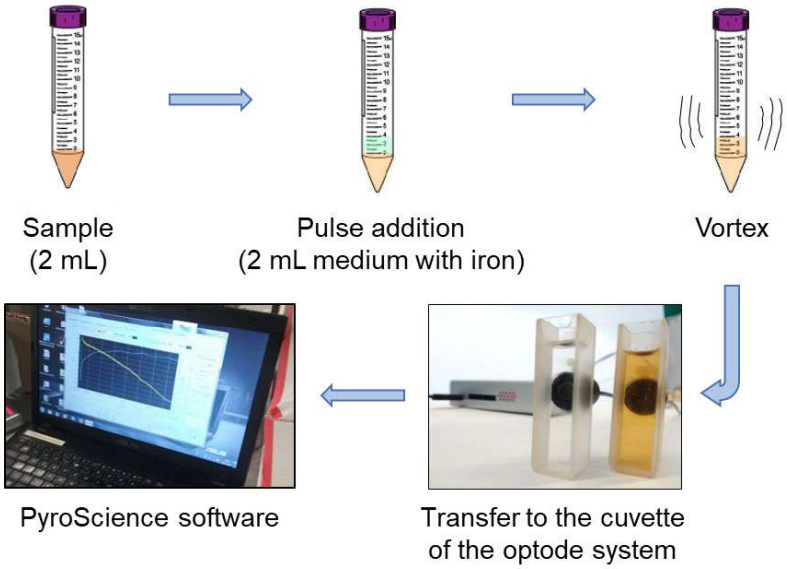

Figure 8.1. Diagram of the procedure to perform microrespirometric measurements in toxic assays in samples without iron.

8.2.4. Cell counting

Cell concentration was determined by the cell enumeration observed by an optical microscope (*BA310LED, Motic, Germany*) using a Neubauer Chamber cell counting. This chamber consists on a glass thick plate of 30 mm width and 70 mm long in which there were 2 counting areas, both divided into nine 1 mm x 1 mm grids each (Figure 8.2). The central grid was also divided into 25 grids of which the ends and the central one were used for cell enumeration. Hence, after placing the coverslip over the counting area, 10 µL of sample were dispensed on the chamber. The optimal concentration for the correct counting is 10^6 cell/mL, so dilutions were needed when the

concentration was higher. In order to obtain the concentration in cell/mL after the cell counting, the Eq.8.3 was applied.

$$Cell\ concentration\ (cells/mL) = \frac{\sum Cells}{D_G \cdot S_G \cdot D_F} \quad (8.3)$$

Where:

Cells = cells counting (cells)

D_G = depth of the grid used in the camera (cm), which was $1 \cdot 10^{-2}$ cm in this case

S_G = surface of the grid (cm^2), which was $2 \cdot 10^{-3}$ cm^2 in this case

D_F = Dilution factor, which means the dilution of the sample used

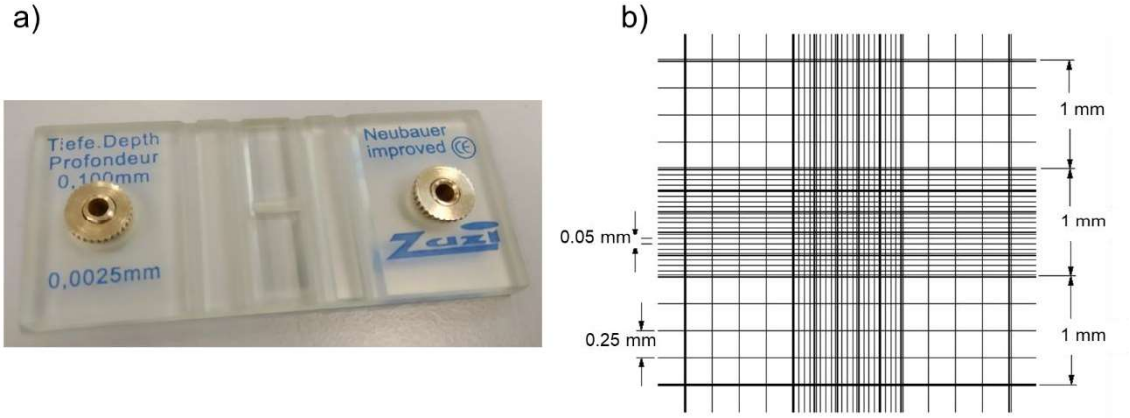

Figure 8.2. Neubauer Chamber cell counting: a) detail of the chamber used in the experiments and b) scheme of the grid observed through the microscope to perform the cell counting (adapted from Lara et al. (2016)).

8.3. Results and discussion

8.3.1. Toxicity assays of heavy metals for *Acidithiobacillus ferrooxidans* by microrespirometries

Some authors have focused on the resistance of *Acidithiobacillus ferrooxidans* to high heavy metal concentrations. For instance, Cho et al. (2008) studied the maximum tolerance of these microorganisms to Cu (II), Cd (II), Zn (II), Ni (II), which were 142, 440, 690 and 850 mM, respectively. In addition, David et al. (2008) found that the maximum tolerance of *Acidithiobacillus ferrooxidans* to Pb was 2.41 mM whereas 0.05 mM of Hg completely inhibited their activity. As it has been explained in the introduction, it has been reported that e-waste contain toxic materials that can affect biological activity in

bioleaching studies (Brandl, Bosshard, and Wegmann 2001). In the present work, the toxicity effect of three different metals (copper, nickel and aluminium) was studied at different concentrations each one, using the microrespirometric measurements, following the methodology described in section 8.2.3. The selection of the metals studied was based on the results obtained in the previous chapter, in which copper and nickel were two of the valuable bioleached metals in greater quantity. Moreover, aluminium was also selected since it has been also bioleached in the process but its toxicity has been scarce studied in the literature.

Copper was the first metal evaluated in this work, since this is the metal found at higher concentrations in bioleaching solutions (Bas et al. 2013; Chen et al. 2018b; Rodrigues et al. 2015; Wu et al. 2018; Yang et al. 2009). In addition, there are a lot of bioleaching studies that performs the process in one step which means that the biooxidation of iron(II) and the leaching of metals takes place in the same flask/reactor (Joshi et al. 2017; Mrážiková et al. 2016; Willner and Fornalczyk 2013). This fact leads to accumulate the solubilised copper in the liquid, which could affect the efficiency of the process and so limiting the recovery of the metal. In order to study the toxicity of copper six different concentrations of this metal were prepared (0.01, 0.05, 0.60, 0.80, 1.00 and 1.20 M). The results shown in Figure 8.3 were expressed as a percentage of relative loss of activity, which means the activity loss by the microorganism in the presence of the copper in relation to their activity without the copper addition.

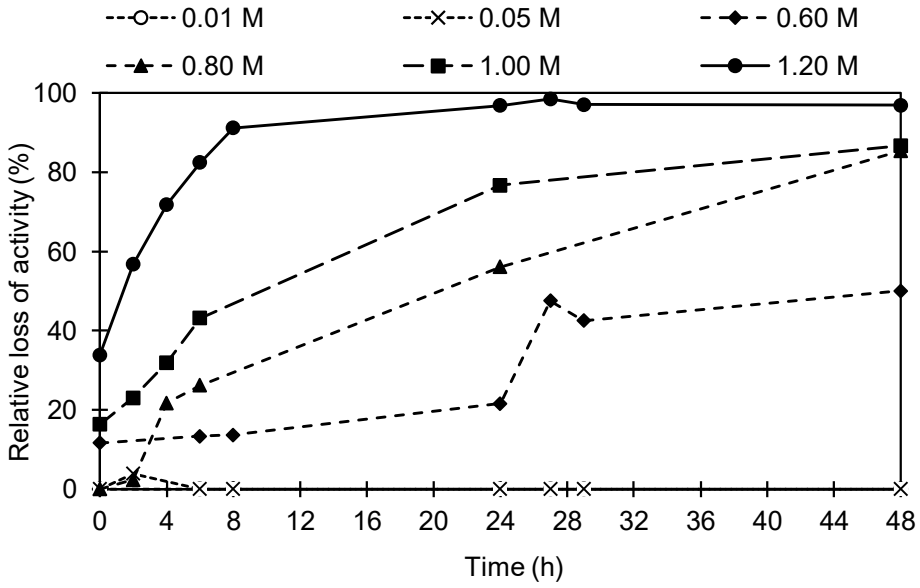

Figure 8.3. Toxicity evaluation of copper on activity of *Acidithiobacillus ferrooxidans* at six different concentrations. The relative loss of activity is calculated as: $((A_c - A_T) / A_C) \times 100$; where A_C means the activity of the control sample and A_T the activity of the sample with the toxic concentration.

In general, an increasing on the relative loss of activity was observed when copper concentration increases. It means that the higher the copper concentration, the more toxic for microorganisms. This behaviour is reflected through the 48 hours of the experiment. Moreover, it is noteworthy that the toxic effect was immediate at metal concentrations over 0.60 M whereas at concentrations below 0.05 M there was no effect on biological activity. Taking into account that the lowest concentration tested (0.01 M) was the copper concentration obtained in the previous bioleaching experiments, it was confirmed that this concentration had no negative effect on microbial metabolism after 48 hours. Nevertheless, it should be considered for those cases when the biological oxidation and the leaching steps are taking place in the same place, since in these cases the copper concentration will be continuously increased until reaching concentrations that affect negatively the microbial activity. For instance, in a bioleaching process in which 0.05 M of copper has been extracted, although this concentration was not toxic to the microorganisms, after 24 days the copper concentration reached could begin to be toxic to them, being able to be completely toxic after 48 days (considering steady-state copper extraction). However, the limiting time that a metal concentration becomes toxic will mostly depend on the metal extraction obtained in each case. Leduc et al. (1997) concluded that the inhibitory concentrations depend on the specific strain of *Acidithiobacillus ferrooxidans*. Despite of that, Cho et al. (2008), who based their study on the inhibition effect of copper on the rate of iron oxidation of *Acidithiobacillus ferrooxidans*, found that the maximum tolerance concentration for copper was 0.142 M due to concentrations over this one completely inhibited the biological iron oxidation during the 42 hours experimental hours. This result is in agreement with the results obtained herein, although the methodology used for the measurements of the loss of activity were completely different. Whereas Cho et al. (2008) evaluated the iron (II) concentrations along time to define the limits of tolerance, in this work the oxygen consumption was directly measured on the biological sample in order to observe the effect of excess iron to them.

Similar assays were performed to evaluate the potential toxic effect of nickel on the activity of the iron-oxidizing microorganisms. In this case, six different concentrations of the metal were also prepared (0.0005, 0.05, 0.10, 0.30, 1.00 and 1.50 M). Figure 8.4 showed that nickel was also toxic to microorganisms after 48 hours, especially at concentrations over 0.3 M in which the effect was immediate. Although in this case the experiment was extended until nearly 300 hours, the main changes and tendencies occurred during the first 48 hours, so this was the period showed to discus and to compare with the other toxicity assays in this section. In addition, it is noticed that the

relative loss of activity increased along time at concentrations over 0.3 M, which indicates that not only the metal concentration affects the biological activity since the time exposure also greatly affects the biological activity. Moreover, the results demonstrated that those concentrations of nickel described in the literature as natural tolerance, which is 0.30 M according to Magnin et al. (1998), were not completely innocuous for microorganism when the contact with the solution was prolonged for several hours. Although microorganism metabolism has not been disrupted after one cycle of leaching, operation strategies for reducing time on continuous mode, as the strategy developed in column experiments in Chapter 9, should take into account these evidences avoiding long contact times by previous separation operations. The toxicity effect of nickel observed was in agreement with Cho et al. (2008) who reported that the inhibitory effect of the metal was produced on concentration over 1.02 M, which is the concentration when the toxic effect was visible from the beginning herein. However, this work demonstrated that depending on the total time contact, the effect could be effective over 0.30 M of nickel instead of 1.02 M as it was reported.

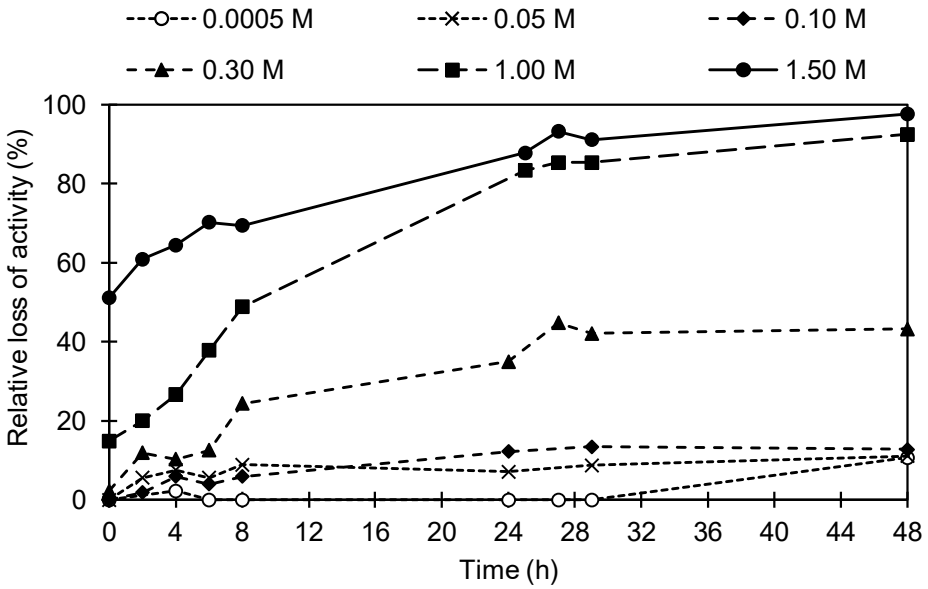

Figure 8.4. Toxicity evaluation of nickel at six different concentrations on activity of *Acidithiobacillus ferrooxidans*. The relative loss of activity is calculated as: $((A_c - A_T) / A_C) \times 100$; where A_C means the activity of the control sample and A_T the activity of the sample with the toxic concentration.

Finally, the toxic study was completed with the effect of aluminium ion. Hence, six different concentrations (0.001, 0.05, 0.10, 0.20, 0.35 and 0.50 M) were also prepared. As can be observed, aluminium resulted toxic at all the concentrations tested (Figure 8.5). However, this toxicity increased as the metal concentration also increased.

The toxicity was observed from the beginning of the experiments in all the assays, including the bioleaching concentration (0.001 M). After 4 hours, no loss of relative activity was observed in any case since it remained constant for the next 44 hours. This means that the toxic effect of this metal was practically instant whereas the other two previous metals studied showed an increase of their toxic effect over time. This revealed that aluminium was potentially toxic despite it has not been included in published toxicity studies (Cho et al. 2008; David et al. 2008), despite this was speculated by Brandl et al. (2001). Therefore, aluminium represents an important metal to take into account if it is bioleached from the scrap due to its toxic effect to microbial activity over 0.001 M, being more noticeable over 0.05 M.

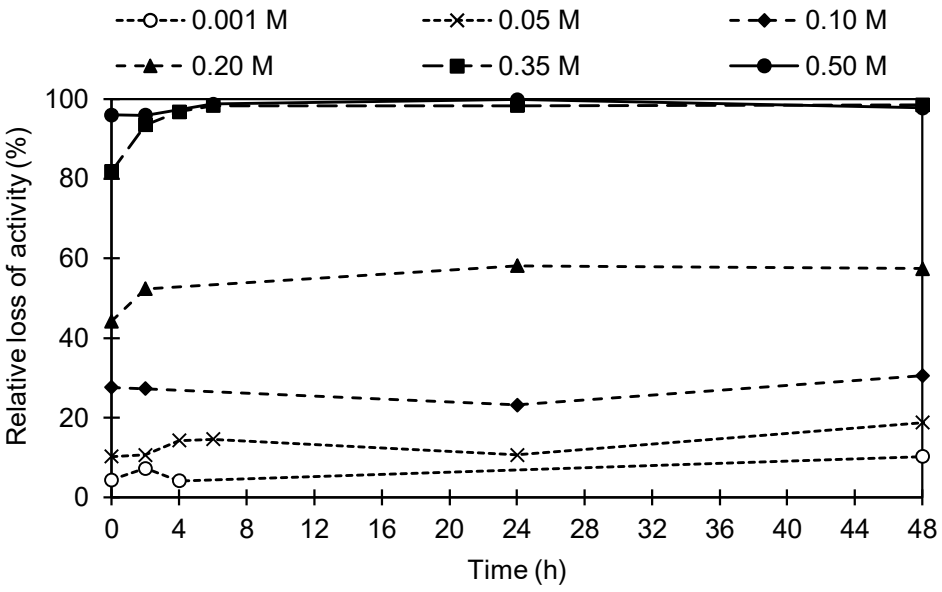

Figure 8.5. Toxicity evaluation of aluminium at six different metal concentrations on activity of *Acidithiobacillus ferrooxidans*. The relative loss of activity is calculated as: $((A_c - A_T) / A_C) \times 100$; where A_C means the activity of the control sample and A_T the activity of the sample with the toxic concentration.

In order to compare the toxicity effect of the three metals studied, a comparison of them at three different ion concentrations was considered. On the one hand, the bioleaching concentration, which was the average concentration of the metal obtained after the bioleaching process, these being 0.05, 0.0005 and 0.001 M for Cu, Ni and Al, respectively. On the other hand, the concentration referring to the natural and limit tolerances, which tolerances were described by Magnin et al. (1998) for copper and nickel, whereas the resistance for aluminium was studied by Fischer et al. (2002). According to these Magnin et al. (1998), the natural tolerance represents the metal concentration not resulting in stunted growth, whereas the limit tolerance represents the

maximum metal concentration that does not inhibit bacterial growth. The comparison for the three metals is shown in Figure 8.6.

There are noticeable differences between the three metals. Regarding the natural tolerances, although previous authors described that these concentrations did not affect the activity of the microorganisms, this work demonstrated that the concentrations reported as a natural tolerances produced a negative effect on the biomass in the case of nickel and aluminium, especially after 48 hours of contact when the microorganisms lost 43% and 19% of their activity, respectively. Nevertheless, copper natural tolerance concentration did not reduce their activity, which means that they can tolerate it during their growth. In the case of limit tolerances, they have a negative effect on the bacteria for all metals, losing 50, 57 and 93% of the activity for Cu, Al and Ni, respectively, after 48 hours. It means that nickel was more toxic than aluminium to the microorganisms, which in turn was more toxic than copper at this concentration. However, it is noticeable that aluminium was the most toxic metal during the first 10 hours at the limit tolerance concentration, although negative effect of this metal was visible from the beginning of the experiment in all cases. Therefore, it can be affirmed that the contact time between the metals and the microorganisms was crucial during their growth. In this sense, the higher the exposure time, the more toxic the metal. For this reason, reducing the experimental time of the bioleaching process, as well as the development of the process in two different steps are very important to maintain the microorganisms at their optimal conditions. Regarding bioleaching concentrations, the results demonstrated that they were not toxic for the microorganisms, since the relative loss of activity was less than 10% in 48 hours in all cases. However, as stated previously, metal accumulation could eventually cause inactivity to the microorganisms. Therefore, this aspect should be taken into account in these cases.

Given that the average concentration obtained in previous bioleaching experiments was 0.05 M of copper in 48 hours, if this process is extended for more days it would be toxic for the microorganisms when bioleaching takes place in only one step. This occurs since a concentration of 0.60 M of copper (which begins to be toxic for the microorganisms), could be reached after 24 days, leading to their complete inactivation after 48 days when the copper concentrations could raise to 1.20 M, in accordance to the results obtained in the toxicity assays. In the case of nickel and aluminium, although they resulted toxic at certain concentrations (over 0.05 M for aluminium and 0.30 M for nickel), the concentrations obtained after bioleaching are too small (less than 0.001 M in both cases) to affect the microorganisms during the process. Nevertheless, it is important to control the concentration of the metals in the leaching solution in one-step bioleaching

as it is above mentioned, since an unexpected increased may imply the inactivation of the microorganisms and, eventually, the finish of the extraction process.

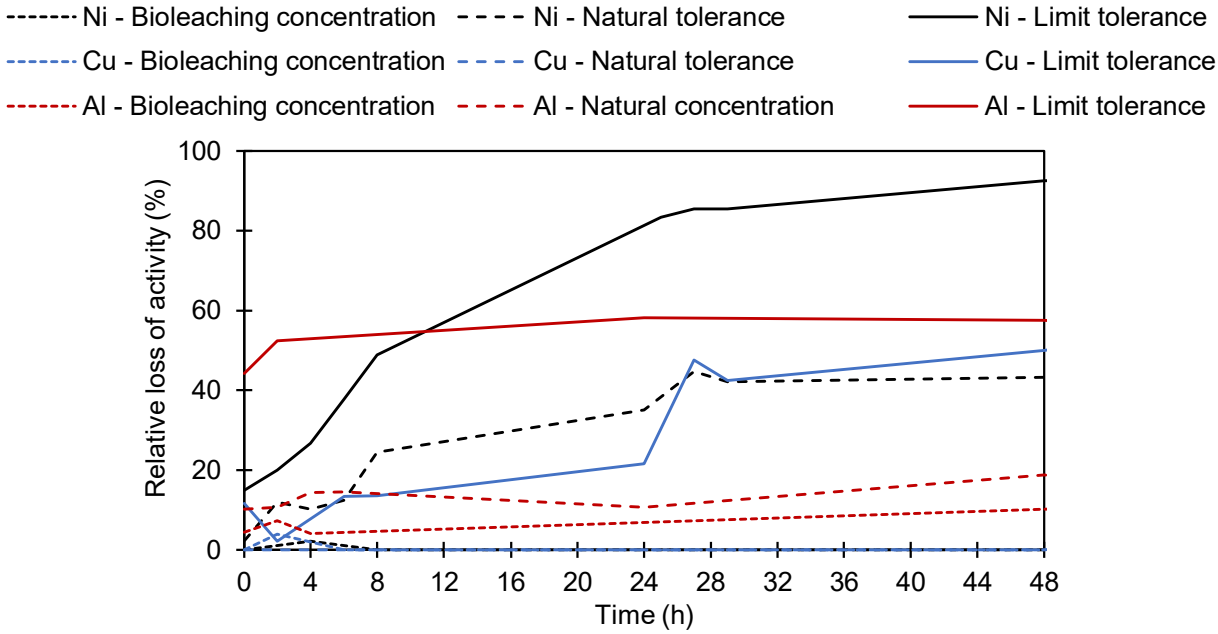

Figure 8.6. Comparison of the toxicity measurements of copper, nickel and aluminium for *Acidithiobacillus ferrooxidans*. The natural tolerance represents the metal concentration not resulting in stunted growth, whereas the limit tolerance represents the maximum metal concentration that does not inhibit bacterial growth.

8.3.2. Study of inhibition by iron substrate for *Acidithiobacillus ferrooxidans*

In this section the inhibitory effect of iron (III) to the *Acidithiobacillus ferrooxidans* culture was studied. In order to perform the experiment, the methodology used was the same than the toxicity assays, but in this case, iron was considered the potential inhibitor. Hence, six different iron concentrations were prepared (0.10, 0.20, 0.35, 0.50, 0.75 and 1.00 M) in which the metal was added as $FeSO_4 \cdot 7\ H_2O$. Results are showed in Figure 8.7. As in previous toxicity tests (section 8.3.1), the results were expressed as a percentage of relative loss of activity which means the activity loss by the microorganism in the presence of the iron in relation to their activity without the iron addition. On the contrary, a negative value in the relative loss of activity indicates an increase of the microorganisms' activity.

Figure 8.7 depicts that the iron presence can cause microorganisms to lose activity depending on the concentration in the solution, indicating that inhibition is produced. In particular, excepting the 0.35 M iron concentration, when iron concentration was over 0.20 M, 40% of the relative activity of the microorganisms was lost at the beginning of the experiment whereas 0.10 M increased the activity 6%. This indicates

that iron reduced the biological activity at high metal concentrations, inhibiting their metabolism. However, after 6 hours all the tendencies were changed. On one hand, the highest iron concentrations (0.75 and 1.00 M) produced a complete loss of activity, corroborating the inhibitory effect of the iron substrate. On the other hand, the lowest iron concentrations (0.10 and 0.20 M) steadily climbed to reach its highest increase of activity after 24 hours, being lower the increase achieved at greater iron concentrations. This activity enhancement could be associated to the acclimation of the microorganisms to the new conditions in which they were. This fact could imply an improvement on the iron (III) production which results interesting for bioleaching process. However, the oxidation rate should be studied in depth in these cases due to Barron and Luecking (1990) observation. They affirmed that the growth rate was not improved at concentrations over 0.1 M of iron (II), which is also important to maintain the activity of the viable cells as well as to improve the iron oxidation rate along time.

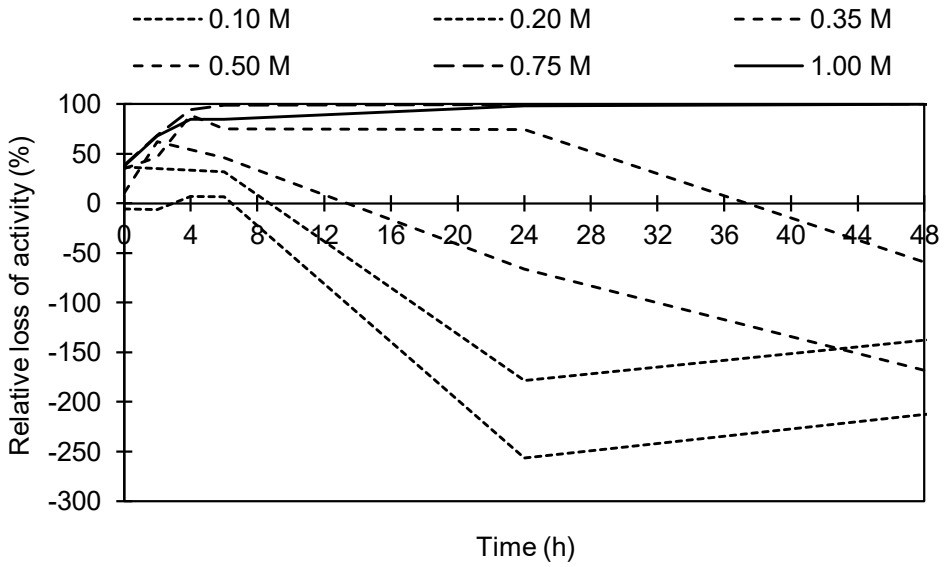

Figure 8.7. Relative loss of activity of *Acidithiobacillus ferrooxidans* at six different iron (III) concentrations.

From the results obtained in the study of inhibition by iron, it was determined that the presence of iron at concentrations over 0.75 M negatively affects the activity of the microorganisms. On one hand these results are in agreement with Nemati et al. (1998) who affirmed that the ability of *Acidithiobacillus ferrooxidans* to oxidize ferrous iron is significantly influenced by the ferrous iron concentration. In the present study, inhibitory effect was observed at concentrations over 0.75 M of iron (II), in accordance to Kelly and Jones (1978) who also obtained substrate inhibition above 0.7 M of iron (II). Nevertheless, conversely to the observations of these authors, at concentrations in the

range of 0.35 and 0.75 M inhibitory effects were also observed but only during the first 6 hours of contact. These differences are associated to the conditions and the methodologies used, since Kelly and Jones (1978) performed their experiments in a chemostat culture without taking into account the time exposure of the microorganisms to the inhibited agent. On the other hand, the results were also in agreement with Pagnanelli et al. (2007) due to below 0.16 M of iron (II) concentrations substrate inhibition was not produced, as it was reported by Barron and Luecking (1990), Kelly and Jones (1978), and Okereket and Stevens (1991). Nevertheless, Barron and Luecking (1990) found that the maximal inhibition of growth was produced at an iron (II) concentration of 20 g/L (0.35 M). Their results differ from those obtained in the present work since at this iron concentration the growth of the cells still occurred. However, the authors did not evaluate how the presence of iron affect their activity along time, so at the beginning of the experiments carried out in this work 0.35 M of iron really inhibits the biological activity as Barron and Luecking (1990) affirmed. This fact implies that the time exposure results an important factor to take into account although most of the studies that focused their research on the effect of iron (II) concentration to *Acidithiobacillus ferrooxidans* did not pay attention to this parameter. In addition, it is noteworthy that the authors usually measured the effect of substrate inhibition by the evaluation of iron (II) concentration along time, assuming that low velocities implied substrate inhibition. However, in this work the evaluation was performed by the direct and instant measurement onto the biomass of their respiration rate. For this reason, the methodology introduced in this work for this kind of assays could supply more information than those techniques used in the studies mentioned above.

8.3.3. Effect of starvation and feeding resumption on *Acidithiobacillus ferrooxidans* culture

The previous section showed that the microorganisms lose their activity depending on the iron concentration of the medium. However, the results were expressed as a relative loss of activity since the microorganisms lose activity during the experiment due to the lack of nutrients. Despite it could be interesting to know the limits of this biomass to be used in systems like bioleaching pilot plants at industrial scale, this aspect has not been studied before. For this reason, the effect of feed lacking (until observing a decrease greater than 90% in the biological activity) was evaluated along time in order to observe how long the *Acidithiobacillus ferrooxidans* can resist without the addition of energy source.

Therefore, a re-feeding sample after energy source shortage was studied in this experiment. This consisted on taking a sample once all iron (II) ions were biologically oxidized. Then, 6 g/L of iron (II) and the rest of salts composing 6K medium were added to the biological sample. The culture evolution after the preceding starvation period and the resumption phase were evaluated by the determination of the respiration rate and the concentration of viable cells along time (Figure 8.8). It is noteworthy that the concentration of viable cells was measured by cell counting with the Neubauer chamber due to it facilitates the observation of changes in cell concentration by this method in long-term experiments.

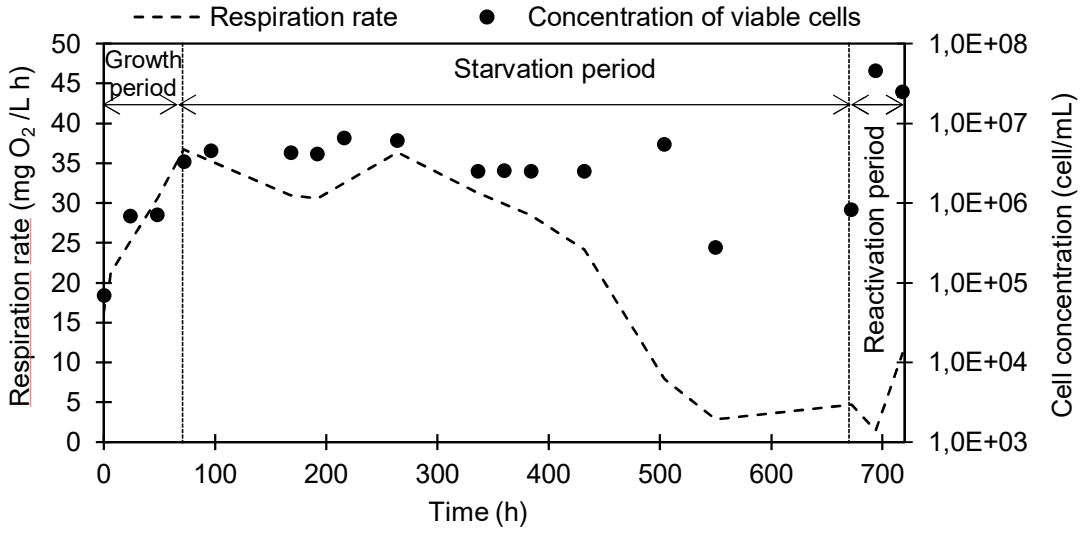

Figure 8.8. Time evolution of cell concentration and respiration rate during the experiment of starvation and reactivation of *Acidithiobacillus ferrooxidans*.

As can be observed in Figure 8.8, the respiration rate increased 15 mg/L h during the first 72 hours when the microorganisms were fed, which corresponds to the growth period. Consequently, the cell concentration also increased in this period from 10^4 to 10^6 cells/mL. Then, on the starvation period it is noteworthy that the respiration rate of the sample obtained the lowest value at 550 h. In this case, this coincided with an important decrease of the cell concentration, which values remained low during the following 122 hours. It means that the culture of *Acidithiobacillus ferrooxidans*, although being affected by the feed lacking along time, they could resist 550 h without the addition of the mineral medium salts. Park et al. (2005) reported that the cell concentration increased after 250 h despite not having iron (II) in the medium to their growth. This also confirmed that the iron-oxidizing bacteria could resist periods without iron addition, maintaining their normal activity. In addition, despite the loss of 90% of the bacterial activity after 550 hours, the

concentration of cells increased again when new mineral medium salts were added. In particular, the concentration of viable cells increased from 10^5 to 10^7 in 48 hours. This increasing was also reflected in the respiration rate, since the bacteria doubled the oxygen consumption during this period of time. Hence, the results demonstrated that the microorganisms could resist until 550 hours without feeding but they could be quickly reactivated again if they are fed after this time.

In this experiment, the evolution of iron (II), iron (III) and total iron concentrations along time were also measured, as shown in Figure 8.9, in order to relate them with the oxygen consumption and the cell concentration. Total iron was only measured during the first 380 hours of the experiment due to the constant results obtained through more than 300 hours. As it was expected, all iron (II) ions were consumed in the sample during the first 50 hours, indicating that the microorganisms oxidized them. This leads to an increase on cell activity and cell concentration (Figure 8.8). After this time, since there was no iron consumption, both the cell concentration and oxygen consumption did not increase. In fact, these two parameters remained constant until 380 hours. Moreover, the total iron concentration resulted in higher concentrations in comparison to the iron (II) concentration of the medium where they growth initially (around 6000 mg/L) due to the addition of more iron salt at the beginning of the experiment.

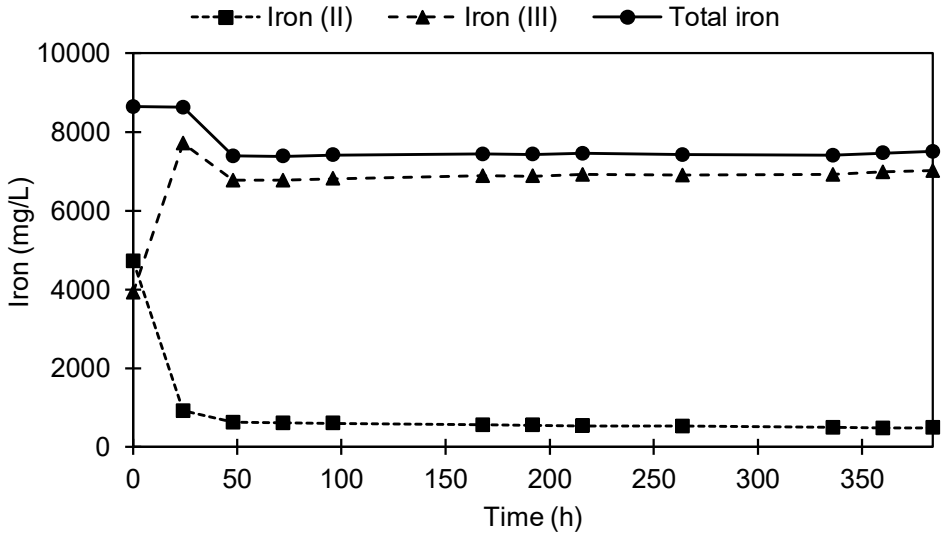

Figure 8.9. Iron concentrations during the starvation experiment of *Acidithiobacillus ferrooxidans*.

According to Molchanov et al. (2007), most of the works published in relation to the growth kinetics of *Acidithiobacillus ferrooxidans* have reported competitive product inhibition based on the Monod model (Eq. 8.4). For this reason, from the data obtained in the experiment, in particular from the iron (II), iron (III) and biomass concentrations, the kinetic model was adjusted.

$$\mu = \frac{\mu_{max}\,[Fe^{2+}]}{K_S\left(1+\frac{[Fe^{3+}]}{K_P}\right)+[Fe^{2+}]} \quad (8.4)$$

Thus, adjusting the experimental values to a Monod model with a competitive product inhibition, it is obtained that the μ_{max} is 0.10 h^{-1} whereas the K_s is 95.2 mg/L of iron (II) and the K_p is 161 mg/L of iron (III). These values are consistent with other studies that also adjusted a competitive product inhibition for *Acidithiobacillus ferrooxidans* (Gómez et al. 1996; Liu et al. 1988). In particular, Gómez et al. (1996) obtained a μ_{max} of 0.14 h^{-1}, and a K_s and K_p of 0.94 g/L for iron (II) and 0.31 g/L for iron (III), respectively. In the case of Liu et al. (1988), they obtained a μ_{max} of 0.11 h^{-1}, and a K_s and a K_p of 0.05 g/L of iron (II) and 0.44 g/L of iron (III), respectively. Although both studies, as well as the present one, adjusted the same kinetic model for *Acidithiobacillus ferrooxidans*, the discrepancies in the results may be associated to the differences in cultivation conditions, despite not being very pronounced. Specifically, Liu et al. (1988) cultivated the strain at initial pH 1.8 and 35 ºC, whereas Gómez et al. (1996) cultivated it at initial pH 2.0 and 30 ºC, and an initial pH of 1.75 and a temperature of 30 ºC were used in the present work.

Regarding to the studies focused on the lack of feed, no more similar studies were found in the literature, may be since the authors, in general, did not focus on this aspect. Nevertheless, this is an important point to consider, if the process is intended to be performed at industrial scale, since maintenance procedures as cleaning tasks or plant breakdowns could cause the feed to lack during some periods. For these cases, results demonstrated that the strain ATCC 23270 *Acidithiobacillus ferrooxidans* responds well to short periods of feed lacking, being resistant up to 550 hours (more than 20 days). In addition, if they are fed again after this time, they could be quickly reactivated without negative consequences to the system.

8.4. Conclusions

From the results obtained in the toxicity experiments, it was concluded that the presence of leached metals affects the activity of the microorganisms. In particular, the presence of copper, nickel and aluminium in the biological solution where the microorganisms were grown, affects their activity, depending on the concentration of those metals as well as on the time of contact. Among the metals studied, aluminium turned out to be the most toxic metal, since its effect on biological activity was higher in shorter time and at lower concentration than that of nickel or copper. Aluminium resulted

in the complete inactivation of the cells at 0.5 M of aluminium in just a few minutes of contact. In contrast, nickel was the least toxic metal in the present study, since the total inactivation was found at 1.5 M of nickel after 48 hours of contact. Copper also was toxic, but in this case, it took 27 hours of contact at a concentration of 1.2 M to inactivate the microorganisms. Hence, aluminium turned out to be more toxic than copper, which, in turn, was more toxic than nickel. In all cases their toxicity was clearly observed after 48 hours, although the most toxic concentrations were noticed from the beginning, especially for aluminium of which all the concentrations turned out to be toxic from the first instant of contact. This aspect has an important effect in the bioleaching process, especially when the leaching solution needs to be recirculated as well as when the biological iron oxidation and the leaching reactions are carried out in the same place. Hence, the results of the toxicity assays reinforce the importance of separating the oxidation step from the leaching one during the bioleaching process.

In the present work an inhibition of *Acidithiobacillus ferrooxidans* by the substrate Fe^{2+} at concentrations over 0.75 M was demonstrated. Moreover, the experimental data was adjusted to a Monod model for competitive product (iron (III)) inhibition, obtaining that μ_{max} was 0.1 h^{-1}, K_s was 9520 mg/L for iron (II) and K_p was 161 mg/L for iron (III). In detail, an iron (II) concentration over 0.75 M inhibited completely the activity of the microorganisms after 4 hours of contact and this lasted for the rest of the experiment. However, at lower iron concentrations the inhibitory effect suffered during the first contact hours was reversed, increasing the biological activity by the use of iron as an energy source for their growth. This means that the biomass showed a high adaptation capacity to changing conditions. Not only toxic metals could cause a decrease of the biological activity, since this work demonstrated that starvation would also produce this effect. From the results, it was also possible to conclude that *Acidithiobacillus ferrooxidans* could stay 28 days (672 hours) without the addition of the mineral medium salts, including iron, without being completely inactivated and incapable to divide. This fact becomes relevant when the biological processes take place at industrial scale because maintenance procedures or technical problems could delay the process and the feeding for long periods of time. The results verified that the limit time for not producing a complete inactivation of the cells was 550 hours. However, despite of this loss of activity, the microorganisms could be reactivated in 24 hours, if they were fed again.

Chapter 9

Column bioleaching to recover copper from e-waste

This chapter is clearly the most ambitious of this thesis, since all the knowledge learned in previous chapters was applied to develop a process based on bioleaching to recover copper from printed circuit boards by a column reactor. This kind of system is interesting since at industrial scale keeping the waste in suspension could be an operation limitation. The first approach was to study the effect of key parameters that can modify the efficiency of the metal recovery in the process such as pH control, the use of packing materials to improve mass transfer and a decrease of operation time. The main objective was to find the best operational conditions to obtain the maximum copper recovery in the minimum time possible performing the process in a column reactor. Moreover, this study will allow to get knowledge of the process for scaling-up purposes and, thus, assess the applicability for an industrial scale.

Abstract

In this chapter, a column reactor was investigated to recover copper from electronic waste by means of a bioleaching technique. In particular, key parameters were studied in order to optimize the process to obtain the maximum recovery rate at minimum time. For this purpose, the study was focused on the effect of pH control, the dosage of e-waste treated, the particle size and the use of packing materials. In addition, two different supports for the waste inside the column were evaluated as well as different operating modes. The results demonstrated that it was possible to recover copper from the scrap using a column reactor, obtaining a significant amount of the metal in less than 48 hours. It was found that a particle size between 0.2 and 1.0 mm of diameter, with a dosage of 7.5 g/L of PCB using a plastic packing material and a porous support for the waste allowed to recover 88% of copper in 48 hours when the pH was adjusted at 1.75. These results improved the efficiencies reported in the literature in column reactors, since the time required for extraction was drastically reduced without losing effectiveness. Additionally, a new strategy was developed to increase the reaction rate and to overcome transport limitations for the leaching solution, achieving copper recoveries of up to 80% in just 6 hours, which has never been reported previously.

A modified version of part of this chapter has been published as:

Benzal, E., Cano, A., Solé, M., Lao, C., Gamisans, X., Dorado, A.D., 2020. Copper recovery from PCBs by *Acidithiobacillus ferrooxidans*: Toxicity of bioleached metals on biological activity. Waste and Biomass Valorization, 11, 5483-5492.

9.1. Introduction

The rapid increase of the e-waste in the world makes necessary the use of an effective industrial methodology to treat them. The bio-heap leaching process is one of the most used in commercial applications, especially for the recovery of copper from low-grade ores and mineral concentrates (Brierley 2001; Olson et al. 2003). Hence, bio-heap could be of a great alternative for the e-waste recycling at industrial scale. Column leaching is used to simulate heap or dumps leaching processes but with the advantage of giving information about what could be expect inside the dump or the heap, allowing the optimization of the process (Ilyas et al. 2013; Muñoz et al. 1995; Qiu et al. 2011). There is not much information on literature about column reactors in e-waste bioleaching studies since most of them are focused on the bioleaching in flasks, and those who focused on the column leaching have much longer operating times. Ilyas et al. (2013) carried out the leaching process in flasks and in column reactors in parallel, studying the best conditions to obtain the highest copper recovery. To achieve 85% of total copper recovery they spent 18 days using flasks and 165 days using the column. Hence, they did not observe any improvement when the column was used but they concluded that their work showed the practicability of biotic electronic scrap leaching using this kind of systems. In addition, Chen et al. (2015) also studied the column leaching process but, in this case, they needed 28 days to recover 95% of the total copper contained in the e-waste treated. It is noteworthy that they carried out the experiments at initial pH 2 using 50 g/L of e-waste and the leaching solution was pumped at the rate of 40 mL/min. However, Ilyas et al. (2013) worked at the same initial pH but with 5 kg/L of e-waste and the rate of the leaching solution was 50 mL/min. Nowadays, as it has been above mentioned, the column leaching is performed at industrial scale to retrieve metals from low-grade ores but the efficiency of the process is low and many days of operation are required (Gu et al. 2018; Pradhan et al. 2008).

As in batch bioleaching, the process could be affected by some parameters when it is performed in column reactor. The main parameters that affects the process include the pH, the PCB dosage, the particle size of the PCB, the operating time as well as the solid-liquid contact system. It has been studied, and also demonstrated in Chapter 6, that the pH control allows a better process when it is carried out in batch conditions since the maintenance of the pH under 2.0 avoids iron precipitation (Baniasadi et al. 2019). In addition, the particle size is also relevant in metal's extraction since small particle size increase the specific contact surface, which improves the recovery yield, but very low particle size could negatively affect the process (Dorado et al. 2012). In this sense, very

low particle size could agglomerate and compact the column, hindering the percolation of the leaching solution through the e-waste. Moreover, crushing into small particles consumes energy which increased the cost of the process (Ahonen and Tuovinen 1995; Gu et al. 2018). There are studies demonstrating that some of the above-mentioned parameters could improve the process in batch conditions, however, in column reactor these factors have not been studied yet. Hence, deepen the effect of these parameters in column bioleaching field is necessary in order to step forward in the investigation.

The aim of the tasks presented in this chapter was to evaluate the bioleaching process to recover copper from the e-waste by a column reactor. In this regard, different parameters were assessed as the effect of the pH control, the performance in a flooding column, the effect of the particle size, the PCB dosage and the use of structuring inside the column reactor. Finally, the best conditions found were implemented in the column reactor to achieve the maximum metal recovery, evaluating the time operation required for this purpose. Moreover, a new strategy overcoming limitations detected was developed in order to reduce the experimental time without losing the efficiency of the process.

9.2. Materials and methods

9.2.1. Electronic scrap

The PCB used for the column leaching experiments come from end-of-life mobile phones collected from the recycling plant Electrorecycling S.A. (*El Pont de Vilomara, Spain*). The plastic components were removed from the mobile phones and the main electronic components (resistors, capacitors, chips...) were separated. In order to obtain the desirable size, the PCBs were crushed and sieved in two different size ranges: particles between 0.2 and 1.0 mm of diameter and particles above 1.0 mm of diameter. As it was mentioned in Chapter 6, Wang et al. (2009) demonstrated that particles lower than 1.0 mm of diameter obtained higher metals extraction than particles over this size in batch bioleaching conditions. Hence, all the experiments were performed with the small particles (0.2 – 1.0 mm), in general, and the biggest ones were used to evaluate the effect of the particle size. In this way, the results will be comparable to those obtained in batch conditions. Moreover, Ahonen and Tuovinen (1995) found that working with a particle size between 1.68 and 5 mm of diameter in a column of 9 cm of diameter achieved the best metal efficiencies in ore bioleaching. Thus, a similar relation was used herein (0.2 – 1 mm of particle size in 3 cm diameter column).

9.2.2. Leaching solution

The leaching solution used in the column experiments was obtained from the biological reactor in which *Acidithiobacillus ferrooxidans* were cultivated where 6K mineral medium was used for microorganisms' growth (for detailed composition, see table 4.2 in section 4.1.1 in Chapter 4). This solution mainly contains bio-oxidized iron (III) among other inorganic salts. However, there were also some precipitates inside. For this reason, the leaching solution was always settled during 2 hours in a beaker before the experiment in order to reduce the interferences that the precipitates could cause.

9.2.3. Bioleaching experiments

All the experiments in this chapter were performed in a column reactor (Figure 9.1). It consists of a PVC cylinder tube of 10 cm height with an internal diameter of 3 cm. The column was filled with the electronic scrap (7.5 or 15 g/L, depending on the experiment) mixed with plastic particles between 1 and 3 mm of diameter as structuring, also depending on the experiment. These plastic particles were obtained by crushing the plastic housing and structure of the mobile phone. In order to keep the scrap inside the column two different methods were used: a net support and a porous support. The net support consists on a mesh of cellulose in which the PCB were introduced. In this way, the leaching solution was forced to pass through the scrap retained in the net. The porous support consisted on a support of polyurethane foam located in the bottom of the column, placing the PCB over it. Hence, the scrap was located freely in the column whereas in the mesh support, the scrap was locked in the net. Moreover, a reservoir was incorporated to facilitate the sampling and the measurements of pH and ORP during the experiments. Hence, the reservoir was filled with 400 mL of the leaching solution. As it was explained, the supernatant of the leaching solution containing the bio-oxidized iron (III), which was previously settled, was used. This solution was pumped inside the column using a spray nozzle (*model EUSPRAY-I1MX3*) at a rate of 54 mL/min by a peristaltic pump (*model 77200-12, Masterflex*), resulting in a leaching rate of 0.13 cm/s. Samples for iron and copper measurements were taken every hour from the reservoir, as well as pH and ORP measurements. Simultaneously, the pH was controlled at 1.75 by the dropwise addition of 3 N H_2SO_4, excepting in the experiment carried out without pH control.

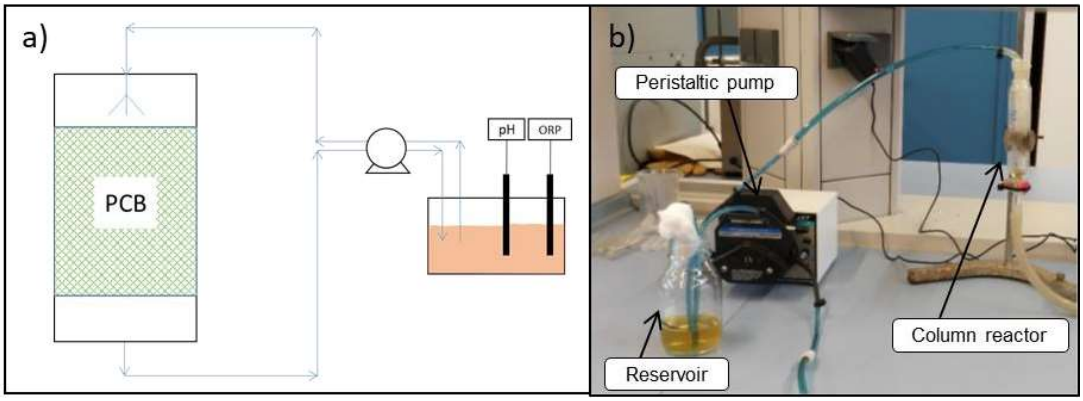

Figure 9.1. (a) Diagram of the column reactor and (b) the column reactor used in the experiments.

9.3. Results and discussion

9.3.1. PCB composition

Chen et al. (2018a) demonstrated that the metal composition of PCBs is very diverse, depending on the device type and its year of construction. In particular, they carried out the characterization of the metals contained in 36 mobile phones from different year and manufacturer. This variability on metal's containing makes the repeatability of experiments difficult. In addition, Khaliq et al. (2014) compared the metal composition of PCBs from different studies, observing, for instance, that the copper content varied from 6.9 to 20% or the nickel content varied from 0.28 to 2%. This fact implies that the composition of the e-waste could not be generalized, so a previous analysis is necessary before the bioleaching process. Therefore, an acid digestion of the PCB used herein and its metal analysis were performed by a microwave apparatus and atomic absorption spectroscopy, respectively (the description of both procedures are described in sections 4.2.3 and 4.2.4 in Chapter 4). The analysis was performed with particle size between 0.2 and 1.0 mm of diameter. With that, the PCB average content of Cu, Ni, Fe, Ag, Au, Al, Pd, In, Sn, Pb, Co and Mn in g/kg was 390.38, 11.51, 1.95, 0.19, 0.80, 1.33, 0.15, 0.12, 28.92, 16.16, 0.14 and 0.58, respectively (Figure 9.2). The total metal content per kilogram in the PCB was 452.23 g and from the data obtained, Cu was found as the major component.

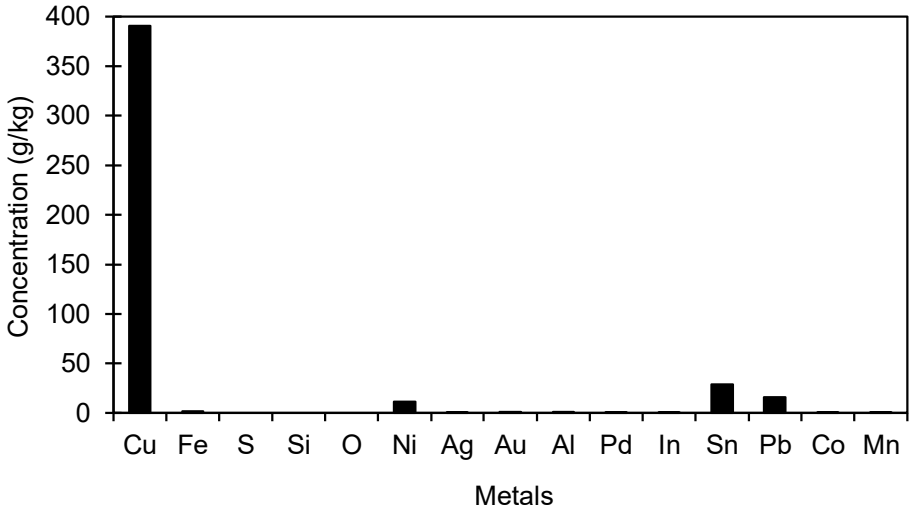

Figure 9.2. Metals composition of the PCB used in the experiments.

Despite the heterogeneity of the PCBs mentioned above, Oliveira et al. (2010) demonstrated that the metal distribution also depends on the particle size fraction. In particular, they concluded that the major elements such as copper, tin and lead had higher concentrations in the particle size range of 0.3 and 1.5 mm and they substantially decrease in lower fractions. In the case of gold and silver, their distribution seems to decrease when increasing the particle size but they did not obtain clear results. However, the authors affirmed that in fine fractions (lower than 0.3 mm) a sort of fluffy material, which mainly contains organic resins, prevailed. In addition, Bizzo et al. (2014) demonstrated that the fraction with particle size below 1.18 mm had a higher inorganic material than the fraction over this size. Moreover, these authors also affirmed that the smaller fractions concentrate larger amount of metal.

Given these results and taking into account the analysis of metal content, this work was performed with the particle size between 0.2 and 1.0 mm of diameter. It was also analysed the copper concentration in the PCB sample when the particle size was between 1.0 and 3.0 mm of diameter, as well as with the particles of 5.0 and 10.0 mm. In this case, only copper was analysed since it is the metal evaluated for extraction in the following experiments. Surprisingly, the results were quite different. Whereas the fraction between 0.2 and 1.0 mm of diameter obtained 39% of copper in their composition, the copper content in size 1.0 - 3.0 mm was only 7%. Nevertheless, the copper content of the particles of 5.0 and 10.0 mm were 19.3 and 29.7% of copper, respectively. These important differences are associated to the heterogeneity of the PCB sample, which indicated that the copper metal is easily crushed. Thus, most of the copper contained in the scrap was obtained in the range size under 1.0 mm. Oliveira et al. (2010)

found that the higher the particle size, the lower the copper concentration, but they performed the experiments until 3 mm. Therefore, the results found in this work are in accordance to Oliveira et al. (2010) in this range size but this work demonstrated that this affirmation was not correct when higher particle size are analysed. For this reason, it is important to analyse the copper content of the particle size used in each experiments for non-erroneous conclusions. As mentioned in section 9.2.1, all the experiments were performed with the particle size between 0.2 and 1.0 mm and just in case to study the effect of the particle size in column reactor, the particles above 1.0 mm were used.

9.3.2. Effect of pH

As it was observed in Chapter 6, pH resulted an important parameter in batch bioleaching experiments since a pH increase could produce the precipitation of metals like iron. Hence, the first approach was to evaluate the bio-extraction process performed in a column reactor, focusing on the effect of pH adjustment. To hold on the scrap inside the column, a mesh support was used. The experiment was performed with a PCB dose of 15 g/L at room temperature, using a particle size between 0.2 and 1.0 mm of diameter. The total iron concentrations are shown in Figure 9.3, as well as the copper recovery during the experiments.

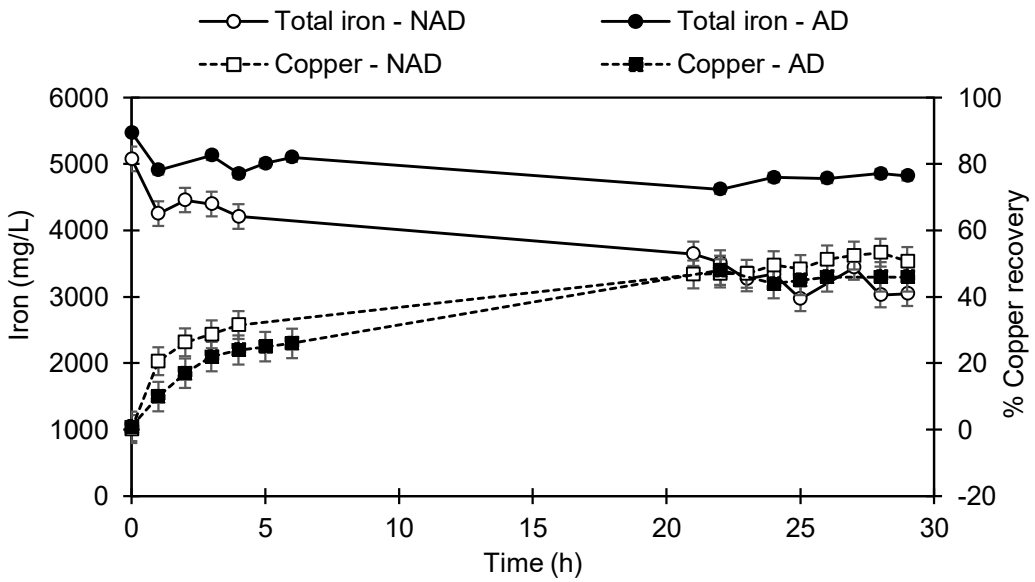

Figure 9.3. Iron and copper evolution in the column reactor when the pH was adjusted (AD) and not adjusted (NAD).

Similar recoveries of copper were achieved after 29 experimental hours (about 50%), although the metal was faster recovered when the pH was not adjusted in the first 5 hours. Moreover, it is noteworthy that in both cases two different tendencies were

observed during copper extraction. In the first hours, copper was recovered faster than the following hours. In particular, about 30% of copper was recovered in just 6 hours whereas the remaining 20% of the recovery achieved was obtained after 23 hours of experimentation. In the case of the total iron concentration, the behaviour was quite different. When the pH was adjusted, the iron concentration remained constant whereas it significantly decreased when the pH was not adjusted. Given bioleaching mechanism, a decreasing on copper recovery when there was less iron in the solution was expected since the iron is the responsible of copper solubilisation. Nevertheless, the recovery of copper followed similar trends, despite observing a decrease in iron concentration in one of them. It is possible that the excess of iron at the beginning of the experiment contributed to, despite the amount of precipitated iron, keep enough iron in the solution to solubilise the copper from the scrap. Regarding iron decreasing, the iron concentration was reduced more than 2000 mg/L when the pH was not adjusted in the solution. This means that iron precipitated because of pH increasing, as it could be corroborated in Figure 9.4 where the pH values along time are represented.

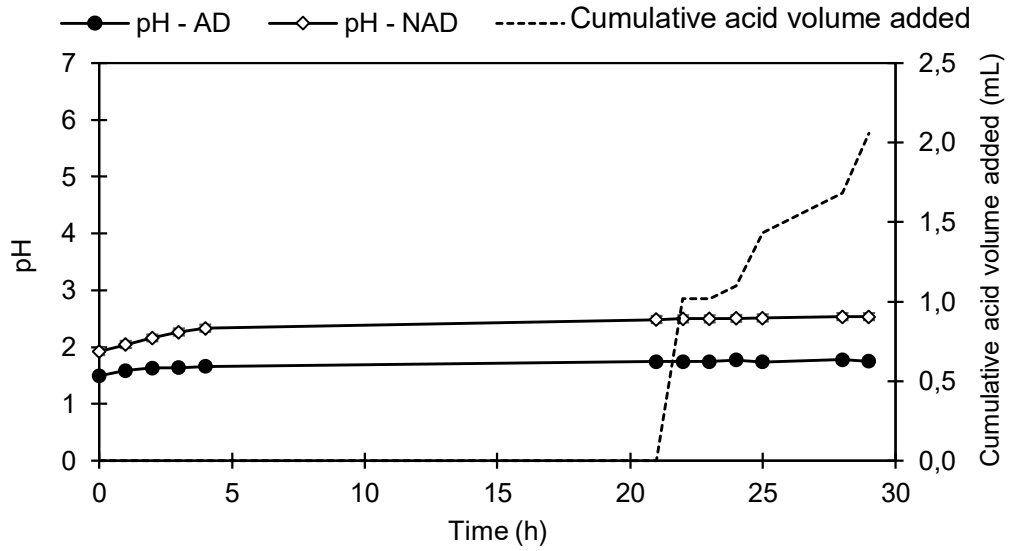

Figure 9.4. Evolution of pH in the column reactor when the pH was adjusted (AD), when it was not adjusted (NAD), and the volume of drop addition of 3 N H_2SO_4 in the case of adjusting pH.

The pH rose up to pH 2.5, when it was not adjusted, producing the iron precipitation. Moreover, although the pH increase was not very high, it is noteworthy that this fact had an important effect on iron precipitation, since almost 40% of the initial iron concentration precipitated. According to Valix (2017) and Baniasadi et al. (2019), an increase of pH over 2.0 results in the formation of iron oxyhydroxides through ferric ion hydrolysis. Moreover, given that there is a lot of sulfate, iron precipitation can be also

caused by the formation of iron hydroxyl sulphates like jarosite or schwertmannite (Liao et al. 2009). As explained in Chapter 6, the pH increase was related to the electronic scrap, since it has been reported to be alkaline in nature although no previous studies have been focused on what component produced the alkalinity observed. Although the oxidative dissolution of elementary copper consume protons from the sulphuric acid, thus alkalinising the medium, this can not be the reason of the alkalisation observed since this oxidative reaction has a low kinetic, occurring after 90 hours of experiment (Bas et al. 2013). However, it was suggested that the depolymerisation of the plastic components of the e-waste could cause the alkalisation (Valix 2017).

Regarding the acid addition when the pH was controlled, it is noteworthy that a total consumption of 0.075 moles of protons were needed to maintain pH below 1.8, which corresponds to a total volume of 2 mL of 3 N H_2SO_4 approximately. It is noted that the initial pH in the experiment when the pH was controlled was nearly 1.5 and despite the pH was increasing during the experiment, it did not rise pH 1.8 until day 22 when the acid addition was needed. Acid addition can contribute to improve the leaching efficiency indirectly by ensuring the iron cycle proceed well since it is important to maintain the iron concentration in solution during the experiment in order to avoid precipitates which decrease the availability of iron (III). Moreover, it also results important to maintain the highest possible iron concentration in solution to allow a cyclic process, which reuses the reduced iron (II) obtained after leaching as feed again for microorganisms, without the need of adding new iron (II) in the process.

As found in batch bioleaching experiments, the pH adjustment results essential to avoid iron precipitation. Moreover, this precipitation was more pronounced when the pH was not adjusted in batch conditions since 23.6% more iron was precipitated in comparison to column experiments without pH control. This fact is associated to the process itself because of the disposition of the e-waste inside the column since the solid-liquid contact is more limited in this case and, thus, the alkalization of the leaching medium by the scrap is also reduced, decreasing the amount of iron precipitated. Nevertheless, under pH control the precipitation of iron was significantly reduced in both cases, obtaining a maximum precipitation of 7.8% and 15.6% of the iron in flask and column bioleaching, respectively. In this sense, both experiments verified that the pH adjustment was necessary in bioleaching processes. Hence, given the reduction on iron precipitation and the fact that no high volumes of acid were needed to achieve a pH

below 1.8, the pH adjustment was applied in the following experiments performed in column reactor.

9.3.3. Evaluation of iron (II) chemical oxidation and its effect on copper extraction

As it has observed in previous experiments, iron (III) is the main responsible of copper extraction in bioleaching processes. In these processes, iron (III) is provided by the biological oxidation of iron (II). However, it might occur that the microorganisms do not oxidize all the iron (II) due to a decrease on their activity or also a decrease on their concentration. This fact would cause the pumping of iron (II) to the column before being completely oxidized. For this reason, copper extraction was evaluated when mineral medium with iron (II) was used instead of mineral medium with only iron (III) as usual. In this way, chemical oxidation of iron (II) was evaluated at the conditions tested in the column reactor, which would imply the oxidation of the metallic copper from the e-waste. Hence, two experiments were carried out, one with mineral medium and iron (III) and the other replacing iron (III) by iron (II). In both cases, the pH was adjusted between 1.7 and 1.8 by the dropwise addition of 3 N H_2SO_4 and the mesh support was used. As in previous experiments, 15 g/L of PCB was used with a particle size between 0.2 and 1.0 mm. The evolution of iron (III) concentration and copper recovery along time are shown in Figure 9.5.

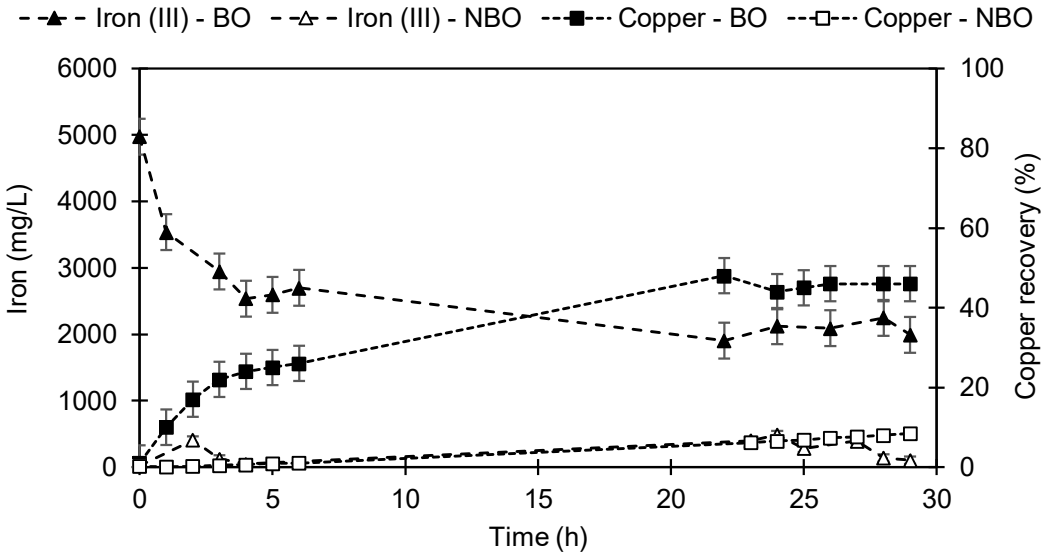

Figure 9.5. Evolution of iron (III) concentration and copper recovery over time in the column reactor when bio-oxidized iron (III) was used in the leaching solution (BO) and iron (II) was used (NBO).

When iron (II) was used instead of iron (III) in the leaching solution insignificant oxidation was produced because the concentration of the ferric ion did not practically

increase. Nevertheless, the copper recovery obtained in this case was 8.4%. On the contrary, when bio-oxidized iron was used, it was noticed that its concentration decreased, especially at the beginning of the experiment when the copper recovery was more pronounced. This experiment corroborated that iron (II) was not chemically oxidized at this pH in the column reactor and, in consequence, no copper was retrieved. The 10% of copper recovered in this experiment was associated to the remaining iron (III) present in the solution. Hence, it was considered that iron (III) was the main responsible of copper extraction in column bioleaching at the conditions tested.

Regarding again the copper recovered when iron (II) was used, 8.4% of copper was extracted in 30 hours, which corresponds to a copper concentration of 455 mg/L. It is noteworthy that a maximum of 488 mg/L of iron (III) was oxidized during the experiment but this iron could only solubilise 277 mg/L of copper in accordance to the stoichiometry of the leaching reaction (Eq. 9.1).

$$2\,Fe^{3+} + Cu^{0} \rightarrow 2\,Fe^{2+} + Cu^{2+} \qquad (9.1)$$

Hence, the oxidation of copper, and so its extraction, was produced by other factors apart of the iron (III). Torres and Lapidus (2015) affirmed that copper is solubilized by the presence of oxygen in the acidic medium. Moreover, Bas et al. (2013) also corroborated this effect in their experiments. Nevertheless, none of them performed the experiment in the absence of oxygen to proof this statement. For this reason, 100 mL of mineral medium without iron at pH 1.75 and 0.75 g of e-waste were introduced in a glass vial and the oxygen was removed by sparging nitrogen gas into the vessel. In this way, the nitrogen gas allows to displace the oxygen found inside it. Then, the vessel, previously sealed, was incubated at 30 ºC for 30 days, analysing the copper concentration in the liquid after this time. Results showed that 0.44% of copper was extracted from the scrap in the experiment, which means that the acidic medium was not able to solubilise the copper without the oxygen presence. Thus, this experiment confirmed that the oxygen in acidic medium is the responsible of copper extraction when there is no iron (III) in the leaching solution. Nevertheless, Bas et al. (2013) observed that the kinetics of copper oxidation by this reaction are very slow since they recovered 18% of copper in 90 hours at pH 1.75 and 30 ºC. Therefore, despite the presence of oxygen in acidic medium, copper will be firstly leached by the effect of iron (III) when the process takes place in short periods of time since this reaction will predominate at these conditions.

9.3.4. Improvement of solid-liquid contact

In batch conditions, it was possible to achieve around 90% of the total copper contained in the PCB whereas in the column reactor it did not exceed 51% of copper extraction. One of the main differences between these systems is the distribution of the e-waste and their contact with the leaching solution. Thus, it is possible that mass transfer limitations occurred in the column system used since the solid-liquid contact area in this case is less than in batch conditions using flasks in which the scrap is continuously submerged in the leaching solution. Assuming that there is a mass transfer limitation between solid-liquid contact due to the scrap disposition, the column reactor was evaluated in the conditions used in batch experiments. This means that the column was flooded with the mineral medium while the leaching of PCB takes place. So that, 15 g/L of PCB (0.2 – 1.0 mm of diameter) were introduced in the column reactor using the mesh support to hold the scrap. In this case, the pH was also adjusted between 1.7 and 1.8. Figure 9.6 gives information about copper recovery and iron (III) concentration along time during the experiment.

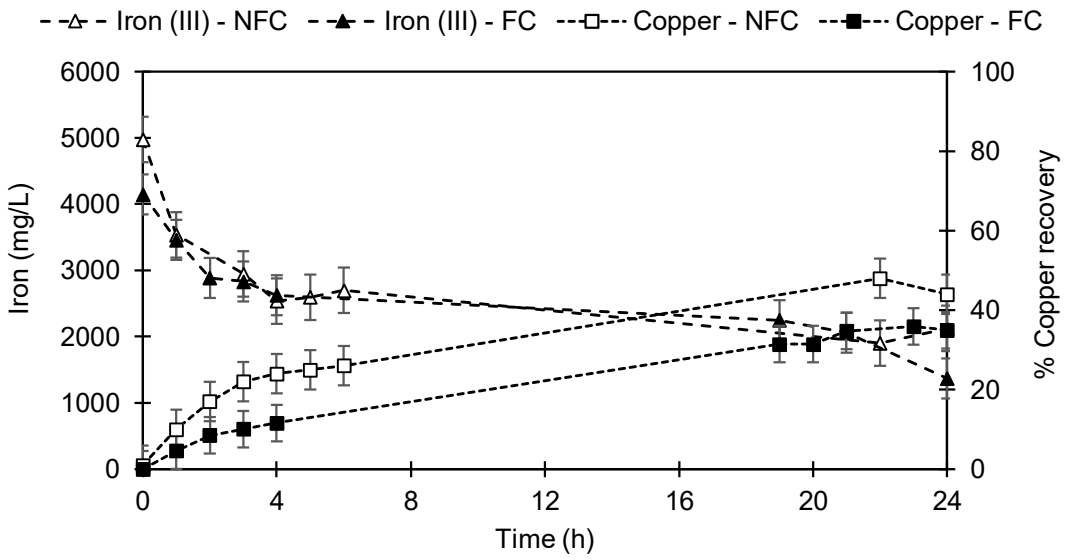

Figure 9.6. Copper recovery and iron (III) concentration over time when the effect of flooding the column was investigated (NFC, not flooding column; FC, flooding column).

Figure 9.6 shows that copper recovery increased in both cases but the flooding column obtained less copper extraction from the beginning. In particular, 44% of copper was recovered in the usual column whereas 35% of copper was recovered in the flooding column in the same period time. On the contrary as it was though, flooding the column did not contribute to obtain better results. This means that the solid-liquid contact wat not enough and some parts of the scrap were still inaccessible for leaching solution, so not

all copper was extracted. This fact is related to the possible compaction of the e-waste inside the column since when the bioleaching takes place in flasks, they are continuously stirred during the whole process, making it impossible to compact the waste in this case. However, in column reactor the PCBs are immobilized in the mesh in which the scrap is hold. As it has observed, not all copper was extracted and this was also noticed on iron (III) evolution since it could be appreciated that it remained non-reacted iron (III) in the solution, indicating that there was a contact limitation between the iron and copper ions. Given that the process is based on a superficial phenomenon, the PCBs compaction would limit the contact between ions, thus the iron could not react with the copper found in the deepest parts of the treated waste. Additionally, the difference on the initial iron (III) concentration was associated to the use of biological leaching solution that come from the biological oxidation of iron. It is assumed that, despite the mineral medium to feed the microorganism contained 6 g/L of iron (II), not all the iron was oxidized when it was used. Nevertheless, the iron behavior during the experiment in both cases were quite similar and so the amount of iron (III) consumed. This fact would mean that similar copper extraction has to be obtained, however, more copper was extracted in the case of non-flooding column. From a stoichiometric point of view, the iron (III) consumed in both cases could extract 1600 mg/L of copper approximately, which corresponds to the total amount of copper recovered in the flooding column. On the contrary, in non-flooding conditions it was retrieved more copper than it should have been obtained by iron. It is assumed that, under non-flooding conditions, the mass transfer of oxygen to the medium easily occurred due to the movement of the leaching solution itself through the column. This incorporation of oxygen in the liquid favors the reaction between it and the copper because of the acidic conditions in which the process takes place (Bas et al. 2013; Torres and Lapidus 2015).

This experiment leads to continue improving the mass transfer limitations detected when a column system is used. For this reason, the experiments were focused on how to improve the holding system for the scrap inside the column. Until now the experiments in the column reactor were performed using the net support (Figure 9.7a). However, after observing that the extraction was not completed, even in the case of flooding the column, a new hold on system was evaluated. In this case, a porous support (polyurethane foam) was used (Figure 9.7b). In order to maintain the same conditions than the previous experiments, the pH was adjusted to pH 1.7 – 1.8 and 15 g/L of PCB with a particle size between 0.2 and 1.0 mm were treated.

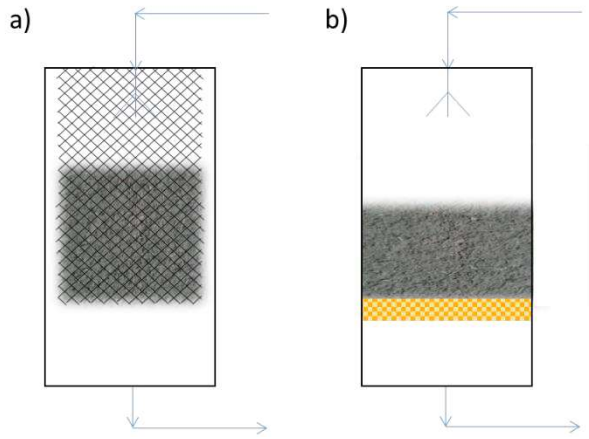

Figure 9.7. Diagram of (a) the net support and (b) the porous support (b) used to hold the scrap inside the column reactor.

As Figure 9.8 depicts, better recovery was achieved when the porous support was used in just 6 hours. In a closer inspection, 37% of copper was obtained from the PCB when the porous support was used while 26% was achieved with the mesh one. Despite not having a big difference on metal recovery, it is noteworthy that all the iron (III) from the leaching solution reacted when the porous support was used, decreasing from 3585 mg/L to 280 mg/L in 6 hours. In the case of the mesh support, only 2250 mg/L of iron (III) reacted in this period of time. Hence, although the iron (III) was in solution when the net support was used, it did not completely react. This means that the contact between iron and copper was not enough and, thus, there was some internal points in the scrap in which iron could not access. The improvement in the contact between the leaching solution and the e-waste is related to the hold system itself. As shown in Figure 9.7b, the porous support forces the leaching solution to pass through the bed of PCB whereas in the net support the leaching solution may pass around the bed, thus preventing the leaching agent from accessing all the particles treated. Moreover, the fact that all the iron (III) has reacted with the copper contained in the PCB when the porous support was used means that no more copper could be recovered. Hence, the copper recovery using this support was the maximum that can be recovered at the conditions tested, since the reaction was chemically limited and, consequently, the complete extraction of copper was not achieved. Moreover, in this experiment the difference on the initial iron (III) concentration was more pronounced, but, as mentioned above, it is likely due to the use of the leaching solution before the complete biological oxidation of iron (II). It clearly demonstrated that the porous support allowed improving the contact between the leaching agent and the scrap, although further experiments are still necessary to achieve better recoveries.

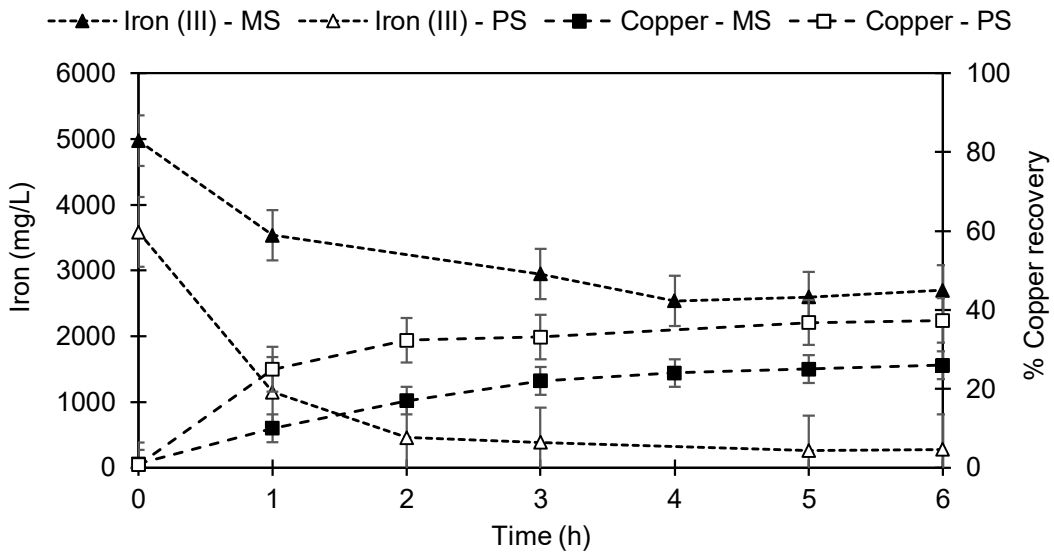

Figure 9.8. Copper recovery and iron (III) concentration when two different supports for the e-waste were used in the column reactor: the mesh support (MS) and the porous support (PS).

9.3.5. Effect of the particle size

The porous support improved the contact between the iron and the copper contained in the scrap. However, the contact still was not enough since not all copper was retrieved. So that, the limitation of the extraction process is the contact between copper and iron ions. For this reason, it is necessary to focus on this aspect and investigate strategies to improve such contact. In batch bioleaching it has been observed that one factor that can influence the process is the particle size (Shah et al. 2015; Wang et al. 2009; Zhu et al. 2011). In general, the percentage of metals solubilized into leaching solution increases when the particle size is reduced due to the increase of the surface area per unit mass (Wang et al. 2009; Zhu et al. 2011). This implies that the mass transfer improves and, thus, the bioleaching rates are enhanced (Lotfalian et al. 2012). Nevertheless, when the particle size is too small, the metal extraction decrease due to the development of thick slurry, which increases the apparent viscosity of the medium created by the small particles (Shah et al. 2015). It has to take into account that the size reduction not only requires a great amount of energy for crushing process since it also depletes a considerable amount of steel due to the deterioration of grinding media and machine lines, which increases the cost of the process (Lotfalian et al. 2012; Zhou et al. 2019). Hence, it is important to find the optimum grain size to improve the bioleaching recoveries but also considering the economic aspects. In addition, as mentioned in section 9.3.1, the copper content varies from one size to another. This could also affect the recovery of the metal, since not all the size fractions contain the same amount of copper. Therefore, this is another important factor when selecting a particle size.

In this sense, the use of particles between 1.0 and 3.0 mm of diameter as well as particles of 5.0 and 10.0 mm was evaluated, comparing the results to those obtained in previous experiments in which only particles between 0.2 and 1.0 mm of diameter were used. In order to test the use of big particles as a packing material to improve the solid-liquid contact inside the column, the use of the PCB with a particle size between 0.2 and 1.0 mm (50%) mixed with particles between 1.0 and 3.0 mm of diameter (50%) was also evaluated. Results of copper recoveries in these experiments are given in Figure 9.9.

Copper was recovered in all the assays tested, although the extraction was produced mainly in the first two hours. This may be due to solid-liquid contact limitation. Despite of that, differences on copper recovery were observed when different particles sizes were used. In particular, more copper was obtained when particles between 0.2 and 1.0 mm were used during 6 hours (37%) comparing to the recovery obtained with the other sizes. In these cases, particles between 1.0 and 3.0 mm obtained an extraction of 15.7%, particles of 5.0 mm extracted 14.4% of copper and particles of 10.0 mm obtained 8.3% of extraction during the same period of time. However, regarding the mixed particles, it means 50% particles between 0.2 and 1.0 mm and 50% particles above 1.0 mm, it could be observed that the copper recovery increased in comparison to use only the biggest ones. For this, mixed particles obtained higher recovery than the use of 100% of particle size between 1.0 and 3.0 mm but less than the use of 100% of particle size between 0.2 and 1.0 mm. This fact implies that the small particles are the ones that achieve higher recoveries. The results were in accordance to Shah et al. (2015), who reported that metal extraction increased when the particle size decreased. Nevertheless, as it was above-mentioned they found that the metal extraction was negatively affected when the particle size was too small since it creates an increase in the apparent viscosity of the medium which difficult metal solubilisation. Wang et al. (2009) also studied the particle size effect, concluding that particles between 0.5 and 1.0 mm allowed recovering more copper than particles between 1.0 and 3.0 mm. Their results are in agreement with those obtained in the present research, in which better recovery was obtained with the smallest particles (0.2 – 1.0 mm). Zhu et al. (2011), who also observed this behaviour, affirmed that the surface are per unit mass of the scrap is increased by decreasing the particle size. Moreover, they justified that the differences on bioleaching efficiencies when different particle size are used occurred due to particles below the critical level may increase the extent of particle-particle collision, imposing severe attrition on the cells. Nevertheless, in the present study most of the cells have been previously separated from the leaching solution by sedimentation, thus this fact could not be associated to the differences observed in copper recovery with different particle sizes studied herein.

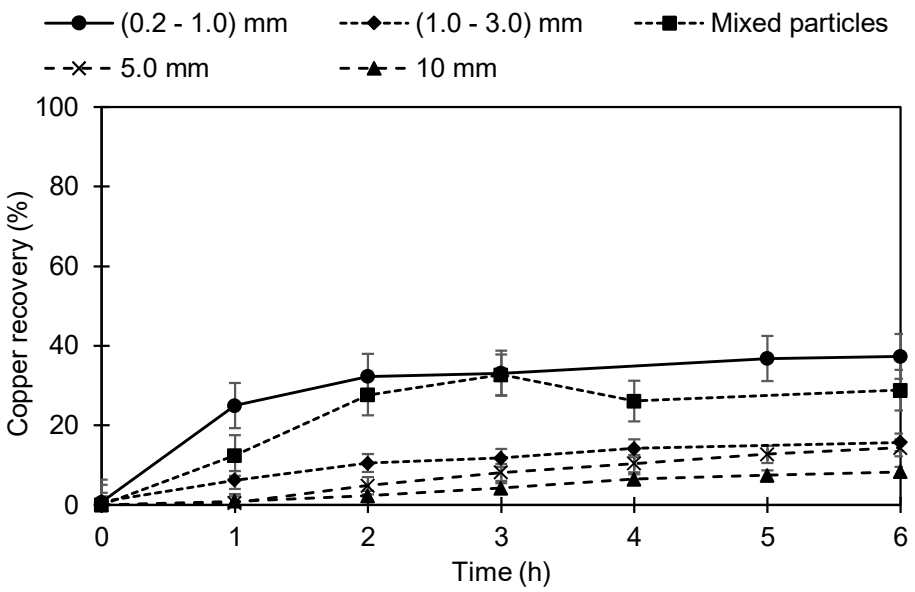

Figure 9.9. Copper recovery obtained when different particle sizes were used in the column leaching reactor.

9.3.6. Comparison of two different PCB concentrations

According to Xiang et al. (2010), increasing the amount of e-waste treated in bioleaching processes hamper the rates of copper extraction. They affirmed that the limitation of air distribution and oxygen mass transfer are the reasons why high PCB dosage resulted in low copper extraction when the microorganisms and the PCB are in contact during the whole process. Moreover, although the leaching agent is regenerated to be used again for more copper extraction, the velocity of copper oxidation is higher than that for the iron bio-oxidation. Hence, when an important amount of PCB wants to be treat, it is important to take into account the stoichiometry of the leaching reaction in order to assure enough availability of soluble iron (III) to extract all the target metal.

Almost all the studies focused on the PCB dosage have been carried out in batch conditions but this parameter has not been studied in column reactor. For this reason, two different PCB dosages were tested (7.5 and 15.0 g/L) in column reactor, based on the volume of the reservoir (see Figure 9.1). The experiments were performed at the best conditions found in the previous experiments that is adjusting the pH, small particle size (0.2 – 1.0 mm) and using a porous support, expecting the highest copper recovery possible. Iron (III) concentration and copper recovery at two different PCB dosages are shown in Figure 9.10.

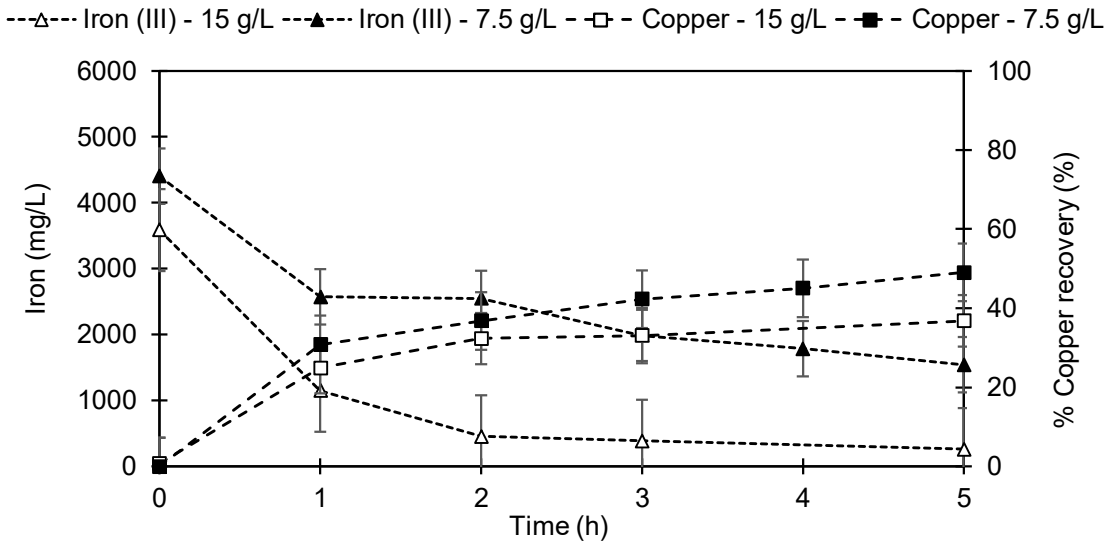

Figure 9.10. Iron and copper recovery over time when 7.5 and 15.0 g/L of PCB were treated in the column leaching reactor.

The copper recovery increased along time in both PCB dosages studied. However, 10% more copper was obtained when 7.5 g/L of scrap was treated during the 5 hours in which the experiment lasts. This behaviour was related to the iron (III) availability, since all the soluble iron (III) reacted before copper was completely removed from the e-waste when 15.0 g/L of PCB was treated. This means that in the case of a PCB dosage of 15.0 g/L the leaching reaction was chemically limited because the initial iron (III) was not enough to allow the total recovery of the copper contained in the scrap. Zhu et al. (2011) focused its study on the effect of the PCB concentration, testing 4, 8, 12 and 16 g/L of PCB in batch bioleaching. They found that the highest recovery (97.5% in 8 days) was achieved using 4 g/L of PCB, whereas the lowest one (73.3% in 8 days) was obtained with 16 g/L. Nevertheless, Adhapure et al. (2013) found that 20 g/L of PCB was the highest concentration in biological leaching processes that can be used. On the contrary, Brandl, Bosshard, and Wegmann (2001) concluded that high PCB dosages could be used when the microorganisms have been previously adapted, reaching treated concentrations up to 100 g/L of e-waste.

One must take into account that most of the microorganisms have been previously separated from the leaching solution used herein for copper extraction in the column reactor. Therefore, the iron (III) that allows oxidizing the copper from PCB was only that initially found in the leaching solution since iron could not be re-oxidized in this stage. For this reason, when the process takes place without the continuous entrance of fresh leaching solution, as in this study, the reaction will be chemically limited by the availability of iron (III). According to the stoichiometry of the leaching reaction between

copper and iron, when 6 g/L of iron (III) are used in the medium, no more than 3.4 g/L of copper could be extracted. Nevertheless, given the copper content in the PCB, when 7.5 and 15 g/L are treated, the highest copper concentration that could be achieved in the solution are 3.3 and 6.6 g/L of copper, respectively. Therefore, it is not possible to exceed 51% of copper extraction when 15 g/L of PCB are treated if a concentration of 6 g/L of iron (III) is used since it is consumed before all the copper was retrieved (Figure 9.10). For this reason, no more than 7.5 g/L of PCB could be treat using 6K mineral medium in the column reactor when it operates in a discontinuous mode. Therefore, a dosage of 7.5 g/L of PCB was selected for the following experiments.

9.3.7. Utilization of the plastic structure from mobile phones as packing material

It has been demonstrated that packed columns increase the efficiency of different processes, so it is extensively used in industrial chemical process such as distillation or extraction, among others (Wang et al. 2005). Regarding to packing materials, these could be of natural or synthetic origin. For instance, natural packing materials are commonly used like compost or wood, but also synthetic ones as polyurethane foam (PUF) in biofiltration field (Dorado et al. 2010). For this, the use of packing material was evaluated in the column bioleaching reactor. Taking into account that the plastic structure of the cell phones must be previously separated from the PCB to recover the metals by bioleaching, the use of these plastics as a packing material inside the column was tested. The idea is to take advantage of part of the waste that has not been treated in the bioleaching process. This would have the benefit of avoiding to separate the plastic materials from the mobile phones treated by bioleaching, which would allow the use of the raw materials matrix itself as a packing material in the process.

For the experiment, the plastic structure of the mobile phones was crushed and sieved, obtaining a particle size between 1 and 3 mm of diameter. Then, the plastic and the PCB particles were mixed before being introduced in the column reactor (Figure 9.11). In this sense, copper recovery was compared with and without packing material introduction (Figure 9.12).

Similar copper recovery and similar behaviour in both cases were observed, regardless of whether the packing material was used or not inside the column during the process. In particular, 50% of copper was extracted in 5 hours. Regarding to the iron (III) concentration, its value decreased as copper recovery increased in both cases, following the same behaviour. Nevertheless, 400 mg/L more iron (III) was consumed in the packed column although similar recoveries was achieved. Despite not having found studies focused on the use of packing material in column leaching reactors, it has been demonstrated that its use improves the contact between particles in other fields such as biofiltration or distillation (Cai 2018; La et al. 2018), as it has been mentioned at the beginning of this section. This improvement is based on the increase of the solid-liquid

contact, overcoming the mass transfer limitations that occur when no packing material is used. Moreover, the use of packing material facilitates the percolation of the leaching solution through the e-waste during the experiment, which corroborates the improvement of the solid-liquid contact and so the efficiency of the process expected.

Figure 9.11. Packing material and PCB particles used in the experiment in which the plastic structure of the mobile phones was evaluated as a packing material in the column reactor.

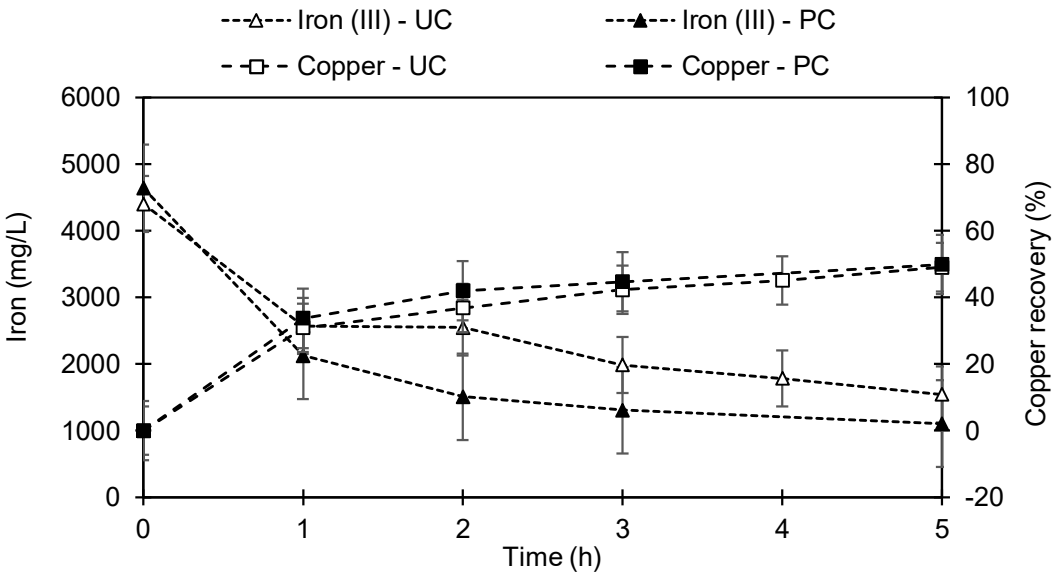

Figure 9.12. Evolution of copper recovery and iron (III) concentration during the column bioleaching using a packed column (PC) and a column without packing material (UC).

9.3.8. Effect of the contact time

An important factor in bioleaching processes is the experimental time. While its value should be reduced at industrial scale to increase the productivity, a minimum time is needed to complete the process. Until now, 6 hours has been usually tested since it has been observed in Chapter 6 that after this time lapse, no significant changes were

discerned in batch conditions. However, what happens in the process at higher times was tested in order to observe if there are any positive changes performing the bioleaching in the column reactor at the best conditions found. These conditions include pH control (between 1.7 and 1.8), the use of a porous support for the e-waste inside the column reactor as well as packing plastic material and a particle size between 0.2 and 1.0 mm of diameter with a pulp density of 7.5 g/L of PCB. Copper and iron (III) concentration were measured during 48 hours, and the experiment was repeated twice to prove its repeatability (Figure 9.13).

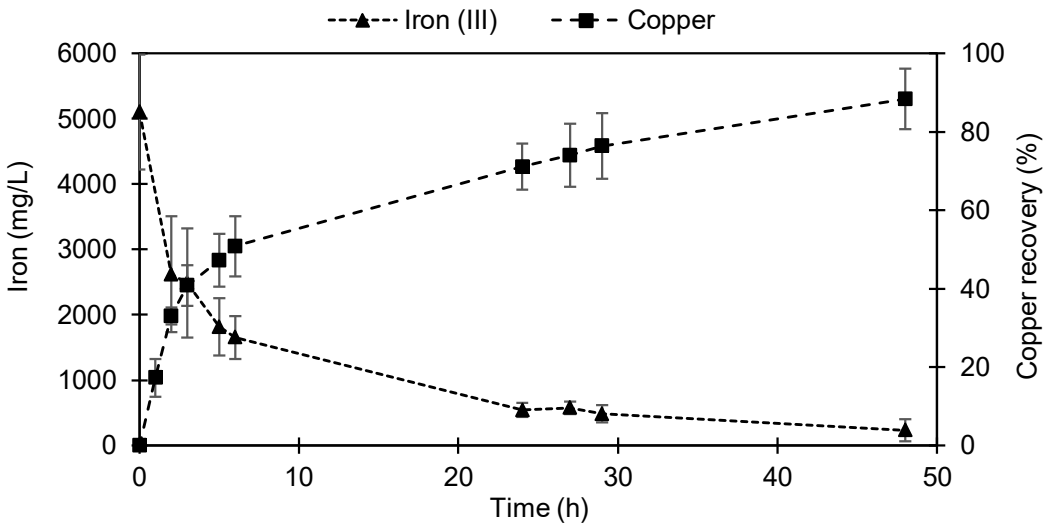

Figure 9.13. Evolution of copper recovery and iron (III) concentration in column reactor leaching with a particle size between 0.2 and 1.0 mm operated for 48 hours when the pH was controlled and packing material was used.

Almost all the copper contained in the e-waste was recovered in 48 hours. However, two clear tendencies were observed: in the first 6 hours 50% of copper was extracted at a rate of 258 mg/L of copper per hour, which extraction was quite similar than those found in previous experiments. Then, in order to reach 88% of copper recovery, 42 experimental hours were needed. In this case, the rate of copper extraction was only 29 mg/L of copper per hour. Hence, 50% of copper was recovered in 6 hours whereas it took 42 hours to recover 38% of the remaining copper from the scrap since the rate of copper extraction during the first hours was 8.9 times the rate after this time. Adhapure et al. (2013) also obtained similar copper recovery by bioleaching from printed circuit boards, requiring long time exposition. In particular, they spent 10 days to reach 96% of extraction. This time was reduced by Nie et al. (2014) who obtained the complete extraction of copper in 7 days. Hence, as many other authors, they need some days to recover almost all the copper contained in the scrap (Awasthi et al. 2016; Liang et al. 2013; Willner and Fornalczyk 2013). Nevertheless, Hong and Valix (2014) considerably reduced the experimental time, achieving high recoveries in 24 hours although they used high temperatures (90 ºC) and very low pH (1.0). It is worth noting that all these studies

have been bioleached in flasks which operational conditions are quite different from those used in the column reactor. Hence, present results demonstrate that column reactor achieved better results than those obtained in flasks, which is associating to the operation itself since in column reactor the liquid flows over a static surface, optimizing the contact between the waste and the leaching agent. The most similar study performed in column reactor with *Acidithiobacillus ferrooxidans* to recover copper from the e-waste was carried out by Chen et al. (2015), who spent 28 days to recover 94.8% of the metal. In this way, although the recovery of almost all the copper took 48 hours in the column reactor developed, the experimental time was significantly reduced more than 14 times.

Regarding iron (III) concentration, this decreased along time, being practically consumed in its entirely after 48 hours. Nevertheless, as for copper recovery, different tendencies on iron consumption were observed. In particular, three different trends were appreciated. The highest consumption rate was achieved during the first 6 hours of the process when it was consumed at a rate of 529 mg/L per hour. Then, the rate of consumption drastically decreased, being consumed at a rate of 61 mg/L per hour in the following 18 hours. After this time, the consumption was even slower since the rate was 14 mg/L per hour until its total consumption. It is noteworthy that the highest the iron concentration, the higher the consumption rate. This fact also implies that the bioleaching process accelerates because the greater the iron consumption, the greater the extraction of copper. Therefore, if the bioleaching's time want to be reduced without losing efficiency, it could result interesting to focus on when the iron concentration is high and thus, its consumption rate is also high.

The variance of the experimental data observed in some points is associated to the inhomogeneity of the e-waste since, as discussed in section 9.3.1, the metal composition of PCB varies, making difficult the repeatability of the experiments. This fact was also observed in all the experiments performed previously.

9.3.9. Improvement of bioleaching process to overcome previous limitations: cyclic operation

After different experimental tests, the best conditions were achieved since it was possible to recover almost all the copper contained in the PCB by the column reactor. Nevertheless, although the time was drastically reduced herein in comparison to the results reported in the literature (Awasthi et al. 2016), a new strategy was developed to try to further shorten the experimental time. In this sense, the study was focused on the bioleaching process in cycles based on the observation of two different kinetics in copper recovery. As mentioned above, half of the total copper recovered was obtained in 6 hours whereas the rest of the copper was extracted in 42 hours. Taking this into account, the new strategy consisted of doing the bioleaching in cycles of 6 hours. For each cycle, the

waste inside the column was stirred to avoid dead zones and stagnant regions and the leaching solution, containing iron (III), was renewed to avoid limiting reagent concentrations during the metal extraction. First, the process was performed in two cycles of 6 hours each and the experiment was done in duplicate.

As Figure 9.14 depicts, copper recovery increased to 48% in the first cycle whereas 40% of copper was recovered in the second one, achieving a total metal recovery of 88% in just 12 hours. This indicated that the waste stirring and the change of the leaching solution considerably reduced the experimental time, since the iron (III) could access to almost all the copper contained in the PCB. In addition, its concentration was always enough to oxidize the metal of interest. The iron (III) concentration decreased because of its reaction with copper, but when the next cycle started, the iron concentration was again the initial concentration of iron in the fresh leaching solution. In this sense, the iron concentration was always in excess during the leaching process.

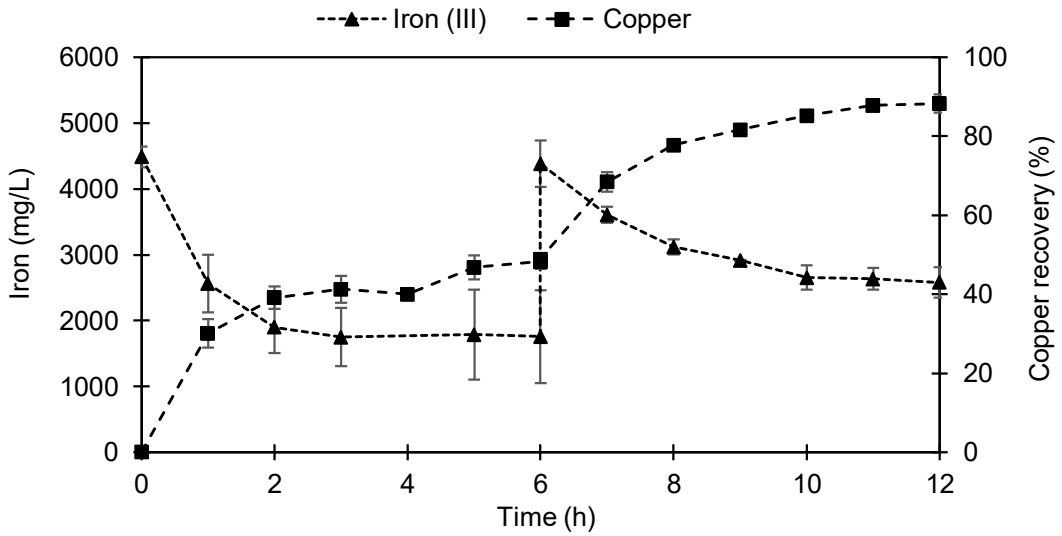

Figure 9.14. Evolution of copper recovery and iron (III) concentration during bioleaching in the column reactor performed in 2 cycles. A new cycle was started by stirring the material in the column and by using renewing the iron (III)-containing leaching solution.

It is should be noticed that similar amount of copper was recovered in 1 cycle of 48 hours (see Figure 9.13) in comparison to the copper retrieved in 2 cycles of 6 hours. However, when 2 cycles were done two different tendencies in copper recovery was also observed in each cycle as it was perceived in 1 cycle. In particular, an important part of the metal was extracted during the first 2 hours in comparison to the next 4 hours for each cycle. Therefore, the reduction of the cycle time to 2 hours instead of 6 hours was investigated. This meant that the waste was stirred and the leaching solution was renewed every 2 hours in order to observe, if the time required for this purpose could be reduced even more.

Figure 9.15 shows that the copper recovery steadily climbed to reach 80% of their extraction in 6 hours. On the contrary, iron (III) concentration decreased in each cycle as it occurred in the previous experiment and their concentration raised to its highest value at the beginning of each cycle. These results demonstrated that the strategy developed to bioleach in cycles allow to bioleach an important part of the copper contained in the PCB in just 6 hours. As in the previous experiments, the initial iron (III) concentration was not 6 g/L, as it was expected. This means that despite the iron (II) added in the medium was 6 g/L, not all of this iron was biologically oxidized and, consequently, the concentration of the metal at the beginning of the process was under 5 g/L. This fact could cause the chemical limitation of the reaction between iron and copper due to their stoichiometry (see Eq. 9.1). However, the leaching process performed in cycles avoid this limitation assuring the sufficient availability of the iron to recover all the copper. Therefore, this methodology allows high recoveries despite the fluctuations on iron (III) concentration in the leaching solution since it is maintained in excess during the whole process.

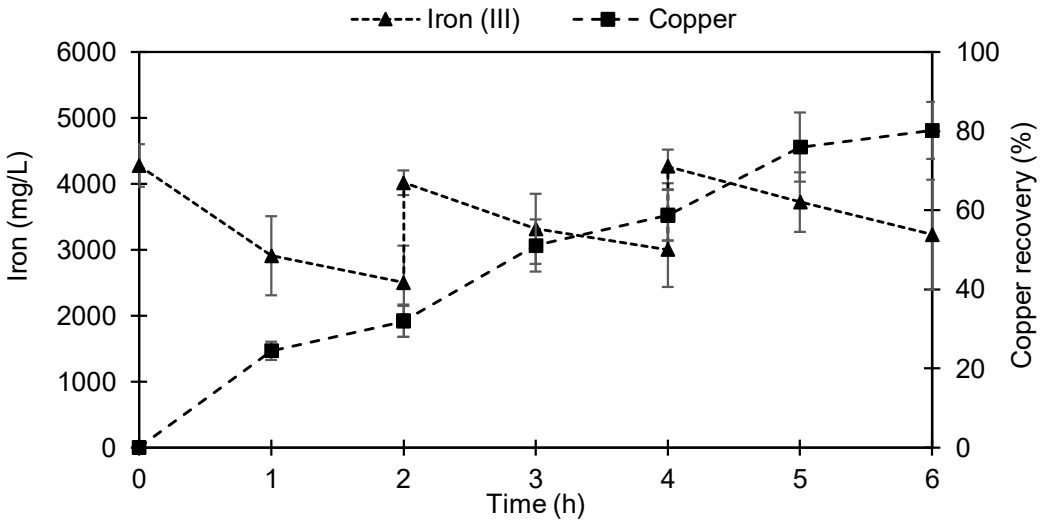

Figure 9.15. Evolution of copper recovery and iron (III) concentration during bioleaching in the column reactor performed in 3 cycles. A new cycle was started by stirring the material in the column and by using renewing the iron (III)-containing leaching solution.

In order to facilitate the comparison between the amount of copper obtained in the different bioleaching experiments performed in cycles, the copper recovery along time for each experiment has been represented in Figure 9.16. Despite similar extractions were obtained in all cases, the use of the new strategy developed allows to reduce the experimental time from several hours to just few ones. Nevertheless, it must be taken into account that the more number of cycles, the more dilution of the soluble copper since more leaching solution was used. This could be a handicap for the following processes where the metal has to be recovered from the solution. In the case of

electrolysis or cementation, the dilution of the metal does not difficult the metal recovery process but the use of high volumes may increase the time needed and the cost of the operation. Despite this fact, the new strategy developed is an important finding, since it allowed reaching attractive amounts of copper at competitive period time, being very promising the scale-up of the technology as an alternative to conventional processes.

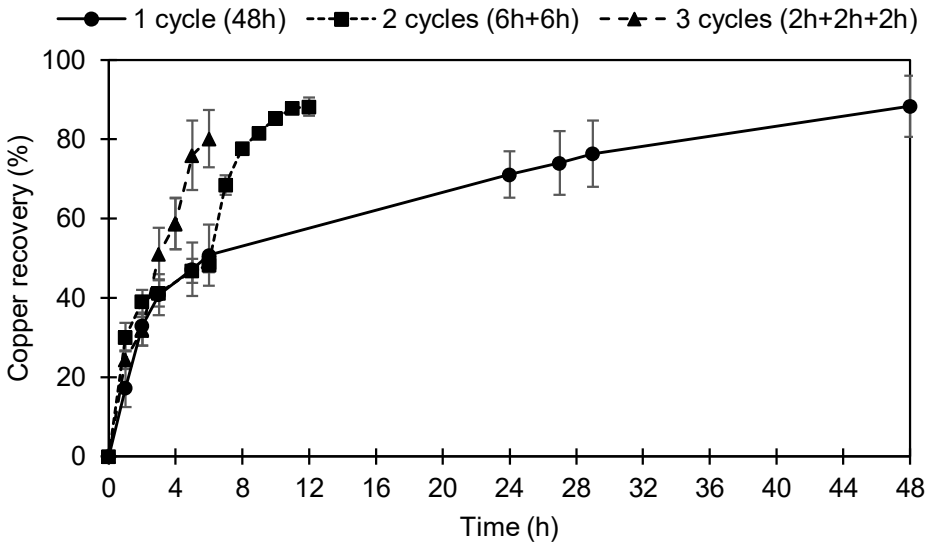

Figure 9.16. Comparison of the copper recovery over time in column leaching performed in one, two and three cycles. Data from Figures 9.13, 9.14 and 9.15 were replotted on the same timescale for comparison.

Since two procedures were performed in each cycle (waste stirring and leaching liquid renewed) the main effect that increased copper recovery and reduced the experimental time was investigated. For this reason, the procedure to bioleach in 3 cycles of 2 hours each was repeated, but in one case, the waste was not stirred, whereas in the other case, the leaching solution was not renewed. By comparing these two cases with the previous experiment in which both procedures were implemented, it should be possible to elucidate which methodology improved the metal recovery. Results of copper recoveries in this experiment are shown in Figure 9.17.

Despite having extracted copper in all cases, important differences on the metal recovery were observed. When only the leaching solution was renewed, without stirring the waste, the copper extraction rose to 49% in 6 hours. This result was quite similar to those obtained before the implementation of the new strategy consisting of bioleaching in cycles in which 50% of copper was obtained in the same time. Hence, no improvement on metal recovery was observed in the latter case. In contrast, when the waste was moved, although the medium was not renewed, the recovery of the metal increased, achieving 73% of the extraction during the same period of time. This fact indicated that the stirring of the waste improved the extraction, suggesting that the movement of the

waste allowed the leaching solution to access to all the dead zones or inaccessible parts of the scrap. From the results observed, it can be concluded that the application of the two procedures (renovation of the leaching solution and moving the waste) really improved the copper extraction since 80% of the copper contained in the PCB was retrieved in just 6 hours. Therefore, although the movement of the waste resulted more efficient than the renovation of the leaching solution in terms of copper extraction, the use of both procedures together had a better response in the process, increasing the recovery of copper over 80% in only 6 hours. The results obtained herein significantly improved the copper recovery and drastically reduced the time required for this purpose in comparison to the studies found in the literature focused on column bioleaching. For instance, Chen et al. (2015) spent 28 days to recover 94.8% of copper, Ilyas et al. (2013) needs 165 days to achieve 85% of copper recovery and Jagannath et al. (2017) recovered 63.5% of copper in 5 days. Nevertheless, it is noticed that all of them used different operational conditions than those applied in the present work, but no similar studies were found in the literature for a more detailed and comparative discussion.

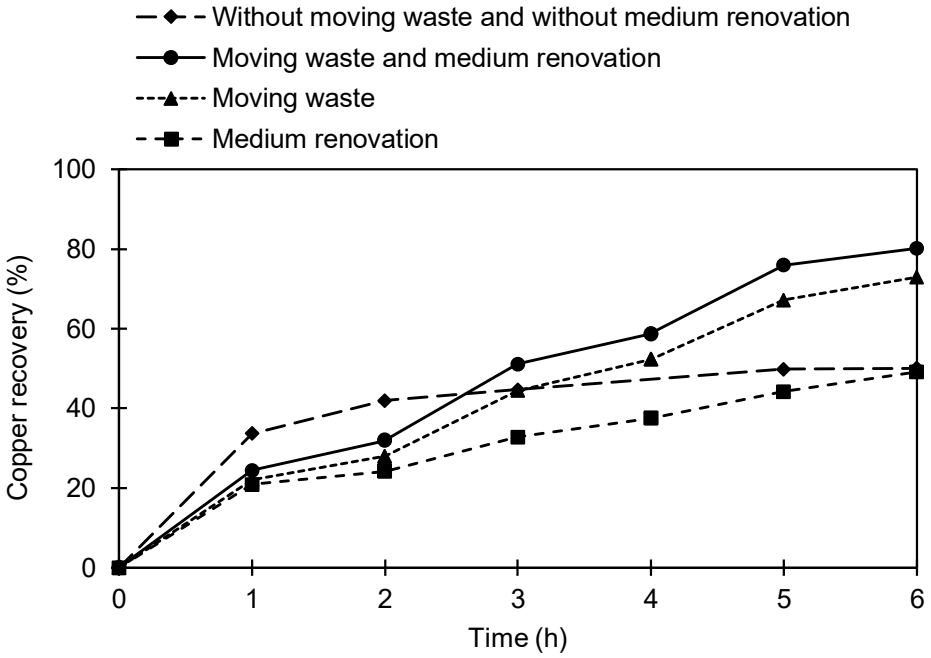

Figure 9.17. Effect of moving the waste and/or renewing the leaching solution during column bioleaching in 3 cycles.

9.4. Conclusions

After testing different conditions and evaluating different parameters during the bioleaching of copper from printed circuit boards in a column reactor, it was concluded that the performance of this process is feasible and improves the copper extraction

obtained in batch experiments. However, in order to obtain the highest metal recovery, it is important to maintain specific operating conditions.

Regarding the parameters studied, it was concluded that, despite not having better recoveries when the pH was adjusted, pH control is essential to assure the solubility of the leaching agent (iron). However, this work demonstrated that iron (II) was not oxidised at the conditions tested in the column, which means acid pH and room temperature, so only previously oxidized iron (III) could solubilise the copper contained in the PCB. The fact that in the first experiments performed in the column reactor copper recovery did not exceed 50% of extraction in 30 hours was mainly associated to the mass transfer limitation between the leaching solution and the e-waste. For this reason, different strategies were implemented in order to improve the contact between the leaching solution and the scrap placed inside the column.

It was concluded that using a flooding column did not improve the process, since only 35% of cooper was obtained with this system. However, better hydrodynamic characteristics were observed when a porous support was used instead of a mesh support, achieving 11% more recovery with the former in only 6 hours. In addition, this work demonstrated that particles between 0.2 and 1.0 mm of diameter allowed higher recoveries than those over 1.0 mm of diameter, achieving 37% with the smallest size tested. The use of mixed particles (50% between 0.2 and 1.0 mm and 50% over 1.0 mm) was also tested with the purpose of using the large particles as a packing material, thus, facilitating the contact between the leaching solution and the e-waste. Unfortunately, the results did not show an improvement when the mixed particles were used (28% of copper recovery). This was associated to the different copper concentrations observed for the different sizes, the small particles having the size with the highest copper concentration (around 39% of copper).

This chapter also demonstrated that the PCB dosage is an important factor for the process. Whereas 7.5 g/L of PCB allowed 50% of copper extraction in 5 hours, 15 g/L of PCB only recovered 37% of the metal in the same period. In addition, it was concluded that concentrations over 7.5 g/L of PCB could not be used in column bioleaching when it operates in discontinuous mode, since the amount of iron contained in the leaching solution would not be enough to oxidize all the copper contained in the scrap. In the case of continuous operation, however, this impediment would be avoided by the constant entry of fresh leaching solution. Although no improvement was observed by the use of large particles as a packing material, the use of plastic particles was tested with the same aim. Similar recoveries were obtained regardless of using packing material

or not, but it was noticed that the use of packing material gives better percolation, thus avoiding the caking of the waste inside the column. This is an important finding, especially when the process is intended to be scale-up to treat high volumes of waste.

Finally, after observing that the improvements observed did not achieve the complete extraction of copper, the operation over longer time was evaluated, since almost all the previous experiments had been carried out in 6 hours. Hence, it was concluded that 48 hours were required to recover 88% of the copper at the best conditions previously found. These conditions included pH control at 1.75, porous support to hold the PCB, particles between 0.2 and 1.0 mm, 7.5 g/L of PCB dosage, and plastic particles as a packing material.

The new strategy developed in the present work to bioleach in cycles allows reaching attractive amounts of copper in less time. In particular, it was observed that 88% of copper was recovered in 12 hours (2 cycles of 6 hours each). However, this result was even improved by reaching 80% of copper in only 6 hours (3 cycles of 2 hours each). However, in order to obtain the highest extraction, it was important to stir the waste as well as to renew the leaching solution between cycles, since the application of only one of these two procedures did not obtain such good results. This behaviour occurred since the particles of the e-waste treated inside the column are static while the leaching solution is irrigated. This makes it more difficult for the leaching agent to access the internal parts of the scrap. So moving the waste improves its accessibility. In addition, due to the medium renovation, the concentration of the leaching agent was also increased, thus accelerating the rate of metal recovery.

This led us to the conclusion than the bioleaching process performed in the column reactor, especially when the strategy developed herein is used, makes this technology very promising as an alternative to conventional processes at industrial scale.

Chapter 10

Recovery of copper from leaching solutions and semi-continuous bioleaching pilot plant proposal

The main motivation of this chapter was to investigate a technique to recover leached copper from the solution and, thus, to close the global process of bioleaching, separating the metal from the matrix in which it is found initially. In this sense, the bioleaching process investigated in this thesis could be concluded by recovering metallic copper from the e-waste. This completes the study of copper recycling by recirculating the solution to close the overall process as a cyclic system without the need of constant reagents addition. The cementation process was the technique tested to recover the bioleached copper for its simplicity and its low-cost. In addition, based on the knowledge acquired, in this chapter a process diagram is proposed as basis for bioleaching of copper from e-waste, including all the steps to perform the recovery in an automated and semi-continuous-operation pilot plant.

Abstract

In this chapter, a cementation technique was investigated to recover copper from a solution in its metallic state. Preliminary assays were performed using a synthetic copper solution and scrap iron to test the efficiency of the process. Then, the technique was proved using leaching solution. The results revealed that the cementation procedure allows recovering 100% of the copper from the liquid solution as a metal in just 2 hours. In addition, these good results were also obtained when the leaching solution was used. It means that the other bioleached metals in the solution did not affect the efficiency of the process. Therefore, the cementation was considered a good process for its high velocity and its simplicity. However, the copper powder obtained after the cementation was not so pure (below 70%), which would reduce the number of possible applications of this material, though the main impurity obtained together with the copper powder was iron. To increase the purity other processes, such as solvent-extraction and electrowinning, should be carried out instead of cementation. Average values of 2.96 g of copper powder from one litre of bioleaching solution were obtained. Finally, in this chapter a pilot plant diagram is proposed to continuously recover copper from PCBs and convert it back to metallic copper. In this proposal, the global process of bioleaching was divided in four different steps: the biological oxidation of iron (II) by the microorganisms, the separation of the biomass from iron (III) solution by a settler, the extraction of copper

from e-waste using a column reactor and the recovery of metallic copper from the leaching solution by cementation.

A modified version of part of this chapter has been published as:

Benzal, E., Solé, M., Lao, C., Gamisans, X., Dorado, A.D., 2020. Elemental copper recovery from e-wastes mediated with a two-step bioleaching process. Waste and Biomass Valorization, 11, 5457-5465.

10.1. Introduction

After the leaching process, the metals are found in aqueous solution in their ionic forms. There are different methods to recover these metal ions in their metallic state (Agrawal and Kapoor 1982; Khattab et al. 2013; Zhang et al. 2010). Among the methods, one of the simplest and cheapest one is cementation, which consists of precipitating a metal ion from a liquid solution by a more reducing metal (Jhajharia et al. 2016). This metholology has been extensively used to remove toxic metallic ions from solutions and is still used in hydrometallurgy, surface waste treatment and electrolyte purification (Djoudi et al. 2007). Although the abovementioned advantages, some authors affirmed that the main disadvantage is excess sacrifical metal consumption (Agelidis et al. 1988). However, the cementation is commonly performed by using metals scrap (Tzaneva et al. 2016), which also reduces the amount of this kind of waste.

According to Dib and Makhloufi (2004), cementation of copper is usually performed by iron. This affirmation was also supported by Stefanowicz et al. (1997) who affirmed that this fact ocurred due to the difference between the standard reduction potential of these metals (Eqs. (10.1) and (10.2)).

$$Cu^{2+} + 2\,e^{-} \rightarrow Cu^{0} \quad (E^{o} = +0.34\ V) \qquad (10.1)$$

$$Fe^{2+} + 2\,e^{-} \rightarrow Fe^{0} \quad (E^{o} = -0.44\ V) \qquad (10.2)$$

Hence, iron reacts spontaneously with copper ions following Eq. (10.3) (Anastassakis et al. 2015).

$$Cu^{2+} + Fe^{0} \rightarrow Cu^{0} + Fe^{2+} \qquad (10.3)$$

Therefore, iron cementation could allow to close the process to recover copper from the bioleached solution. On one hand, several authors have studied the bioleaching process but avoiding the final metallic copper recovery step (Annamalai and Gurumurthy 2019; Işıldar et al. 2019; Priya and Hait 2017). On the other hand, there are other authors focused on the cementation process to recover copper, as it was abovementioned. Although few authors have been studied both processes together (Agate and Khinvasara 1986; Rossi et al. 1986), no articles were found proposing laboratory or pilot plants operating in continuous or semi-continuous mode for this purpose. In general, these authors have been studied the process at laboratory scale using flasks.

The aim of the work presented in this chapter was to test the viability of cementation process to recover copper from the leaching solution. The first approach was performed by the study of copper cementation from a copper (II) sulphate dissolution to test the viability of the process. Then, the methodology was applied to recover metallic

copper from the leaching solution obtained in the previous experiments. In addition, the obtained copper powder was analysed to determine its purity as well as its morphology by a SEM microscope. Finally, in this chapter a possible diagram for a continuously operated pilot plant to recover copper by bioleaching process is proposed. The system includes from the biological oxidation of iron (II) to the cementation to obtain metallic copper, going through the leaching of the e-waste in a column reactor and the biomass recirculation.

10.2. Materials and methods

10.2.1. Scrap iron

The scrap iron (actually steel was used) used for cementation tests was provided by the Mechanical Engineering Department from Universitat Politècnica de Catalunya. The iron scrap was obtained from the residues produced by a lathe machine. The composition of the iron used was 97.26% iron, 0.40% carbon, 0.32% silicon, 0.80% manganese, 1.03% chromium and 0.19% molybdenum (analysis performed by Centre Tecnològic de Manresa, Spain). The scrap iron was placed in contact with the leaching solution in small pieces of 10 mm length and 1 mm of diameter, approximately.

10.2.2. Cementation experiments

For the preliminary cementation assays the process was tested using a solution of $CuSO_4 \cdot 5\ H_2O$ and scrap iron. In particular, a solution of 0.4 M of $CuSO_4 \cdot 5\ H_2O$ was used, corresponding to a copper concentration of approximately 2500 mg/L and 1.0 g of scrap iron in pieces of 10 mm length and less than 2 mm of diameter. The experiments were performed in triplicate.

For cementation experiments, 100 mL of the bioleaching solution were placed in a 250 mL flask with 4 g/L of scrap iron in small pieces. Considering that the average copper concentration obtained after bioleaching was 3000 mg/L, 4 g/L of iron filings were used since an amount greater than what is stoichiometrically needed improves the velocity of the process (Dib and Makhloufi 2004). The process was performed in an incubator (*SI500, Stuart, United Kingdom*) at 130 rpm by orbital agitation and at room temperature. Samples of the liquid were taken every hour for copper and iron analysis as well as for pH and ORP measurements. When the cementation process finished, the solution and the solid copper were separated by decantation, drying the solid copper by

a laboratory heater and analyzed for its composition by Energy-Disperse x-ray Spectroscopy (EDS) analysis and atomic absorption spectroscopy (performing a previous acid digestion of the powder sample). The experiments were performed in triplicate.

10.3. Results and discussion

10.3.1. Preliminary assays

10.3.1.1 Copper recovery from synthetic solutions by cementation

The suitability of the cementation process to recover metallic copper by the addition of iron has been investigated, according to Eq. (10.3). In this sense, the preliminary cementation experiments were done by using a solution of $CuSO_4 \cdot 5\ H_2O$ and scrap iron. Results of iron and copper concentration evolution during the experiment are shown in Figure 10.1a. It was observed that that reaction between scrap iron and copper (II) was very fast since almost all the soluble copper was reduced to metallic copper in only one hour. As a consequence, iron (II) concentration increased but remained constant after 2 hours when all the copper has been reduced. It is noticed that iron was oxidized at this conditions after 4.5 hours, but the amount of iron oxidized after the first 2 hours when the cementation process took place was insignificant. A pH increasing was also observed in the process (Figure 10.1b). In particular, the pH increased from 2 to almost 5, which could cause iron precipitation since no pH control was carried out. Nevertheless, the precipitation was not appreciated in the analysis since the concentration of iron (II) observed after 4.5 hours was the corresponding to the stoichiometry of the cementation reaction (Figure 10.1a). The cementation has the advantage that the solution obtained after the process contains high amount of iron (II), which could be used as an energy source for the microorganisms again (Xiang et al. 2010). However, if the solution obtained after the cementation needs to be re-introduced in the bioreactor, it is important that the cementation solution maintain acidic pH values to reduce the interferences that this fact could cause to the bioreactor. Therefore, it is more interesting to stop the cementation process after 2 hours since the main reaction was completed at this time, avoiding pH increasing and also precipitation problems. Regarding ORP measurements, this parameter constantly decreased until reaching negative values (-85 mV). Then the signal slightly increased to 20 mV after 4.5 hours. This behaviour has not been observed before in the literature since the authors focusing on cementation studies did not measure this key parameter (Moradkhani et al. 2011;

Stefanowicz et al. 1997). Nevertheless, the decrease of ORP was associated to the increasing concentration of iron (II) and the low iron (III) concentration in the solution (the higher concentration of reduced species, the higher the signal of the ORP, Silva et al. 2015) in leaching studies. Therefore, this parameter can be used to control de process. In this case, when the cementation process was completed and no more copper was in the solution, the iron (III) concentration slightly increased and so, the ratio between iron (II) and iron (III), which was also related to the increase observed in ORP measurements after the first 2 hours.

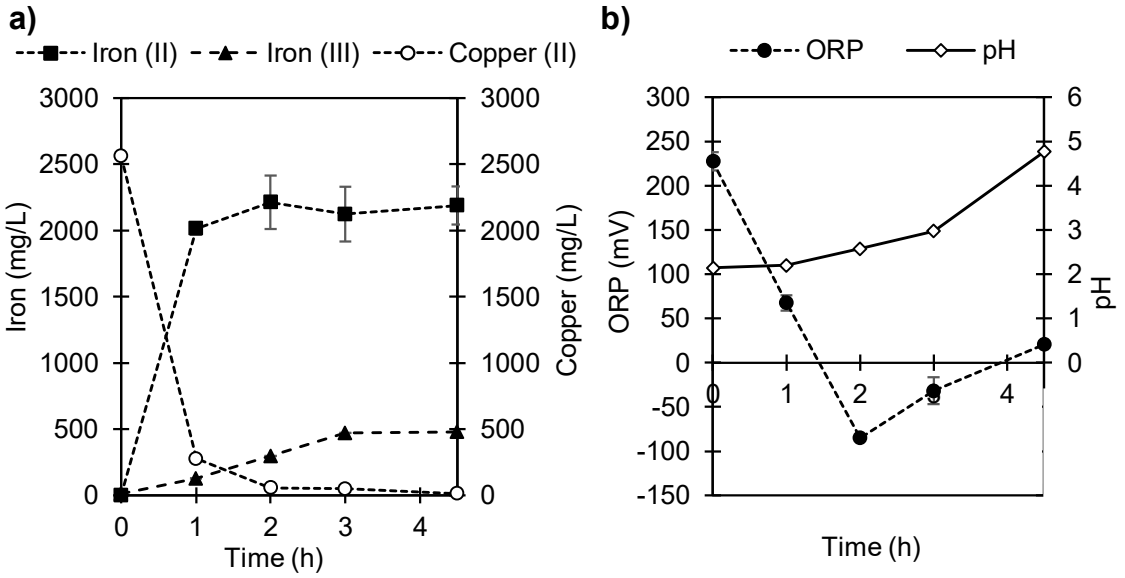

Figure 10.1. (a) Evolution of iron and copper concentrations and (b) evolution of pH and ORP in the preliminary tests of cementation to recover metallic copper from copper sulphate solution.

10.3.1.2. Copper recovery from bioleached solutions by cementation

The aim of the cementation process was to recover metallic copper from the leaching solution. However, this leaching solution did not only contain copper, since many other substances could be also found in the leachate as it was observed in Table 7.1 (Chapter 7). Nevertheless, the analysis carried out in Chapter 7 was done after 16 days of experimentation with a particle size of 0.5 cm^2. Hence, after observing that the leaching was completed in less time when it was performed in the column reactor using a particle size between 0.2 and 1.0 mm of diameter, the leaching solution in that case was also analysed. The results were resumed in Table 10.1.

Table 10.1. Concentration of the metals analysed in the leaching solution after leaching of PCBs in the column reactor and before to be used in the cementation process.

Metal	Concentration (in mg/L)
Al	3.00
Pd	< 0.01
In	< 0.01
Sn	0.15
Pb	2.25
Ni	28.4
Cu	3140
Fe	4100

As can be observed, the leaching solution basically contains iron and copper, as it was expected, since the iron (III) generated by the *Acidithiobacillus ferrooxidans* is mainly used to extract copper at the conditions in which bioleaching takes place (Hubau et al. 2018). Also other metals were leached in the process such as nickel (28.4 mg/L) or aluminium (3.00 mg/L), although their concentrations were much lower than that of copper.

Although the same waste was treated in Chapters 7 and 10, different metal concentrations were obtained in the leaching solutions, which is related to the use of two different systems (CSTR and column) as well as the use of different particle size and different experimental time. The main difference was the amount of copper extracted since 1607 mg/L was obtained when the CSTR was used whereas the column reactor allowed extracting 3137 mg/L of copper. This fact implies that the column reactor extracted more copper than the CSTR but the difference on the efficiency was mainly related to the particle size. As in Chapter 9 was observed, when the effect of the particle size was studied, small particles have a greater surface area and thus, greater accessibility of the leaching agent to the e-waste, which increases the efficiency of the extraction. Nevertheless, regarding the other solubilized metals during the leaching process, similar concentrations were found in both analyses since all of them were found as trace elements at low concentrations.

The preliminary assays carried out with synthetic chemical solutions (section 10.3.1.1) allowed to test the cementation technique and its efficiency. For this reason, further step consisted of using the solutions obtained from previous leaching experiments, which composition was described in the Table 10.1. This experiment was carried out in 2 hours according to the experiment described in 10.3.1.1. Copper and iron

concentrations along the experiment are presented in Figure 10.2a whereas the pH and ORP measurements are presented in Figure 10.2b.

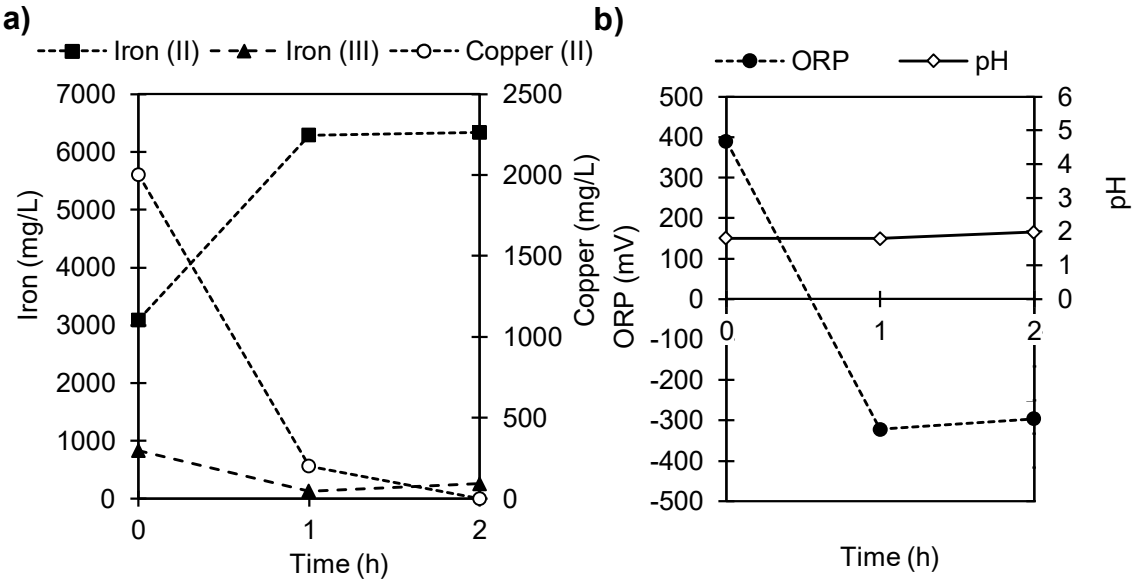

Figure 10.2. (a) Evolution of iron and copper concentrations and (b) evolution of pH and ORP measurements over time during the cementation of the leaching solution.

As it can be observed in Figure 10.2a, copper concentration decreased from 2000 mg/L to 0 mg/L in only 2 hours. It means that all the soluble copper reacted with the scrap iron, obtaining metallic copper which is insoluble. As it was observed in the last cementation experiment, the iron (II) concentration increased as a consequence of the reaction between the scrap iron with copper (II), which produces the oxidation of the scrap. The iron (II) concentration was found to be 6300 mg/L after the cementation process. On one hand, 3000 mg/L were initially in solution and come from the bioleaching process in which the ferric ions were reduced to ferric ones by their reaction with the PCB. On the other hand, 3300 mg/L were obtained by the oxidation of the metallic iron. It is noteworthy that iron (II) was not practically oxidized at the conditions tested since the iron (III) concentration remained below 850 mg/L during the whole experiment.

Regarding pH measurements (Figure 10.2b), it was observed that it remained constant at pH 2 during the 2 experimental hours. It means that the cementation reaction did not alkalinize the solution in this period of time, unlike the previous experiment in which pH changes were observed after the first hour. This is mainly associated to the time contact since the main pH changes occurred after 2 hours in the preliminary assays. This fact favours the use of the solution obtained as a feed for the bioreactor due to its high iron (II) concentration, although the concentration of toxic metals has to be evaluated before its recirculation as it was explained in Chapter 8. In relation to ORP measurements, a similar behaviour to the preliminary cementation assays was

observed. The oxidation-reduction potential decreased from 400 mV to near -300 mV in the first hour whereas it remained constant at this negative value in the following hour, when the cementation reaction was almost completed and the iron (II) and iron (III) concentrations remained constant. As it was abovementioned, the ORP measurements were related to the oxidation and reduction species in the solution. Hence, its value decreased when the ratio between iron (II) and iron (III) also decreased (Silva et al. 2015).

Comparing the preliminary results to these obtained when leaching solution was used, it could be observed similar behaviours since the chemical reaction between the scrap iron and the copper was achieved in the same period of time. It means that the rest of the chemical compounds that could be found in the leaching solution did not affect the cementation reaction and, as a consequence, the efficiency of the reaction was unchanged. In addition, stopping the process after 2 hours improve the process in relation to pH changes, because at this time the pH did not change significantly. Hence, the cementation resulted efficient to recover metallic copper from leaching solutions, allowing to recirculate and take advantage of the obtained iron (II), which is a key factor in the bioleaching process.

10.3.2. Closing the loop: copper recovery from PCB to copper powder

Finally, the cementation process was tested with leaching solution but starting the monitoring of the process from the first step, that is, from the biological oxidation of iron by the microorganisms. Nevertheless, the steps before the cementation was not evaluated along time, but the solutions were analysed at the beginning and at the end of each stage. In addition, in this experiment the purity of the metallic copper obtained was evaluated and its morphology by SEM microscope was also observed.

The first stage was the oxidation of the iron (II) from the 6K mineral medium by the activity of *Acidithiobacillus ferrooxidans*. In this case, the iron (II) and iron (III) concentrations were measured just before to take the solution to be used in the leaching stage. In particular, it was obtained that the average iron (II) and iron (III) concentrations after the biological oxidation were 61 and 5150 mg/L, respectively. Therefore, these were the initial iron concentration in the leaching step. After the biomass separation from the iron solution by sedimentation, it was put in contact with 15 g/L of PCB powder in a 500 mL Erlenmeyer flasks. The initial pH of the solution was 1.76 and the ORP was 554 mV and the leaching experiment was performed in 6 flasks, in order to evaluate the repeatability of the process. The flasks were incubated at 30 ºC and 130 rpm during 6 hours. Results demonstrated that 2871 mg/L of copper were recovered during the leaching step with a standard deviation of 54 mg/L. At this time, the solution also contains

4973 mg/L of iron (II) and 495 mg/L of iron (III). The pH was 2.14 and the ORP was 347 mV. Finally, the cementation was carried out. In this case, results of the iron and copper concentrations along time are shown in Figure 10.3a whereas the pH and ORP measurements are shown in Figure 10.3b.

As can be observed in Figure 10.3a, the behaviour of the iron and copper concentration was the same than the previous experiments. Nevertheless, it is noticed that the iron (II) concentration at the beginning was higher than the concentration observed in the earlier cementation. However, the increased was virtually the same since 3000 mg/L of iron (II) was produced in the cementation reaction. It occurred because the initial copper concentration in both experiments was the same, so stoichiometry the same amount of iron (II) was obtained during the process. In addition, as it occurred before, the pH remained quite constant around pH 2 and the ORP measurements decreased until negative values.

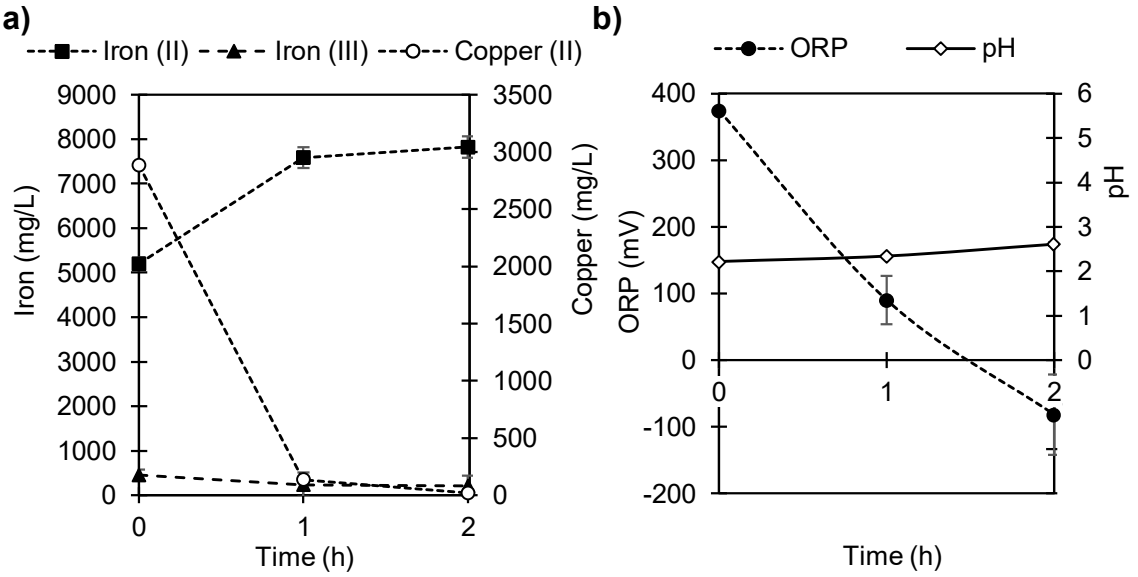

Figure 10.3. (a) Evolution of iron and copper concentration and (b) evolution of pH and ORP measurements over time in the cementation process after the development of all the bioleaching steps.

As expected, the last two experiments obtained very similar results since, although the different steps of the global bioleaching process were performed at different times or sequentially, the metal concentrations at the beginning of the different stages were comparable.

Despite not having detailed before, mechanical friction produced by the stirring between the copper powder and the metallic scrap iron, makes the copper to break off from the layer deposited on the iron metal. This fact was also observed by Stefanowicz

et al. (1997) who affirmed that the mechanical friction between plates results in scraping of the copper layer deposited on the iron plates. In addition, these authors suggested that this behaviour favour a continuous cementation route.

For that, copper was obtained as a fine brown powder during the cementation (Figure 10.4). As it was above-mentioned at the beginning of this section, this powder was analysed by AAS after its acid digestion, revealing that the content of copper, nickel, iron, gold, silver, aluminium, palladium, indium, tin, lead, cobalt and manganese in mg/kg was 648000, 57, 208879, 11, 57, 33, 5, 5, 59, 150, 11 and 1414, respectively. As it was found in the original PCB used in bioleaching, the highest metal concentration was copper, followed by iron. It was assumed that the iron found in the copper powder was related to the iron scrap used during the cementation that could not be separated correctly from the metallic copper obtained. As a consequence, this iron could be mixed with the copper.

Figure 10.4. Metallic copper obtained as a fine brown powder by cementation.

After cementation, from a litre of bioleaching solution 2.96 g of metallic copper with a purity close to 70% were obtained, which means that other impurities have been also cemented, as it has been explained above. This fact could affect the final use of the copper as raw material (Alers et al. 2004). Nevertheless, cementation is a very low-cost and simple process to obtain metallic copper from a solution. Even though, if more purity is necessary for its application, other techniques after cementation could be applied such as solvent-extraction or electrolysis (Sinha et al. 2018; Zhang et al. 2010).

In Figure 10.5, the morphology of the powder obtained by SEM microscope is observed. The images demonstrate that copper was crystallized as small spherical structures. In addition, the sample was also analysed by EDS (Figure 10.6). The EDS spectrum revealed the presence of copper and iron, essentially. These results are in

accordance to the results obtained with the acid digestion, in which copper and iron were the majority components. Hence, conducting a good separation of the remained scrap iron and the copper powder could improve the purity of the copper after the cementation.

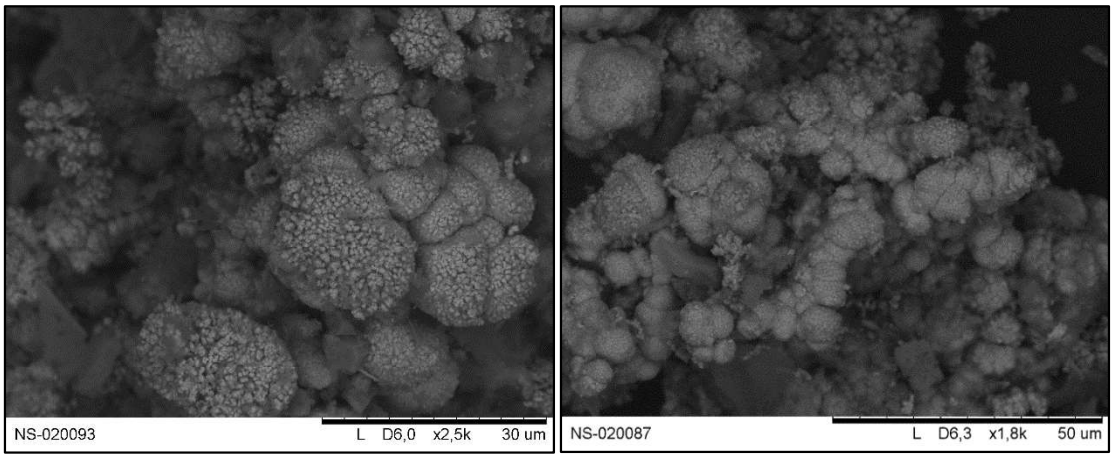

Figure 10.5. SEM images of copper powder obtained by cementation at 2500x (left) and 1800x (right) magnification.

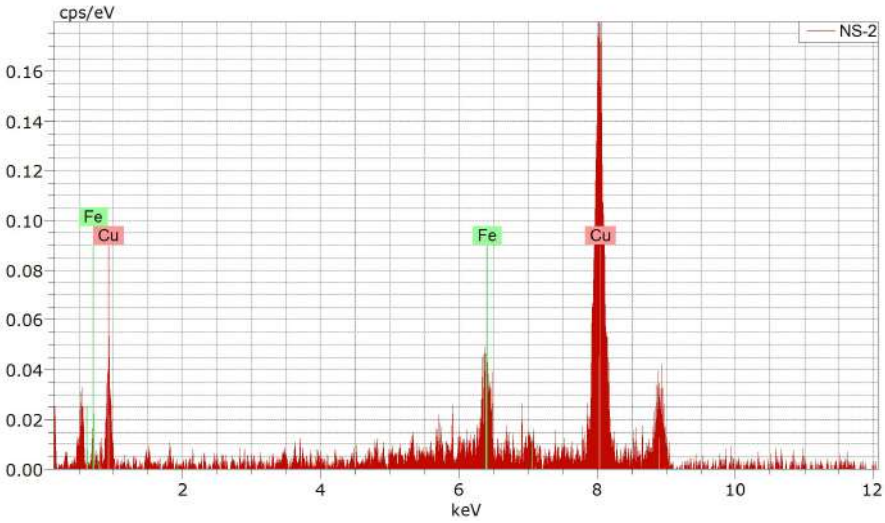

Figure 10.6. EDS analysis of the copper powder recovered by cementation.

10.3.3. Bioleaching pilot plant integration

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10.4. Conclusions

In this chapter, the efficiency of cementation to recover copper as a metal from copper solution has been tested. The preliminary assays revealed that the cementation was a very fast method to recover metallic copper, which needed only 2 hours for 3000 mg/L of copper to react with the iron. However, these results were obtained using a synthetic solution of copper (II) sulphate. Hence, the same procedure was performed using the leaching solution from previous experiments. It was concluded that the process took place at the same velocity and with the same efficiency. Thus, the rest of the metals found in the leaching solution obviously did not interfere in the process.

In the experiments on copper recovery from PCBs to copper powder, a similar behaviour was obtained as in the cementation in preliminary assays, since the iron and copper concentrations obtained after the leaching step were similar to those of the bioleached solution used in the preliminary cementation assays. This experiment demonstrated that the efficiency of the process is not affected by the fact the process took place sequentially or in different time intervals. In this experiment the copper powder obtained after cementation was also analysed by measuring the metal content of the digested copper powder. The major metal concentrations found in the digestate were copper and iron. This was explained by to an inadequate separation of the copper powder from the scrap iron used for cementation. In the current experiment, the purity of the copper powder was below 70%. However, if the separation between copper and iron could be improved, higher copper purities could be obtained with a cementation process. Hence, this technique could be an efficient technique to recover bioleached copper due to its simplicity and its low operational cost. At the same time cementation allows to recirculate the obtained iron (II) to be used again as an energy source for the biomass in the bioleaching process.

Finally, in this chapter a scheme for a pilot plant is proposed, which allows metal recovery in semi-continuous mode from PCB. In this pilot plant it could be appreciated that the global bioleaching process has been separated in four different steps: the biological oxidation of the iron (II), the separation of the biomass from the iron (III) solution, the leaching of the PCBs and the recovery of metallic copper from the bioleached solution by cementation. By the proposed diagram, the intention is to propose a new methodology based on the experiments performed herein to recover copper or other metals from PCBs in a semi-continuous mode. From this point, the process could

be scale-up to take place in an industrial environment under semi-continuous and automatic control.

Chapter 11

General conclusions and future work

11.1. General conclusions

This thesis is focused on the investigation of the bioleaching process to recover valuable metals, especially copper, from metal-containing materials. In order to achieve this objective, the basis of the methodology was established and different conditions and procedures were analysed and implemented. As a result, several experiments were performed under different conditions and in different bioleaching systems such as flasks, stirred-tank reactors and columns, thus allowing to find the highest copper recovery in the shortest period of time. Moreover, biological parameters were also studied such as the effect of some bioleached metals to the microorganisms involved in the process or the effect of substrate inhibition.

In general, this thesis led to conclude that a bioleaching process performed in a column reactor, especially when the cyclic strategy developed is used, makes this technology very promising as an alternative to conventional processes at industrial scale. However, the presence of leached metals affects the activity of the microorganisms, depending on the concentration of those metals as well as the contact time. In this sense, microrespirometry turned out to be an efficient methodology to monitor biomass activity, especially when the biotechnological process takes place, avoiding a limitation of traditional methods such as precipitation effects. The optode methodology allows using a small sample volume (less than 2 mL) to obtain the oxygen consumption of the biological sample directly at real time. Finally, this thesis allowed to conclude that cementation is a very fast method to recover metallic copper from the leaching solution, since the rest of the bioleached metals do not interfere in the cementation process.

More specifically, the conclusions derived from this thesis are listed below.

- A mixed consortium of microorganisms obtained from a lab-scale gas-phase biotrickling filter treating high loads of H_2S has proved efficient for copper bioleaching from chalcopyrite, although a previous adaption of the culture allowed to nearly double the amount of copper obtained. The medium used affects the recovery obtained, achieving greater recoveries (up to 25 times) with a medium with higher sulphate content.

- High-grade ore allows to recover a greater amount of copper (47 mg/L) in comparison to a low-grade ore (11 mg/L) in the same period of time. This is likely caused by the matrix of the ore, which comprises some components capable of inhibiting the activity of the microorganisms. Therefore, there may be a limitation to the applicability of bioleaching for some copper ores due to the composition of the matrix where the metals are contained.

- After 60 days of experimentation of bioleaching of a chalcopyrite sample, an increase of the pH of the medium produced the formation of jarosite. This fact produced a reduction on copper recovery (from 3.6% to 0.5%), since the formation of jarosite may have locked some of the extracted copper.

- Although a mixed consortium of microorganisms obtained from a lab-scale gas-phase biotrickling filter allowed to bioleach ores, a pure culture of *Acidithiobacillus ferrooxidans* increased the recovery rate, reaching nearly 8 times the amount of copper recovered by the adapted mixed consortium.

- A bioleaching process can be applied to recover copper from PCBs under batch conditions, but performing the process in two separate steps can reduce the time required to extract copper from several days (around 22 days) to just 48 hours.

- In two-step bioleaching the separation of the biomass through sedimentation after the first step may allow to obtain higher copper recovery in the second step (e.g. 90%) in comparison to the copper recovery obtained when the separation is done through filtration (e.g. 70%). This is related to the biological oxidation of iron (II) resulting in the leaching step, since the sedimentation does not separate all the biomass and the remaining microorganisms oxidize again the resulting iron (II) from the leaching reaction, so more copper can be oxidized.

- The performance of bioleaching in a stirred-tank bioreactor allows to recover copper from PCBs, but similar recoveries were obtained during the biotic (56%) and abiotic with iron (II) (52%) experiments after 16 days of experimentation. The similar results obtained are related to the methodology used in the biological assay, because the recirculation of the solution between the oxidation and the leaching reactors without biomass separation made the process act as a one-step process, resulting in a slower and less efficient process. Nevertheless, during the biotic experiment the copper extraction began earlier since the bio-oxidation of iron (II) is faster than the chemical oxidation. In this way, a higher concentration of the leaching agent is achieved earlier in the biological assay, which implies that the leaching of PCBs began earlier.

- Apart from copper, the biological leaching performed in a CSTR also recovered many other metals such as tin, nickel, manganese, silver, aluminium, cobalt, indium, gold, palladium, and osmium. As a consequence of these recoveries, a loss of 23.6% of the initial weight of the PCBs was observed. In particular, a loss of 21.3%

was due to the extraction of copper, whereas the remaining 2.3% were due to the set of the other extracted metals.

- A column system to bioleach PCBs is feasible and improves the copper extraction obtained in batch experiments. The best conditions to obtain the highest copper recovery in column reactor were found to be: pH control at 1.75 to ensure the solubility of the leaching agent (iron); the use of a porous support for PCBs to improve hydrodynamic characteristics; the use of small particles (between 0.2 and 1.0 mm of diameter); PCBs concentration not over 7.5 g/L when the column is operated in a discontinuous mode; and, the use of packing material to get better percolation. Under these conditions, 88% of copper was recovered in 48 hours.

- A new strategy consisting of cyclic bioleaching allows reaching high amount of copper in less time (in comparison to the previous column system), e.g. recovering 88% of copper in 12 hours (2 cycles of 6 hours each one) and up to 80% of copper in just 6 hours (3 cycles of 2 hours each one). However, stirring the waste as well as renewing the leaching solution at the beginning of each cycle are obviously needed to obtain the highest extraction. In this strategy, the leaching agent can access to more internal parts of the scrap, improving its accessibility and, moreover, the increase of the concentration of the leaching agent accelerates the rate of metal recovery.

- Metal toxicity may have an important effect in the bioleaching process. Among the metals studied, aluminium turned out to be the most toxic one, producing the complete inactivation of the microorganisms at 0.5 M in just a few minutes of contact. Nickel, in contrast, was the least toxic one, bringing about a total inactivation after 48 hours at 1.5 M of nickel. Copper, the metal with one of the highest concentrations in bioleaching solutions, is also toxic, but it took 27 hours of contact at a concentration of 1.2 M to inactivate the microorganisms.

- The *Acidithiobacillus ferrooxidans* strain used during the experiments presented a substrate inhibition at iron (II) concentrations over 0.75 M. Nevertheless, at lower iron concentrations the inhibitory process is reversed and the biological activity increases by the use of iron as an energy source for their growth. Thus, the biomass shows a high adaptation capacity to changing conditions. Moreover, this strain could operate 550 hours (23 days) without nutrient addition, including iron, without complete inactivation, and they can be reactivated in 24 hours, if they are fed again, which reinforces their high adaptation capacity previously observed.

- Cementation is an efficient technique to recover bioleached metals due to its simplicity and low operational cost, but the purity of the copper obtained (as a fine powder) is only around 70%. Apart from copper, the major metal found in the resulting powder is iron, which is associated to the inadequate separation of the powdered copper from the scrap iron used for the cementation.

11.2. Future work

In this thesis, an extensive knowledge of bioleaching processes to recover copper has been acquired, especially oriented towards industrial application. However, further investigation is required in order to implement the methodology at industrial scale.

Regarding the biological recovery of copper, the following considerations in the process should be performed in order to scale-up to an industrial environment:

- The major part of the experiments in this thesis has been performed with PCBs from end-of-life mobile phones, but the technology could be applied to PCBs from other electronic devices to recover valuable metals from them.

- A pre-treatment of the e-waste before the bioleaching process should be studied to evaluate if it increases the metal recovery.

- Different metals apart from copper, especially rare earth metals and precious metals should be evaluated to be extracted through bioleaching of PCBs due to their high economic value and their increasingly high demand.

- At industrial scale, it is proposed to use the continuous mode, so further experiments are required in column reactors when the leaching solution is continuously flowing down the column. In this sense, the best conditions found in this thesis have to be tested and adapted to the new operation mode.

- Cementation turned out to be an efficient technique to recover the metal from the leaching solution, but the obtained purity is not high and, moreover, it results in a fine powder. Therefore, other techniques (e.g. solvent extraction plus electrowinning) should be evaluated to increase the purity of the metal obtained and to obtain the metal in other forms (e.g. metal foil) that could be interesting depending on the application for which it is intended.

- A bioleaching process presents many advantages over the traditional methods, especially in the environmental field. However, a thorough economic study is recommended before its implementation at industrial scale to verify the viability of this technology.

With respect to the microorganisms involved in bioleaching and their activity during the process, the following considerations should be made in order to optimize the biological process:

- After observing that some metals from the leaching solution affect the microbial activity, an extensive toxicity study should be performed with all the metals found in the leaching solution.

- In this thesis, the microorganisms have been cultivated in suspension. However, their immobilization should be evaluated, since this may reduce biomass displacement along the different process steps and, thus, avoid the concentration loss when the biomass is inactivated by the contact to the toxic bioleached metals.

- In order to reduce the precipitates during the biological oxidation of iron (II) without losing microbial activity, the mineral medium used in the process could be optimized, reformulating its composition.

Chapter 12

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