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# Biotechnologies for metal recovery from wastes

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## CONTENT

# Dissertation outline

## 1. Overview

In the last decades, the technology advancement and innovation has been very significant and fast. This aspect is evident in the electric and electronic equipment (EEE), where every year new devices are produced. The manufacture development can be translate in two main criticality: the highest metal required and the highest waste management (Baldé et al., 2017; Kyser et al., 2015; Zhang and Xu, 2016).

In detail, the increasing metal demand is due to not only by the rising new equipment production, but also by the technology innovation; an example is the evolution of the printed circuit boards (PCBs), they are gone to be composed from 11 in the 1980s to 60 elements in the 2000s (Johnson et al., 2007). The annual growth rate for PCB manufacturing increases by 8.7%, mainly in China and Southeast Asia (Huang et al., 2009). The continue increasing on metal demand is a huge problem, and for this reason the European Commission, every three years, draws up a list of “critical raw material”. The most recent one (2017) includes twenty-four materials (antimony, baryte, beryllium, bismuth, borates, cobalt, coking coal, fluorspar, gallium, germanium, hafnium, helium, indium, magnesium, natural graphite, natural rubber, niobium, phosphate rock, phosphorus, scandium, silicon metal, tantalum, tungsten, vanadium) and three groups of elements (platinum group metals, heavy rare earths, light rare earths) (European Commission, 2017). Nevertheless, other metals, that are not included in this list, could be critical in the next future. For example, the scientists demonstrated that the global copper ore deposit will be exhausted by 2030 (Bonnin et al., 2015; Wang et al., 2019). Therefore, the mining industry is focusing on the low-grade ores, often located in remote areas or at great ocean depth, with significant environmental and economic impacts (Hein et al., 2013; Petersen et al., 2016).

The second criticality is due to the short lifespan of the new technologies. For example, the PCBs are designed for durability of 500,000 h, but the average end-of-life of electronic components is 20,000 h (2 years) or less than the 5% of durability lifespan (Kaya, 2019). The shortest lifespan of the EEE than that designed is determined now mainly by the fast technological advancement and marketing strategies (R. A. Mesquita et al., 2018).

To solve this double criticality, the old linear management method, in which every product became a waste at the end of its life, evolves in a circular approach following the principle “resource-product-regenerated resource”, where municipal waste is converted in a resource (urban mining) (D’Adamo et al., 2019; Ljunggren Söderman and André, 2019). This kind of waste management allows to combine environmental protection, resources conservation and economic advantages.

## 2. The electronic waste: the new mines

The WEEE constitutes the 8% of the total municipal waste, and it continues to increase by 4-5% every years (Kaya, 2016; Widmer et al., 2005). The average generated WEEE per inhabitant is 5.9 kg in the world per year, and the highest record per inhabitant is produced in Europe (15.6 kg/y) (Baldé et al., 2017; Evangelopoulos et al., 2019).

The Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal in the 1989 classified WEEE as hazardous waste and so they should not be treated like ordinary commercial goods (landfill or incinerator disposal) (Baldé et al., 2017). The Basel Convention has been transposed by European Parliament and the objective of the European Directive 2002/96/EC on WEEE (WEEE-directive) was to prevent the WEEE landfill or incinerator disposal and to support the reuse and recycling, to preserve the primary resources, the energy and the environment (European Parliament, 2003; Hagelüken, 2005). The European Directive has been transposed into Italian legislation in the 2014 (Italiana, 2014). The definition of WEEE reported in the European Directive is: "Electrical or electronic equipment which is waste... including all components, sub-assemblies and consumables, which are part of the product at the time of discarding." (European Parliament, 2012, 2003).

There are four main sources of WEEE: small/large home appliances, hospital medical equipment, office machines, and industrial equipment/machines (Kaya, 2019). The PCBs are the brain part of EEE, and they constitute the 3-5% of the WEEE amount (Faraji et al., 2018; Vermeşan et al., 2020). The PCBs are constituted by three main layer: the structure (composed by fiberglass and Cu), and upper and lower compound units (composed by fiberglass, solder joints, conductive tracks, contacts, and solder mask) (Kaya, 2019). Typically, the average content of metals, ceramics and plastic are estimated of about 40%, 30% and 30% (w/w), respectively (Evangelopoulos et al., 2019; Jadhav, U. Su, 2015; Rocchetti et al., 2018a; Tiuc, 2020; Zhang and Xu, 2016). As regards metals, the PCBs are composed by 20% Cu, 5% Al, 1% Ni, 1.5% Pb, 2% Zn, 3% Sn (w/w) with 250 ppm Au, 1000 ppm Ag and 110 Pd (Baldé et al., 2017; Birloaga and Vegliò, 2016; Faraji et al., 2018; Kaya, 2019). The PCB "mineralogy" is completely different from mineral ores for metal refining. Natural ores are composed by oxidized or sulphured mineral, on the other hand, PCB contains pure metal or metal alloys in their structure (Kaya, 2019). The Cu and Au concentration in the PCBs is 20-40 and 25-250 times higher than the concentration reported in the natural ores, respectively (Hsu et al., 2019; Li et al., 2007; Qiu et al., 2019; Xiang et al., 2010). The potential value on metal recovery from PCBs changes from 1000 to 25000

Euros per ton. After Au and Pd, the Cu is the next highest value metal recovering from PCBs (Awasthi and Zeng, 2019; Kaya, 2019; R A Mesquita et al., 2018).

## 3. Current technologies for metal recovery from electronic waste

The technologies for metal extraction from PCBs are totally different for the metal recovery from minerals (Weeden et al., 2015; Zhang and Xu, 2016). Currently, various industries process and recover metals from PCBs, including Umicore (Belgium), Noranda (Canada), Rönnskär (Sweden), Dowa (Japan) and Aurubis (Germany) (Ma, 2019; Park and Kim, 2019).

The PCB recycling process is divided in four main steps (Maragkos et al., 2013):

- Collection and presorting
- Pretreatment (including dismantling, size reduction and separation process)
- Pre-processing (metal extraction)
- End-processing (purification and refining)

In order to enhance the collection, the European Commission sets targets of 65% of all equipment sold or 85% of total WEEE (European Commission, 2017). To improve the WEEE recycling success in a particular country, it is important to change the mentality on the part of the residents (D’Adamo et al., 2019).

The first step is the manual dismantling or mechanical treatment to separate PCBs from the devises. After manual or mechanical dismantling, the PCBs are grinding and the different fraction (metals, plastics, ceramics, flame retardants) are separated with different method. The separation process can be dry (e.g. magnetic separation, eddy current separation, air separating, fluidized-bed separation, electrostatic separation) or wet operation (e.g. sink-float, heavy medium cyclones, shaking table, floatation) (Kaya, 2019).

The second step (pre-processing) aims to leach metals from solid fraction. The main processes used in the current facilities for metal extraction include pyro- and hydrometallurgical techniques (Kaya, 2019; Ma, 2019; Park and Kim, 2019). The pyrometallurgical approaches use high temperature (above 2000°C) to control the chemical reaction as well as melting or smelting process. On the other hand, hydrometallurgical processes require lower temperature than pyrometallurgy (between 90 and 200°C) and lixiviant/chemical reagent (such as HCl, $H_2SO_4$, $HNO_3$, $H_2O_2$, $HCLO_4$). In general, pyro- and hydrometallurgical approaches are used in tandem for metal extraction (Bas et al., 2014; Rocchetti et al., 2018b; Zhang and Xu, 2016). The main disadvantages of these technologies are: toxic gas emission (e.g. dioxins and furans) that requires large investment to protect the environmental by controlling the hazardous gas emission; acid wastewater production that could be corrosive, toxic and irritating both for the human health and the environmental (Zhang and Xu, 2016).

The end-processes (purification and refining) involves cementation, precipitation, solvent (liquid/liquid) extraction, or electrowinning/electrolysis/electrodeposition process. This step is essential for selectivity recovery of the metals and to make them suitable for the market (Kaya, 2019).

Today, the annual Cu production is around 15 million tons and 30% is used for the electric and electronic device (Manjengwa, 2019). The Cu resulting from the recycling is the 36% of the total Cu production (Bonnin et al., 2015). According to the Bureau of International Recycling ("BIR - Non-Ferrous Metals," n.d.) the Cu extracted from e-waste save up 85% of the energy and reduce the $CO_2$ emission around 65% than the primary production (Bonnin et al., 2015).

## 4. Aim and objectives of the research activity during the Doctorate

In the last decades, biohydrometallurgical strategies has gained increasing prominence in this field. Indeed, the microorganism use could be more cost efficient and environmentally friendly than the chemical approaches (Beolchini et al., 2012; Ilyas et al., 2013). Furthermore, the bio approaches allow to solve the main limits of pyrometallurgical methods due to the request of high temperatures. Several works investigated the acidophilic bacteria ability for metal extraction from PCBs, mainly using: *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. High efficiencies, greater than 95% for Cu, were achieved by ferrous iron concentration between 4 and 10 g/L, a solid concentration between 0.78 and 2.8% (w/v), a leaching time from 2 to 10 days (Annamalai and Gurumurthy, 2019; Arshadi and Mousavi, 2015; Choi et al., 2004; Hubau et al., 2018; Ilyas et al., 2013; Ilyas and Lee, 2014; Joshi et al., 2017; Liang et al., 2013; Wang et al., 2009; Wu et al., 2018; Yang et al., 2009, 2014; Zhu et al., 2011), also using pyrite, as ferrous iron energy source for bacteria metabolism (Bas et al., 2013). Comparable extraction results were obtained by the microbial community recovered from pyrite mine or activated sludge taken from municipal sewage treatment plant (Liang et al., 2010; Xiang et al., 2010). On the other hand, cyanogenic bacteria (*Chromobacterium violaceum, Pseudomonas fluorescens and P. aeruginosa*) showed an affinity to Au, with recovery efficiency of 10%, with a solid concentration between 0.5-1% (w/v) (Chi et al., 2011; Pham and Ting, 2009; Pradhan and Kumar, 2012; Yuan et al., 2019, 2018).

Fungi strains are investigated as an alternative for the metal extraction from PCBs, mainly *Aspergillus niger* and *Penicillium semplicissimum*. Nevertheless, the greatest Cu extraction efficiency was around 70% with PCB concentrations between 0.1 and 0.5% (w/v) (Brandl et al., 2001; Faraji et al., 2018). Jadhav et al. (2016), increased the solid content up to 1% (w/v) adding the hydrogen peroxide to the fermentation medium, to increase the metal extraction by organic acid produced by *A. niger*.

The main criticalities highlighted by the literature are the low PCB concentration to treat and the long time to complete the metal extraction. Consequently, the research activity had as general goal the development of an innovative high efficiency biotechnology for Cu and Zn recovery from end-of-life PCBs. The target is the increase of the treated waste concentration, limiting the metal toxicity on both bacteria (*At. ferrooxidans* and *L. ferrooxidans*) and fungi (*A. niger*) metabolism. Specific targets are:

- The identification of the best conditions for a complete Cu and Zn extraction from end-of-life PCBs;
- The development of a Cu and Zn selectivity recovery process from the extraction solution;
- The chemical reaction between ferric iron and Cu, and the bioleaching mechanism and kinetics are studied in detail by mathematical modelling;
- The environmental assessment of different strategies of end-of-life PCB management.

The aim of this research is to identify the best operative conditions to make biotechnology more ecofriendly than pyro- and hydrometallurgy approaches so that can be more attractive for stakeholders and can be applied industrially.

Each one of the following chapters addresses one work-package and can be read independently, since the scientific background and the specific materials and methods are described chapter by chapter.

Chapter 1 describes a new bioleaching process, using both *At. ferrooxidans* and *L. ferrooxidans*, for the Cu and Zn extraction. The high efficiency, higher than 95 and 75% for Cu and Zn, respectively, is achieved using *At. ferrooxidans* with a PCB concentration of 5% (w/v). The multistep process (bacteria growth phase, I step leaching and II step leaching) allows to reach the same reported literature efficiency with the highest PCB concentration. These results demonstrated as the Cu extraction is controlled mainly by the reaction between ferric iron and Cu. Chapter 2 studies in detail the kinetic of this chemical reaction, determining the activation energy. From the determined equation that describe better than other the temporal change of Cu and ferric iron, in Chapter 3 the bioleaching process is studied by a mathematical model and all the characteristic parameters to describe bacteria growth and metabolism are determined. From the determined parameter, it is underlined as the new bioleaching process increase the bacteria tolerance to Cu concentration and as the kinetic of the bacteria metabolism controls the Cu leaching. Starting from the satisfying results of extraction process, Chapter 4 describes a selectivity recovery treatment with high efficiency both for Cu and Zn. In this chapter different recovery techniques are tested (precipitation, cementation, precipitation by a solvent). An analysis of environmental impacts closes both the chapter 1 and 4 by life cycle assessment (LCA) tools. The results of environmental impacts showed as the biotechnological approach has the lower impact than the other hydrometallurgical or bioleaching process reported in the literature but higher than the primary production. In Chapter 5, a preliminary environmental impact study is carried out to identify the best operative condition in order to make the bioleaching process more sustainable than the primary production. Once identified, the experiments are carried out to verify the real application. The Chapters 6 and 7 study the bioleaching with fungi (*A. niger*). The Chapter 6 is an overview of the citric acid bioproduction by *A. niger*. Chapter 7 studies the real application of the fungi to treat PCBs and the effect of the citric acid on the Cu and Zn leaching.

## 5. Products

List of peer-reviewed papers from the research activities within the Doctorate:

- Amato, A.; Becci, A.; Mariani, P.; Carducci, F.; Ruello, M.L.; Monosi, S.; Giosuè, C.; Beolchini, F.; 2019. End-of-life liquid crystal display recovery: Toward a zero-waste approach. *Applied Sciences*, Volume 9, Issue 15. doi:10.3390/app9152985
- Amato, A.; Becci, A.; Birloaga, I.; De Michelis, I.; Ferella, F.; Innocenzi, V.; Ippolito, N.M.; Pillar Jimenez Gomez, C.; Vegliò, F.; Beolchini, F.; 2019. Sustainability analysis of innovative technologies for the rare earth elements recovery. *Renewable and Sustainable Energy Reviews,* Volume 106, Pages 41-53. doi:10.1016/j.rser.2019.02.029
- Becci, A.; Amato, A.; Rodríguez Maroto, J.M.; Beolchini, F., 2019. Prediction model for Cu chemical leaching from Printed Circuit Boards. *Industrial and Engineering Chemistry Research,* Volume 58, Issue 45, Pages 20585-20591. doi:10.1021/acs.iecr.9b04187
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- Becci, A.; Karaj, D.; Merli, G.; Beolchini, F.; 2020. Biotechnology for metal recovery from end-of-life printed circuit boards with *Aspergillus niger*. *Sustainability,* Volume 12, Issue 16, Article number 6482. doi:10.3390/su12166482
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- Becci, A.; Amato, A.; Rodríguez Maroto, J.M.; Beolchini, F., 2021. Bioleaching of end-of-life printed circuit boards: Mathematical modelling and kinetic analysis.

*Industrial and Engineering Chemistry Research,* Volume 60, Issue 11, Pages 4261-4268. doi:10.1021/acs.iecr.0c05566

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# Chapter 1: BIOLOGICAL EXTRACTION OF VALUABLE METALS FROM ELECTRONIC WASTE

## Abstract

The increase of waste from electric and electronic equipment has pushed the research towards the development of high sustainability treatments for their exploitation. The end-of-life printed circuit boards (PCBs) represent one of the most significant waste in this class. The interest for these scraps is due to the high Cu and Zn content, with concentrations around 25% and 2% respectively, combined with further precious metals (e.g. Au, Ag, Pd). Currently, the most common approaches developed for PCBs recycling include pyrometallurgical and hydrometallurgical treatments. On the other hand, biohydrometallurgical strategies are gaining increasing prominence, for the possibility to decrease both the environmental and the economic costs. Nevertheless, these techniques show the main limit due to the possibility to treat low quantities of waste, which makes unsustainable the further scale-up. To overcome this criticality, the present paper introduces an innovative bioleaching process carried out by *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) and *Leptospirillum ferrooxidans* (*L. ferrooxidans*). The developed technology allows to reach high PCB concentration, up to 5% (w/v), thanks to a high efficiency two-step design, able to reduce the metal toxicity on the bacteria metabolism. The treatment uses the ferric iron generated by bacterial oxidation, as oxidant, to leach Cu and Zn from PCBs. The possibility to overcome the solid concentration criticality is combined with high yield of 94% and 70% for Cu and Zn, respectively. The best selected conditions involve the *At. ferrooxidans* bacteria use at: 30°C, solid concentration of 5% (w/v), 10 g/L of $Fe^{2+}$, time of treatment 9 days. The experimental results are further enhanced by the carbon footprint

assessment which proved the environmental advantage, compared to both the reference chemical treatment through ferric iron and literature processes (hydrometallurgical and bioleaching approaches). The analysis explained as the PCBs concentration in the solution allows to decrease the bioreactor size with the consequent reduction of energy and raw material demand. This benefit can be translated into a 4 times reduction of the $CO_2$-eq./kg treated PCB emissions, compared to the bioleaching reported in the literature.

## Content

## 1. Introduction

In the last two decades, the amount of waste from electrical and electronic equipment (WEEE) had an exponential growth (about 45 million tons, in 2016), mainly due to both the new technologies, that have replaced the old and obsolete equipment, and their relatively short replacement cycles (Baldé et al., 2017; Zhang and Xu, 2016). In 2016, Asia was the region that produced most of the WEEE, with 18 Mt, followed by Europe (12 Mt) and USA (7 Mt). Nevertheless, only a scarce fraction, around the 20% of the whole stream, was documented to be collected and properly recycled (Baldé et al., 2017). The illegal WEEE management is mainly due to their content of hazardous components which makes impossible the implementation of traditional methods, such as disposal in landfilling sites or incinerator, for the human and the environmental protection (Leung et al., 2008; Musson et al., 2006; Shen et al., 2008). The end-of-life printed circuit board (PCBs) is a typical example of WEEE, which is the basal and essential component of the electronic industry, since it represents the brain of all the electronic products (Xiang et al., 2010). The PCBs constitute the 3-5% of the WEEE amount and they are one of the most relevant waste of the category since their composition combines many environmental restrictions with several economic advantages for the valuable metal content (Faraji et al., 2018; Priya and Hait, 2017). Furthermore, the quantity and the purity of these metals are higher than those in rich-content minerals (Li et al., 2007; Xiang et al., 2010). In this context, the metal recovery, mainly focused on Cu and precious elements, produces a double advantage: the avoided primary mining and the enhancement of a hazardous waste. The whole effect is a relevant advantage for: the environmental, the human health protection and the economic spheres (Ilyas et al., 2013; Natarajan and Ting, 2015; Veit et al., 2005).

Currently, the most common approaches used for the PCBs recycling include both pyrometallurgical and hydrometallurgical treatments. The first kind of process is mainly based on the smelting technologies that require temperatures between 300 and 900°C (Rocchetti et al., 2018). On the other hand, the hydrometallurgical technologies use chemical solutions for metal dissolution, mainly hydrochloric, sulfuric and nitric acid or/and hydrogen peroxide (Bas et al., 2014; Rocchetti et al., 2018; Zhang and Xu, 2016). For the further Au recovery, the most common leaching agent is cyanide with the consequent production of contaminated wastewater (Behnamfard et al., 2013).

In the last decades, biohydrometallurgical strategies has gained increasing prominence in this field. Indeed, the microorganism use could be more cost efficient and environmentally friendly than the chemical approaches (Beolchini et al., 2012; Ilyas et al., 2013). Furthermore, the bio approaches allow to solve the main limits of pyrometallurgical methods due to the request of high temperatures. Several works investigated the acidophilic bacteria ability for metal extraction from WEEE, mainly using: *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. High efficiencies, greater than 95% were achieved by ferrous iron concentration between 4 and 10 g/L, a solid concentration between 0.78 and 2.8% (w/v), a leaching time from 2 to 10 days (Arshadi and Mousavi, 2015a; Choi et al., 2004; Liang et al., 2013; Wang et al., 2009; Wu et al., 2018; Yang et al., 2009, 2014; Zhu et al., 2011), also using pyrite, as ferrous iron energy source for bacteria metabolism (Bas et al., 2013). Comparable extraction results were obtained by the microbial community recovered from pyrite mine or activated sludge taken from municipal sewage treatment plant (Liang et al., 2010a; Xiang et al., 2010). On the other hand, cyanobacteria (*Chromobacterium violaceum, Pseudomonas fluorescens and P. aeruginosa*) showed an affinity to Au, with recovery efficiency of 10%, with a solid concentration between 0.5-1% (w/v) (Chi et al., 2011; Pham and Ting, 2009; Pradhan and Kumar, 2012; Yuan et al., 2019, 2018).

Fungi strains are investigated as an alternative for the metal extraction from WEEE, mainly *Aspergillus niger* and *Penicillium semplicissimum*. Nevertheless, the greatest Cu extraction efficiency was around 70% with PCB concentrations between 0.1 and 0.5% (w/v) (Brandl et al., 2001; Faraji et al., 2018). Jadhav et al. (2016), increased the solid content up to 1% (w/v) adding the hydrogen peroxide to the fermentation medium, to increase the metal extraction by organic acid produced by *A. niger.* In this context, the present work aims at the development of an innovative high efficiency biotechnology for Cu and Zn recovery from end-of-life PCBs. The target is the increase of the treated waste concentration, limiting the metal toxicity on both *At. ferrooxidans* and *L. ferrooxidans* metabolism. This novelty allows to get beyond the state of the art to solve the limits of the current technologies developed in the literature (Arshadi and Mousavi, 2015b; Liang et al., 2016, 2013; Nie et al., 2015a, 2015b; Priya and Hait, 2018; Rodrigues et al., 2015). Furthermore, following the same approach used in the literature for other kind of WEEE, the life cycle assessment (LCA) tool was used to evaluate the process sustainability (Amato et al., 2017, 2016; Amato and Beolchini, 2018; Latunussa et al., 2016; Rocchetti et al., 2013). More in detail, the carbon footprint of the best developed technology was estimated and compared to the chemical and hydrometallurgical approaches.

## 2. Materials and methods

### *2.1 Preparation of waste printed circuit boards (PCBs)*

The PCBs used in this study were kindly provided by the University of L'Aquila, Italy. The samples of high-grade PCBs, mainly from personal computers, were prepared as follows:

the material was shredded using stainless steel blades and pliers after manually removing the main parts of electronic components (e.g. capacitors, batteries and resistors). Thereafter, the refuse was crushed to obtain a granulometry of less than 0.5 mm which was used for all the bioleaching experiments. A consecutive washing, with saturated water (with NaCl), removed the plastics, toxic for bacteria metabolism, improving the metal extraction efficiencies (Ruan et al., 2018). The literature characterization results report average values of 25% and 2%, for Cu and Zn, respectively. More in detail, the Zn content is in a 0.02-7% range, whereas the Cu data, with a significant variability due to the sample heterogeneity, are reported in Table 1.1.

Table 1.1. Literature about the average Cu concentration in the end-of-life PCBs.

| Cu content (%) | References |
|---|---|
| <20 | (Arshadi and Mousavi, 2015a; Bas et al., 2014, 2013; Jadhav et al., 2016; Natarajan and Ting, 2014; Pant et al., 2012; Pradhan and Kumar, 2012; Priya and Hait, 2017; Veit et al., 2005; Vestola et al., 2010) |
| 20-30 | (Birloaga and Vegliò, 2016; Creamer et al., 2006; Ilyas and Lee, 2014; Wang et al., 2017; Xiang et al., 2010; Yang et al., 2009, 2014; Zhang and Xu, 2016) |
| >30 | (Faraji et al., 2018; Hong and Valix, 2014; Wang et al., 2009; Zhu et al., 2011) |

### *2.2 Microorganisms and culture conditions*

*Leptospirillum ferrooxidans* (DSMZ 2705$^T$) and *Acidithiobacillus ferrooxidans* (DSMZ 14882$^T$), used for the experiments, were obtained from the Deutche Sammlung von Mikroorganismenn und Zellkulturen GmbH, Germany. The DSMZ 882 medium, consisting of three solutions (A, B and C), was prepared as follows; Solution A: 0.132 g of $(NH_4)_2SO_4$, 0.053 g of $MgCl_2 \cdot 6H_2O$, 0.027 g of $KH_2PO_4$, 0.147 g of $CaCl_2 \cdot 2H_2O$ dissolved in 0.950 L of MilliQ water and pH adjusted to 1.6 with a 2 M $H_2SO_4$ solution. Solution B: 49.8 g of $FeSO_4 \cdot 7H_2O$, in 50 mL of 2 M $H_2SO_4$ adjusted to pH 1.2 with NaOH. Solution C (1L): 0.062 g of $MnCl_2 \cdot 2H_2O$, 0.068 g of $ZnCl_2$, 0.064 g $CoCl_2 \cdot 6H_2O$, 0.031 g of $H_3BO_3$, 0.010 g of

$Na_2MoO_4$, 0.067 g of $CuCl_2·2H_2O$, brought to pH 1.8 with 2 M $H_2SO_4$ solution. Solution A and C were autoclaved separately, whereas solution B was sterilized by filtration (Sartolab Filter Systems, polyethersulfone PES 0.22 μm) in order to prevent the oxidation of $Fe^{2+}$ to $Fe^{3+}$. The culture medium was obtained by adding 50 ml of solution B and 1 ml of solution C to the solution A, maintaining the sterility. The bacteria cultures grew in the DSMZ 882 medium and they were incubated, under stirring, at 120 rpm, at 30°C, for 5 days (Stuart, orbital incubator S510).

### *2.3 Bioleaching experiments*

The bioleaching process (Figure 1.1) was conducted in different phases: the growth phase, the first step and the second step processes. The experiments were carried out in 250 mL flasks containing 80 mL of DSMZ 882 medium (10 g/L of $Fe^{2+}$) and 20 mL of bacterial stock culture. After 48 hours of bacterial growth (growth phase), time necessary for the oxidation of $Fe^{2+}$ to $Fe^{3+}$, 5% (w/v) of PCBs were added to the solution of *At. ferrooxidans.* In the first step the bacterial growth was carried out in the presence of PCBs for 48 hours. In the second step, the 80% of medium was removed to reduce the metal toxicity on the bacteria metabolism and the remaining 20%, with the PCBs, was refreshed by fresh medium, to restore the starting bioleaching volume and the Fe concentration, for further 7 days (Cabrera et al., 2005a, 2005b; Tuovinen et al., 1971). After the inoculation, the flasks were kept in the Stuart incubator (orbital incubator S510) at 30°C and 120 rpm, for 11 days. The pH was adjusted, every day, at 1.6 value for both *At. ferrooxidans* and *L. ferrooxidans* by the dropwise addition of 1 M $H_2SO_4$. This condition is optimal for the bacterial growth and to prevent the formation of jarosite precipitation on PCBs, that reduce the reaction kinetic with metals (Boon et al., 1999; Crundwell, 1997; Daoud and Karamanev, 2006). Furthermore, this acidity level does not represent an obstacle in the perspective of an industrial application, as confirmed by Yong Liu et al., (2011) in the registered patent CN10221843. For *L. ferrooxidans,* which has a slower growth rate than *At. ferrooxidans*, the bacterial growth phase was 4 days, after which a 5% (w/v) of the PCBs was added to the solution, carrying out the bioleaching experiment in two steps, at the same conditions of *At. ferrooxidans*.

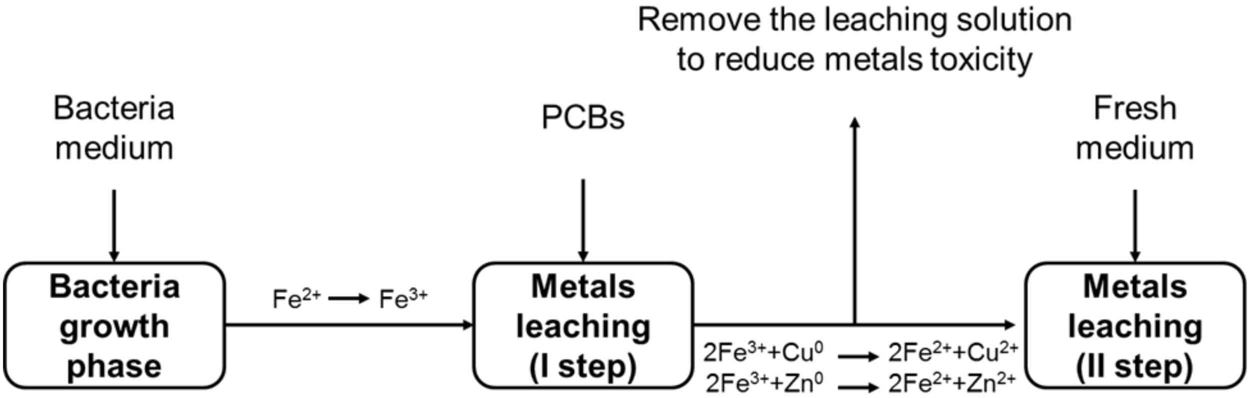

Figure 1.1. Bioleaching scheme.

In order to analyse the bacteria effect on the bioleaching mechanism, a chemical oxidation and an abiotic control test were carried out. More in detail, in the chemical oxidation test, 5% (w/v) of PCBs were added to 100 mL of $Fe_2(SO_4)_3*H_2O$ solution (50 g/L of $Fe^{3+}$) which is incubated, under stirring at 120 rpm, for several hours, until the whole $Fe^{3+}$ was reduced to $Fe^{2+}$. The test was repeated at two different temperatures (30°C and 50°C) to study the effect of this parameter on the kinetic reaction. On the other hand, the abiotic control was carried in two steps using a $Fe_2(SO_4)_3*H_2O$ solution (10 g/L of $Fe^{3+}$) to simulate the biological oxidation, under the same conditions of bioleaching treatment. This control aims at the quantification of the positive effect of the bacteria action.

Samples were collected to analyse the concentrations of $Fe^{2+}$, $Fe^{3+}$, Cu and Zn. Each treatment was carried out in duplicate.

### *2.4 Analytical determination*

The leaching solutions were periodically analyzed for the determination of both Zn and Cu concentration, carried out by an atomic absorption spectrophotometer (AAS) (Varian spectrometer SpectrAA 200). Each sample of 0.5 mL was diluted in agreement with the detection limit of 0.1 mg/L. On the other hand, the quantification of the Fe content was performed by an UV/VIS spectrophotometer (Jasco Model 7850). The determination was obtained by potassium thiocyanate (KSCN) which allows the quantification of $Fe^{3+}$ (at the absorption wavelength of 480 nm). For the measure of the total Fe, the solution reacts with potassium permanganate to oxidize the $Fe^{2+}$ in the sample. Thereafter, the $Fe^{2+}$ concentration was determined as the difference between the total Fe and the $Fe^{3+}$ concentration. The detection limit of this method was 1 mg/L of Fe, and the adsorption measurement was between 0.000 and 1.000 with an accuracy of ± 0.005.

The pH was recorded by a pH metro inoLab Multi 720 (WTW) and it was monitored over the bioleaching period.

### *2.5 Assessment of the carbon footprint*

The determination of the carbon footprint took into account five different scenarios for the treatment of PCBs. The target is the comparison of the impact due to the bioleaching (scenario 1, Figure 1.2) and the chemical approaches (scenario 2, Figure 1.2), described in the present paper, with a hydrometallurgical treatment (scenario 3, Figure 1.2) and two bioleaching approaches (scenario 4 and 5, Figure 1.2), reported in the literature. More in detail, the hydrometallurgical data referred to the process developed by Birloaga and Vegliò

(2016), which includes the use of a solution of sulfuric acid and hydrogen peroxide, at room temperature for 1h, with a solid-liquid ratio of 15% (w/v). On the other hand, the two referenced bioleaching treatments, report a maximum PCB concentration of 2.8% w/v (Liang et al., 2013) and the shortest time of 26 hours (Wu et al., 2018), with Cu extraction efficiency higher than 90%". Table 1.2 summarizes the input and the output flows considered for the assessment, assuming a possible recirculation of water and Fe sulphate of 95%. The micronutrients of scenario 1-4-5 include the agents described in the paragraph 2.2. Considering the lack of data related to the electric consumption of the scenario 2, we referred to Ippolito et al. (2017), which described the exploitation of fluorescent powder at comparable conditions. The values were adapted, on the basis of the reaction time and the solid concentration.

Table 1.2. Input and output flows considered for the carbon footprint assessment of the five scenarios (Functional unit: 1kg of shredded PCBs).

| | Input flow | Products |
|---|---|---|
| Biotechnological treatment (Scenario 1) | Electricity 2.6 kWh<br>$FeSO_4$ 50 g<br>Water 3.4 kg<br>$H_2SO_4$ 0.30 kg<br>NaOH 0.20 kg<br>Micronutrients 8.0 g | Cu 240 g<br>Zn 30 g |
| Chemical treatment (Scenario 2) | Electricity 4.5 kWh<br>$FeSO_4$ 120 g<br>Water 2.0 kg<br>$H_2SO_4$ 0.30 kg<br>NaOH 0.60 kg | Cu 230 g<br>Zn 30 g |
| Hydrometallurgical treatment (Scenario 3) | Electricity 0.30 kWh<br>Water 5.0 kg<br>$H_2SO_4$ 1.1 kg<br>$H_2O_2$ (50% v/v) 0.70 kg | Cu 140 g |
| Biological Treatment (Liang | Electricity 4.2 kWh<br>$FeSO_4$ 20 g | Cu 120 g |

| | | |
|---|---|---|
| et al., 2013)<br>(Scenario 4) | Water 35 kg<br>$H_2SO_4$ 0.17 kg<br>NaOH 0.19 kg<br>Micronutrients 120 g | |
| Biological treatment (Wu et al., 2018)<br>(Scenario 5) | Electricity 2.6 kWh<br>Water 200 kg<br>$H_2SO_4$ 0.98 kg<br>NaOH 1.1 kg<br>Micronutrients 1600 g | Cu 650 g |

The overall system boundaries (Figure 1.2) exclude the steps of: PCB production, use and the pre-treatment of the end-of-life product. All the phases for the Cu and Zn extraction are considered for the analysis, except for the recovery. The functional unit selected for the analysis is 1 kg of shredded PCB.

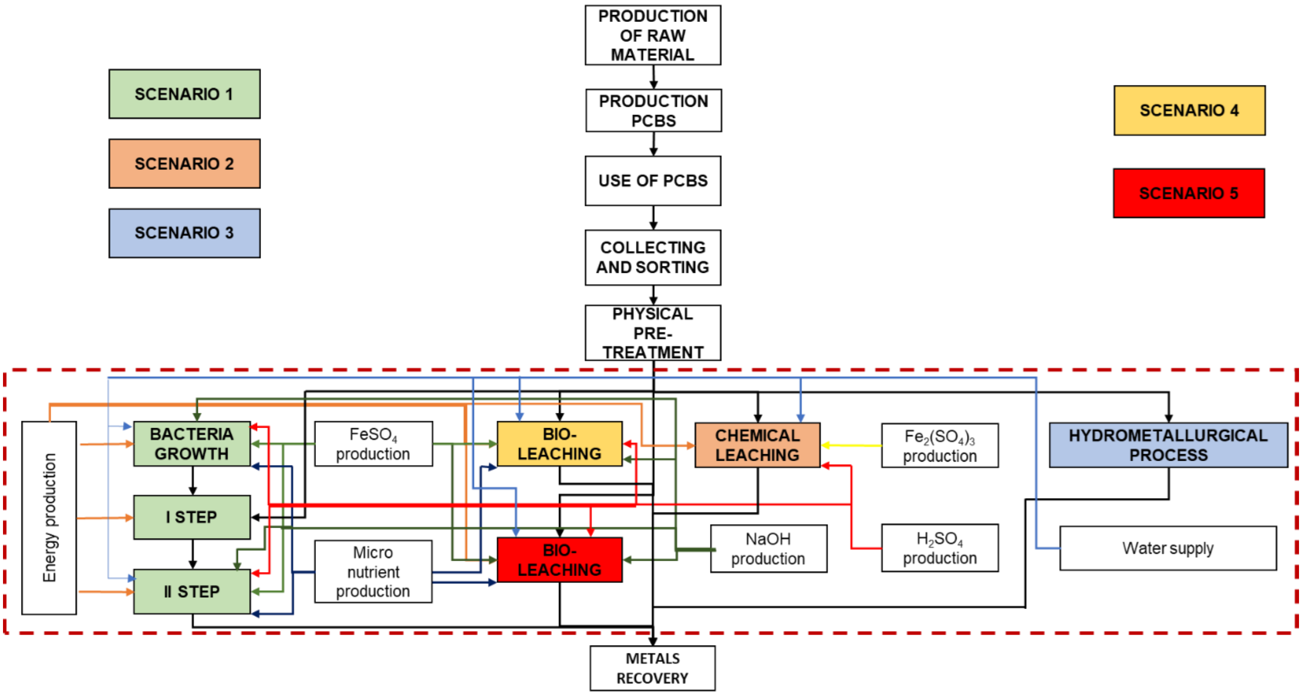

Figure 1.2. System boundaries considered for the carbon footprint assessment.

The thinkstep GaBi software-System and the Database for Life Cycle Engineering (compilation 7.3.3.153; DB version 6.115) were used for the production processes of energy and raw materials and the quantification of the carbon footprint of the assessed scenarios.

## 3. Results

### *3.1 The chemical leaching*

The results in Figure 1.3 show the leaching profiles for time-dependent extraction of Cu and Zn from PCBs and the variability of $Fe^{3+}$ and $Fe^{2+}$ ratio, at the two selected temperatures: 30°C and 50°C. The highest Cu extraction (91%) was achieved at 50°C, after 6 hours of leaching, whereas only the 73% of Zn was leached at the same conditions (Figure 1.3b). It is evident that the temperature rise exerts a significant effect on the leaching kinetics, reducing the time from 24 to 6 hours, and it accelerates the reduction of $Fe^{3+}$ to $Fe^{2+}$. The constant conversion of Fe, during the chemical oxidation process, proves its essential role for the Cu and Zn dissolution. The Cu and Zn mobilization consumes $Fe^{3+}$, a strong oxidant, and the rate of dissolved metals is proportional to the reduced Fe, following the Eqs. 1.1 and 1.2 (Hong and Valix, 2014; Lee and Dhar, 2012; Liang et al., 2010b; Wu et al., 2018):

$$Cu^0 + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} \tag{1.1}$$

$$Zn^0 + 2Fe^{3+} \rightarrow Zn^{2+} + 2Fe^{2+} \tag{1.2}$$

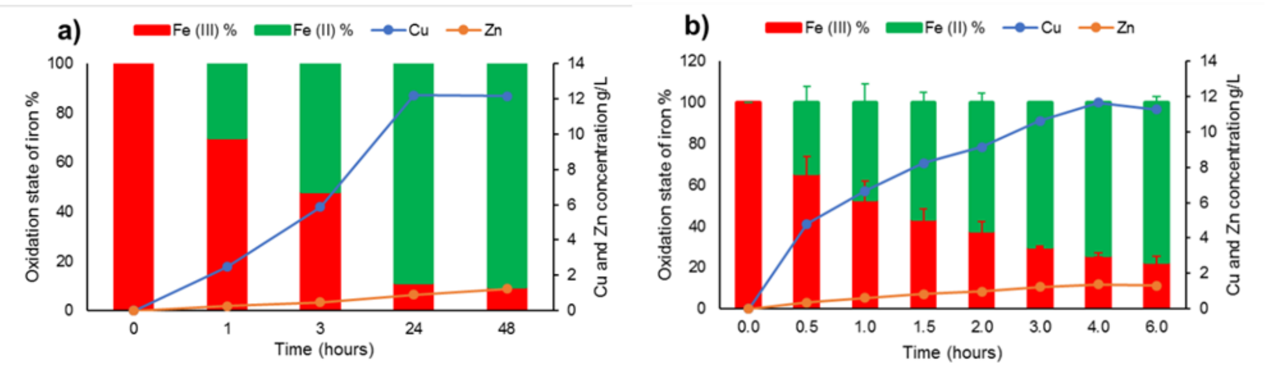

Figure 1.3. Influence of the temperature on the kinetic extraction of Cu, Zn from PCBs and oxidation state of Fe in the chemical control: 50 g/L $Fe^{3+}$, 50 g PCBs at (a) 30°C, (b) 50°C.

### *3.2 The bioleaching process*

The bioleaching takes advantage of the role of bacteria for the $Fe^{3+}$ regeneration, as oxidant, following the Eq. 1.3 (Lee and Dhar, 2012; Rawlings et al., 2017; Xiang et al., 2010). The bioleaching process permitted to use less Fe than chemical reaction, obtaining the same extraction yield. After $Fe^{3+}$ is reduced in the metal dissolution, $Fe^{2+}$ is again available by bacteria metabolism, used as energy source to increase the bacterial population and the metabolism, ready for further metal oxidation (Figure 1.4). This bioleaching process is cyclical and $Fe^{3+}$ has its importance for the leaching reaction.

$$Fe^{2+} + O_2 + H^+ \rightarrow Fe^{3+} + H_2O \tag{1.3}$$

In spite of the chemical results, which supported the highest temperature, the incubation was carried out at 30°C for both the bacteria (*At. ferrooxidans* and *L. ferrooxidans*), since it is the optimal growth condition (Ojumu et al., 2006; Rawlings et al., 2017).

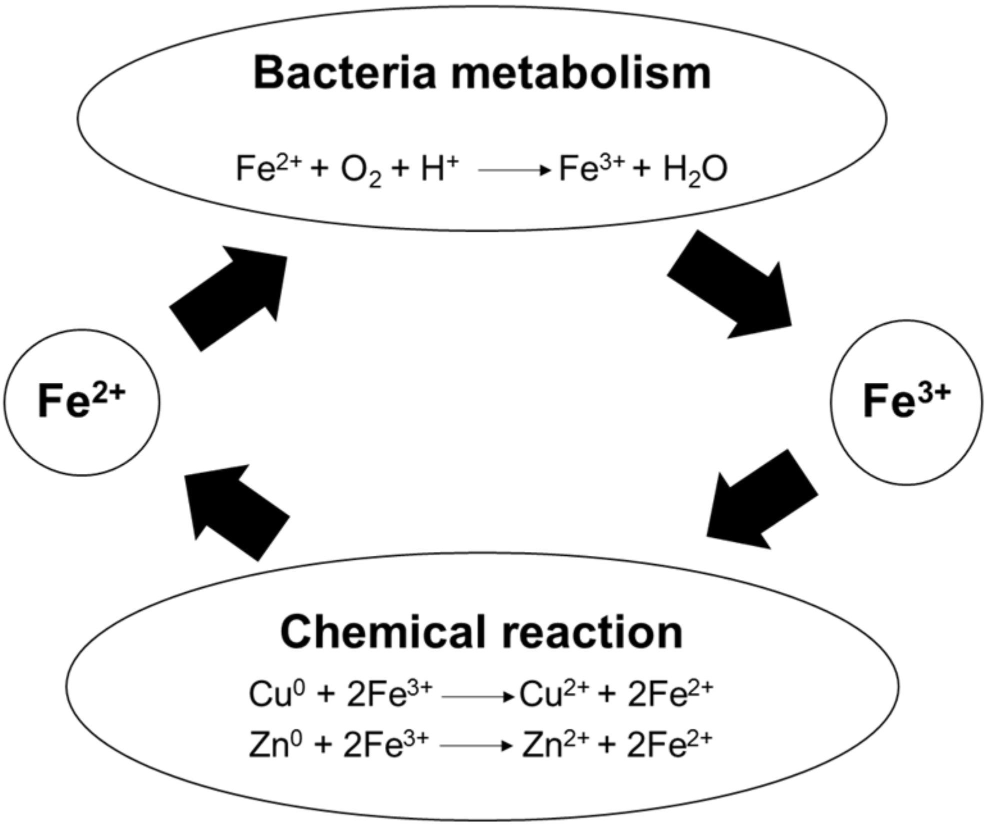

Figure 1.4. Fe cyclical process due to the bacteria metabolism action as supporting to the chemical reaction, showed in the abiotic control.

Figure 1.5 shows the metal (Cu and Zn) concentrations and the variability of $Fe^{2+}$ and $Fe^{3+}$ (%) with the time, using pure culture of *At. ferrooxidans* and *L. ferrooxidans*. During the first two days (bacteria growth phase), the bacteria *At. ferrooxidans* were cultivated under their optimized conditions to obtain a high activity. At the end of this phase, the $Fe^{3+}$ concentration in solution increased, up to 89%, suitable for Cu and Zn leaching, following the oxidation reaction of Eq. 1.3. At the end of the first step, the amount of $Fe^{3+}$ decreased until the 50% of the total Fe, compared to the abiotic control, where, the total $Fe^{3+}$ was converted to $Fe^{2+}$. The amount of Cu recovery was 3.7 ± 0.1 g/L, meanwhile the Zn concentration was 0.38 ± 0.04 g/L in the treatment with *At. ferrooxidans*, more than abiotic control where the Cu and Zn concentration at the end of the first step were 1.78 ± 0.04 and 0.14 ± 0.01 g/L, respectively. These data show a higher efficiency of bioleaching than the chemical technique, thanks to the bacteria activity which oxidized $Fe^{2+}$ to $Fe^{3+}$, during the first step. The removal of 80% of the medium, replaced with fresh solution, causes the further reduction of $Fe^{3+}$, until 12% of the whole Fe (II step). At the end of this step, the Fe is completely converted to $Fe^{3+}$. On the other hand, the abiotic control shows a constant amount of $Fe^{3+}$, 7% of the total, after 24 hours from the beginning of the second step, when fresh leaching solution (10 g/L of $Fe^{3+}$) was added. Nevertheless, as highlighted in Figure 1.5, the additional Cu and Zn leaching is due to both the iron oxidation for the temperature and shaking action and the $H_2SO_4$ activity (Yang et al., 2014). After 7 days, at the end of the two bioleaching steps, 8.9 ± 0.9 g/L and 1.1 ± 0.3 g/L of the Cu and Zn, respectively, were recovered from PCBs samples, with a whole efficiency higher than the abiotic control. Furthermore, the $Fe^{3+}$ concentration in the treatment with *At. ferrooxidans* shows that the Fe oxidation by bacteria activity (Eq. 1.3) was faster than chemical action of Fe, due to the chemical reaction with Cu and Zn (Eqs. 1.1 and 1.2). In addition, the maintenance of the pH at 1.6 limits the Fe precipitation as jarosite (Xiang et al., 2010; Yang et al., 2014). The innovative strategy of medium refresh allowed to increase the treated PCBs amount without a toxicity effect on the bacteria metabolism, solving one of the main criticality of the current bioleaching described in the literature (Liang et al., 2010b; Xiang et al., 2010; Yang et al., 2014; Zhu et al., 2011). Furthermore, an external addition of 10 g/L of $Fe^{2+}$ (II step), produced a positive effect on the dissolution rate of Cu and Zn.

The second bacteria used for the bioleaching is *L. ferrooxidans*, under the same conditions of *At. ferrooxidans*. The only difference, compared to the first bacteria, is the PCB addition, carried out after 4 days of bacterial growth. As showed in Figure 1.6, *L. ferrooxidans* is very sensitive to the toxic effect of metals and the highest achieved recovery value is around 40%

and 20% for Cu and Zn, respectively, as a result of low conversion of $Fe^{2+}$ into $Fe^{3+}$ from bacteria oxidation (Figure 1.5).

This result is also confirmed by the literature, which only reports the *L. ferrooxidans* use in mix bacteria culture (Joshi et al., 2017).

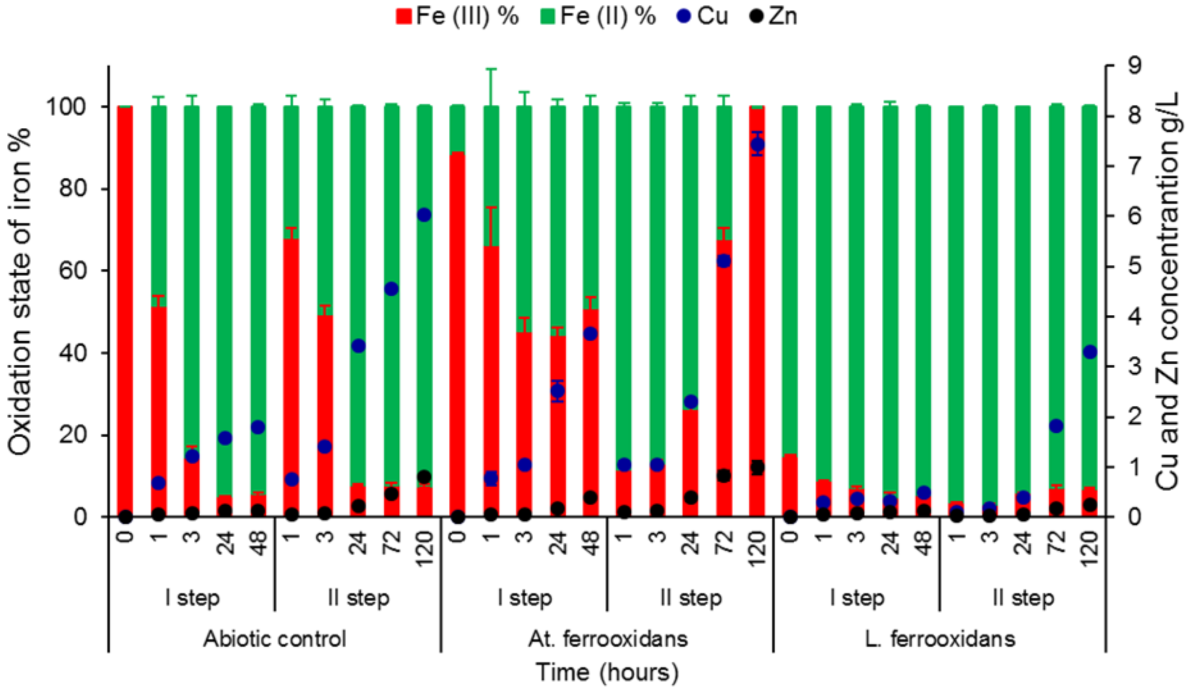

Figure 1.5. The leaching kinetics of Cu and Zn recovery from PCB and oxidation of $Fe^{3+}$ in the different treatment (abiotic control, *At. ferrooxidans* and *L. ferrooxidans*).

Furthermore, the results in Figure 1.6 confirm the highest effectiveness of the microbial leaching (using *At. ferrooxidans* as bacteria) compared to the abiotic control, obtaining an extraction yield around 95% and 70% of Cu and Zn, respectively.

The additional quantification of both As and Cr concentration in the leach liquor showed values lower than established by European Commission for the best available techniques for the direct discharges to a receiving water body (The European Commission, 2018). This relevant information proves the low toxicity of the resulting solution, in the perspective of the treatment implementation on a real scale.

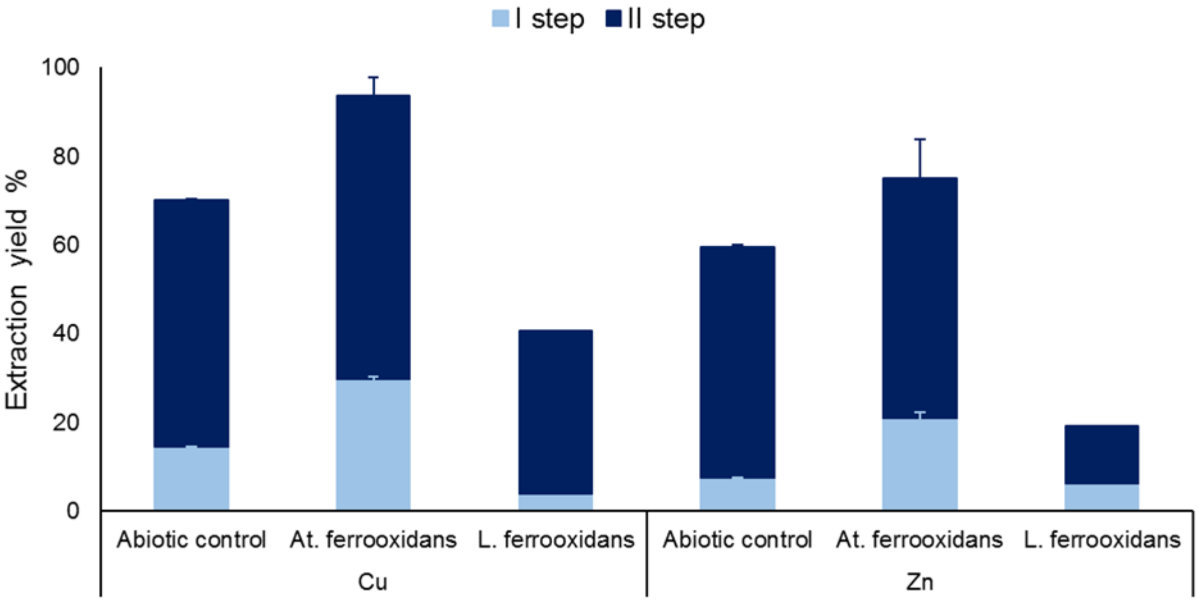

Figure 1.6. Cu and Zn extraction yield from PCB treated with *At. ferrooxidans* and *L. ferrooxidans* by a two steps bioleaching process.

### *3.3 Kinetic of metal recovery*

To better understand the achieved results, the kinetic of metal recovery was studied for all the treatments to highlight the differences between the chemical and the bioleaching approaches. In this regard, the shrinking core model (Eq. 1.4), based on a mechanistic study approach, was used to describe the kinetic reaction for the diffusion through the solid pores (Goto et al., 1996; Yang et al., 2014):

$$kt = 1 - \frac{2}{3}M_t - (1 - M_t)^{\frac{2}{3}} \tag{1.4}$$

Where t is the time, $M_t$ represents the fraction of mobilized metal and k is the constant rate for the shrinking core model. The obtained results (Figure 1.7 a and b), show a reaction kinetic increase of 5 and 12.5 times for Cu and Zn leaching, respectively, in the chemical approaches with 50 g/L of $Fe^{3+}$, thanks to the temperature growth from 30°C to 50°C. The confirmation of the temperature positive effect justifies the time decrease, from 24 (at 30°C) to 6 hours (at 50°C), required for the chemical leaching conclusion. On the other hand, Figures 1.7 c and d compare the behaviour of bioleaching and abiotic control proving the fastest kinetic of the first one, during the two steps of the process. More in detail, the k related to the I step of the biological treatment shows values 4 and 8 times higher than the abiotic control for Cu and Zn leaching, respectively. The difference is less evident during the II step, where the kinetic reactions for Cu and Zn are 1.1 and 2 times faster than abiotic control. The different result obtained for the two steps can be explained by a double reason: 1) in the I step the PCBs were added at the end of bacteria growth phase, when microorganism count and metabolism has already reached the exponential phase. The decrease of the rate during the II step is due to the partial medium replacement, which slows down the whole kinetic for the necessity of a preliminary bacteria growth, essential to restore the same conditions of the I step; 2) the highest metal concentrations in the II steps decreased the specific growth rate and metabolism of the *At. ferrooxidans* (Cabrera et al., 2005a, 2005b; Chisholm et al., 1998; Dopson, 2003).

As concern the comparison between the chemical and bioleaching (at 30°C, chosen as experimental condition), the estimated k values are 4 and 9%, during the I step and 5 and 25% during the second one for Cu and Zn, respectively. The reported percentages are expressed as ratio between the bioleaching and the chemical approaches.

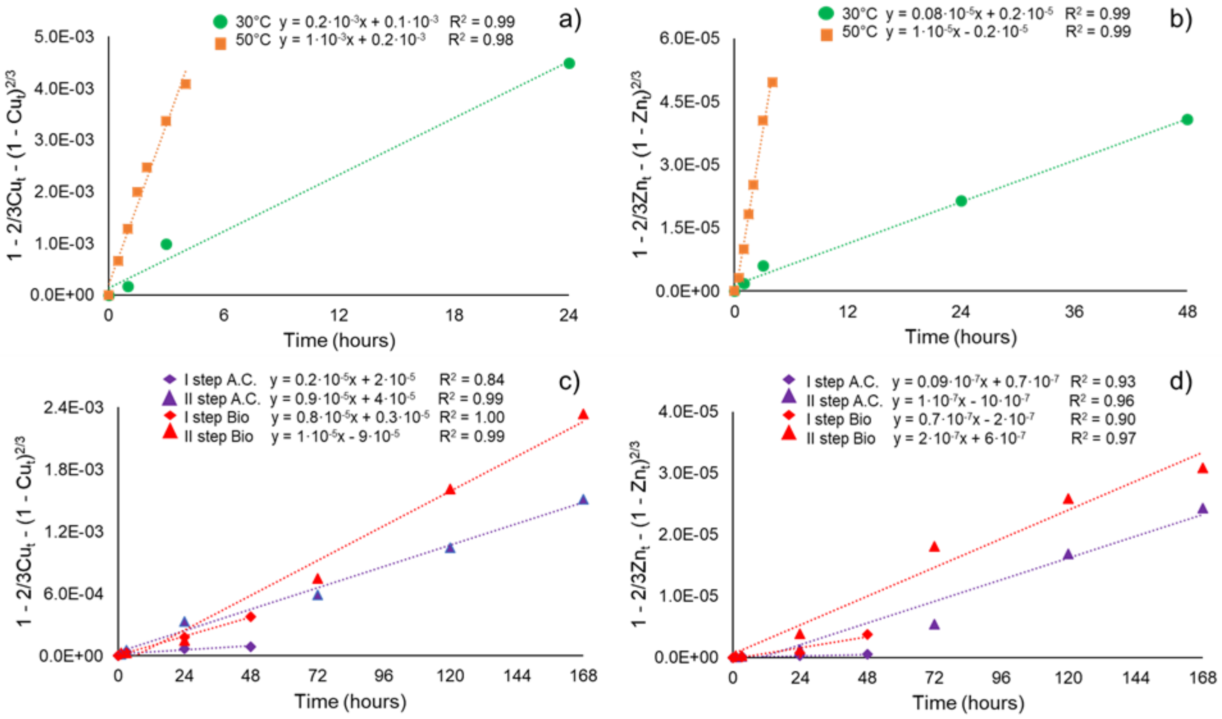

Figure 1.7. Implementation of the shrinking core model for the kinetic study of the recovery of: a) Cu b) Zn by the chemical approach and c) Cu and d) Zn by the bioleaching (Bio) and abiotic control (A.C.).

### *3.4 Assessment of the carbon footprint*

The results reported in Figure 1.8 describe the carbon footprint of the five scenarios selected for the analysis. As concern the innovative bioleaching (scenario 1), the treatment with *A. ferrooxidans* bacteria was selected for the best achieved efficiencies, comparable with those of the other assessed options. Overall, the scenarios 1 and 3 show comparable impacts, with a minimal advantage of the biotechnological approach (0.4 and 0.7 kg $CO_2$-eq./kg treated PCBs, respectively Figure 1.8 a). The positive effect of the innovative process design, proposed in the present paper, is highlighted in Figure 1.8 b, where the environmental impact decreases up to 4 times, compared to the bioleaching approaches reported in the literature. Indeed, the possibility of an increasing PCB concentration during the treatment allows the reduction of both micronutrient and water demands, with the consequent decrease of the bioreactor size and the energy request. This result is confirmed by Wu et al. (2018), which reports an energy requirement of 2.6 kWh (Table 1.2), comparable with that of the proposed innovative process, for the treatment of the same PCB amount, with a bioreactor 10 times greater. In the five options the positive effect of the Cu and Zn recovery (quantified as avoided primary production) allows to partially balance the environmental load due to the energy and raw materials consumption. Nevertheless, this effect is less evident for the third and fourth scenarios since the hydrometallurgical and biotreatment option focuses on the only Cu with a lower efficiency than the options 1 and 2. The effect of electricity consumption is highlighted for both the chemical treatment and the biotechnological approach, due to the high required temperature (scenario 2) and the long reaction time of scenario 1 and 4.

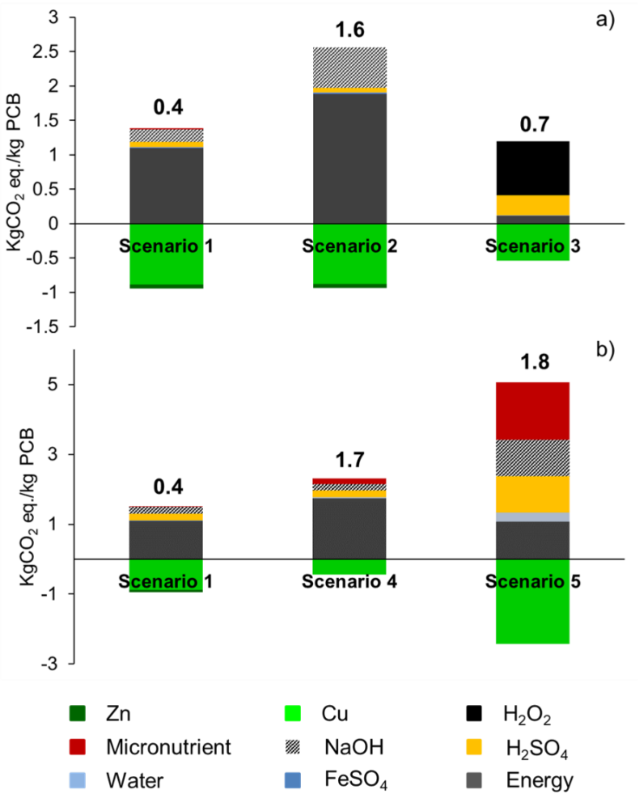

Figure 1.8. Carbon footprint of the five considered approaches. (Function unit: 1 kg of shredded PCBs).

Overall, the environmental credit associated to the metals target is not enough to balance the processes impact. Nevertheless, the possibility of the resulting sludge exploitation could further increase the environmental benefit. In this regard, chemical characterization identified the presence of rare earths (e.g. Sc, Y, La, Ce, Nd, Gd, Lu) (Hubau et al., 2019; Tkaczyk et al., 2018; Yamane et al., 2011); however, as also confirmed by the literature, the low concentration of these elements shift the research attention to the precious metals (e.g. Au, Ag, Pd). These elements combine the significant concentration with a high recovery potential, which could further increase the environmental benefit, in the perspective of the process scale-up.

## 4. Conclusions

In a context of continuous development of biotechnological approaches, the present study describes a high efficiency and high sustainability treatment for the metal extraction from PCBs.

A two-step bioleaching process, using *A. ferrooxidans* bacteria, at the best selected conditions: 30°C, solid concentration of 5% (w/v), 10 g/L of $Fe^{2+}$ conc., for 9 days, allowed to achieve 94% and 70% of extraction yield for Cu and Zn, respectively, with a further decrease of Fe demand higher than the 60%, compared to the chemical treatment. The carbon footprint assessment (0.4 kg $CO_2$-eq./kg treated PCBs) proved the process advantage, with an impact comparable with the hydrometallurgical option. The main biotechnology criticality is the high electricity demand, which could be solved by the implementation of a heap leaching design, able to considerably decrease the necessary energy. This possibility, combined with the high process efficiency and an already low climate change impact, proves the advantage of the biotechnological approach for the exploitation of a WEEE which becomes a secondary source of raw materials, in agreement with the circular economy pillars.

## 5. Products

Results illustrated and discussed in this chapter were used to support a peer reviewed article published on Resources, Conservation & Recycling, Elsevier (Figure 1.9).

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Full length article

An innovative biotechnology for metal recovery from printed circuit boards

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Figure 1.9. Articles published on *Resources, Conservation & Recycling*.

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# Chapter 2: MATHEMATICAL MODELLING OF CHEMICAL EXTRACTION OF VALUABLE MATALS FROM ELECTRONIC WASTE

## Abstract

A kinetic study of the process for the copper leaching from printed circuit board, by $Fe^{3+}$, was reported. A mathematical model, supported by experimental data, considered the mechanisms that control the reaction (chemical reaction, particle or liquid film diffusion). The kinetic of the chemical reaction was studied by two models (pseudo-first and pseudo-second order), with a calculated activation energy of 18-25 kJ/mol. Nevertheless, both the models showed two criticalities: the *Cu* concentration dependence from the $Fe_0^{3+}$ and the $Fe^{3+}$ reducing rate. Therefore, a new mathematical model was proposed to predict the recoverable *Cu* from PCB, by three parameters: the *Cu* content, the $Fe^{3+}$ concentration and the temperature. The high reliability of the model makes it an essential tool for the urban mining process optimization.

## Content

## 1. Introduction

A constant technological development has involved the modern society, with a consequent increase of raw material demand (Kyser, Barr & Ihlenfeld, 2015). The growth of the global electric and electronic equipment production has caused a double problem due to both the required raw materials and the resulting waste generation (Zhang & Xu, 2016). Nevertheless, the waste from electric and electronic equipment (WEEE) could be a secondary metal resource, especially from printed circuit boards (PCBs) that are the brain of electronic products (Xiang *et al.*, 2010). The attention for the metal recovery from end-of-life PCBs is due to the high metal content, mainly *Cu*, which makes them a potential source of secondary raw materials in the perspective of the circular economy implementation (Rocchetti, Amato & Beolchini, 2018; Rodrigues *et al.*, 2015). The positive effect could be further enhanced by the avoided disposal of these components, classified as hazardous waste (Leung *et al.*, 2008; Musson *et al.*, 2006; Shen *et al.*, 2008). The *Cu* leaching from PCBs by biotechnological approaches was studied by several works using iron oxidizing bacteria, mainly *At. ferrooxidans* (Bas, Deveci & Yazici, 2013; Choi *et al.*, 2004; Hubau *et al.*, 2018; Ilyas, Lee & Chi, 2013; Joshi *et al.*, 2017; Liang *et al.*, 2016, 2013, Liang, Mo & Zhou, 2010; Nie *et al.*, 2015a, 2015b; Priya & Hait, 2018; Rodrigues *et al.*, 2015; Wang *et al.*, 2009, 2018; Wu *et al.*, 2018; Xiang *et al.*, 2010; Yang *et al.*, 2009, 2014; Zhu *et al.*, 2011). This bacterial strain is able to oxidize $Fe^{2+}$ to $Fe^{3+}$, a powerful oxidant agent which permits the *Cu* mobilization, following this equation (Eq. 2.1):

$$Cu^0 + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} \tag{2.1}$$

To better understand the nature and the mechanism of the leaching process, the kinetic study represents an essential tool, irrespective of the selected approach. Although the mathematical model cannot include all the possible interactions and secondary reactions, the identification of the best model, able to describe the process, allows to hypothesize the process effectiveness on the basis of the selected operative conditions. In this regard, the literature reports several studies related to the PCB bioleaching (Chen *et al.*, 2015a, Yang *et al.*, 2014) and catalytic bioleaching (Gu et al., 2017a, 2017b). The kinetic of hydrometallurgical approaches is deepened by Kim *et al.* (2011), which study the leaching using electro-generated chlorine in hydrochloric acid solution and Havlik *et al.* (2010) that describes the possibility of a *Cu* extraction after a thermal pretreatment. Alternatively, the kinetic study of Chen *et al.* (2015b) focuses on the use of ionic liquid acids. As confirmed by the literature, the deduction of the best kinetic parameters is essential in the perspective of a plant design (including the possible automatic control), for the treatment optimization and the maximization of the metal recovery (Baniasadi *et al.*, 2019). In this context, the present work aims at the determination of the mathematical model able to predict the kinetics of *Cu* leaching from PCBs by $Fe^{3+}$, applicable on a representative solution resulting from both chemical and bioleaching.

## 2. Materials and methods

### *2.1 Preparation of waste printed circuit boards (PCBs)*

The PCBs used in this study were kindly provided by the University of L’Aquila, Italy, that prepared samples as follows: the material was shredded using stainless steel blades and pliers after manually removing the main parts of electronic components (e.g. capacitors, batteries and resistors). Thereafter, the refuse was crushed to obtain a granulometry of less than 0.5 mm, with an average *Cu* content of 25% (w/w), which was used for all the chemical leaching experiments (Table 2.1).

Table 2.1. Mean metal concentration in PCBs.

| Metals | Concentration % (w/w) |
|---|---|
| Copper | 24.7 |
| Zinc | 3.7 |
| Aluminum | 0.4 |
| Nickel | 0.03 |
| Lead | 1.3 |
| Tin | 2.1 |

### *2.2 Experimental procedure*

The chemical leaching of PCBs was carried out in a 200 mL Becker with 100 mL of a $Fe_2(SO_4)_3$ solution at two different $Fe^{3+}$concentrations and three temperatures (Table 2.2). The PCBs were added, with a concentration of 5% (w/v), when the required temperature was reached. The reaction carried on for a maximum of 6 hours, at pH 1.6, stabilized by $H_2SO_4$ addition. The selected conditions aimed to represent both a chemical and a biological treatment, since a bioleaching by iron oxidizing bacteria produces comparable concentration of $Fe^{3+}$ in solution. Regularly, 1 mL samples were collected for $Fe^{3+}$, total *Fe* and *Cu* concentration determination.

### *2.3 Analytical determination*

The *Cu* and other metal determinations were carried out by an atomic absorption spectrophotometer (Techcomp, AA6000). The $Fe^{3+}$ concentration was determined spectrophotometrically (Jasco Model 7850) by the colorimetric thiocyanate method (Welcher & Hahn, 1964). For the measure of the total *Fe* by atomic absorption, the solution previously reacts with potassium permanganate to oxidize the $Fe^{2+}$ in the sample. $Fe^{2+}$ was determined as the difference between $Fe_{tot}$ and $Fe^{3+}$ analyses.

### *2.4 Kinetic model*

The results obtained from the experimental tests were used to determine the kinetic mathematical model to describe the chemical reaction between $Fe^{3+}$ and $Cu$ in the PCBs. The global mechanism was studied to establish if it was controlled by the chemical reaction (chemical reaction control), by the diffusion of metal ions ($Fe^{3+}$) through the liquid film (film diffusion control) or by the diffusion through the reacted shell both for $Fe^{3+}$ and $Cu^{2+}$ (particle diffusion control) (Figure 2.1).

The chemical reaction was studied by two possible models, pseudo-first order model and pseudo-second order model (Qiu *et al.*, 2009). The process kinetic for the pseudo-first order model (Eq. 2.2) and for the pseudo-second order (Eq. 2.3) are described by the following differential equation:

$$\frac{dCu_t^{2+}}{dt} = k_1(Cu_0^0 - Cu_t^{2+}) \qquad (2.2)$$

$$\frac{dCu_t^{2+}}{dt} = k_2(Cu_0^0 - Cu_t^{2+})^2 \qquad (2.3)$$

Where $Cu_t^{2+}$ is the concentration of $Cu^{2+}$ ions in the solution and $Cu_0^0$ is the maximum concentration expected in solution ant the end of the tests.

Integrating Eq. 2.2 and Eq. 2.3 with the boundary conditions of $Cu_t^{2+} = 0$ at $t = 0$ and $Cu_t^{2+} = Cu_t^{2+}$ at $t = t$, yields (Eqs. 2.4 and 2.5):

$$Cu_t^{2+} = Cu_0^0 - \frac{Cu_0^0}{e^{k_1 t}} \qquad (2.4)$$

$$Cu_t^{2+} = \frac{\left(Cu_0^0\right)^2 k_2 t}{Cu_0^0 k_2 t + 1} \qquad (2.5)$$

Where $k_1$ and $k_2$ are the rate constants given by Arrhenius equation (Eq. 2.6):

$$k = Ae^{-\frac{E_A}{R \cdot T}} \tag{2.6}$$

Where $A$ is the pre-exponential factor, $E_A$ is the activation energy for the reaction (J $mol^{-1}$), $R$ is the universal gas constant (8.31 J $K^{-1}$ $mol^{-1}$) and $T$ is the absolute temperature (K).

In the case of a process controlled by the film diffusion the extent of chemical reaction as a function of time is given by the following equation (Eq. 2.7) (Veglio, Beolchini & Toro, 1998):

$$\chi = k_{F,D} \int_0^t Fe^{3+} dt \tag{2.7}$$

Where $\chi$ is given by the following equation (Eq. 2.8):

$$\chi = \frac{Fe_0^{3+} - Fe_t^{3+}}{Fe_0^{3+}} \tag{2.8}$$

Consequently, if the mechanism is controlled by the film diffusion, a plot of $\chi$ vs. $\int_0^t Fe^{3+} dt$ yields a straight-line relationship and the apparent film diffusion $k_{F,D}$ could be obtained from the slope of the plot.

On the other hand, if the process is controlled by particle diffusion of $Fe^{3+}$, the model is represented by the following expression (Eq. 2.9) (Beolchini *et al.*, 2003; Veglio, Beolchini & Toro, 1998):

$$F_{(\chi)} = 1 - 3(1-\chi)^{\frac{2}{3}} + 2(1-\chi) = k_{P,D} \int_0^t Fe^{3+} dt \tag{2.9}$$

Consequently, in the case of particle diffusion control a plot of a function of $F(\chi)$ vs. $\int_0^t Fe^{3+} dt$ gives a straight-line and the slope indicates the apparent diffusivity ($k_{P,D}$).

An alternative mathematical model to describe a chemical reaction controlled by particle diffusion, is the particle diffusion of the products (Becci *et al.*, 2019; Yang *et al.*, 2014). The model describes the product diffusion, in this case $Cu^{2+}$ recovered from PCBs, through the particle pores, following the Eq. 2.10:

$$tk_{P,D\,2} = 1 - \frac{2}{3}M_t^{2+} - (1 - M_t^{2+})^{\frac{2}{3}} \tag{2.10}$$

Where *t* represents the time and $M_t^{2+}$represents the fraction of mobilized metal.

Table 2.2. Factors and levels investigated for the kinetic modeling of PCBs leaching (PCBs concentration 5% w/v, pH 1.6 and 6 hours of reaction time).

| Factors | Levels | | |
|---|---|---|---|
| Temperature (°C) | 30 | 50 | 70 |
| Initial $Fe^{3+}$ concentration (g/L) | 35 | | 9 |

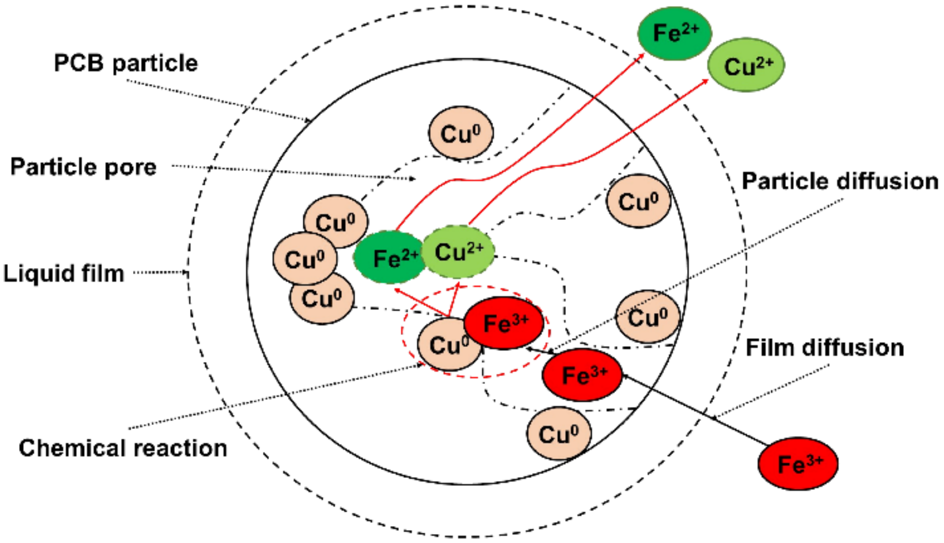


Figure 2.1. A conceptual schema of the possible kinetic control.

## 3. Results

For the activation energy ($E_A$) estimation the factorial plan reported in Table 2.2 was implemented. The reported levels are based on the main conditions identified by Becci *et al*. (2020). Notably, the $Fe^{3+}$ concentration of 35 g/L was higher than stoichiometric to complete the *Cu* chemical leaching; therefore, $Cu_0^0$ in the Eqs. 2.4 and 2.5 was the *Cu* maximum concentration in PCBs (25% w/w, $1.97 \cdot 10^{-1}$ mol in 50 g of PCBs). In the lowest $Fe^{3+}$ concentration case (9 g/L), the $Fe$ amount was not enough to complete the $Cu$ chemical leaching. Therefore, the $Cu_0^0$ in the second case was calculated by the correlation between $Fe_t^{3+}$ and $Cu_t^{2+}$ concentrations during the time (Figure 2.2), at the three temperatures was chosen ($4.49 \cdot 10^{-2}$ mol).

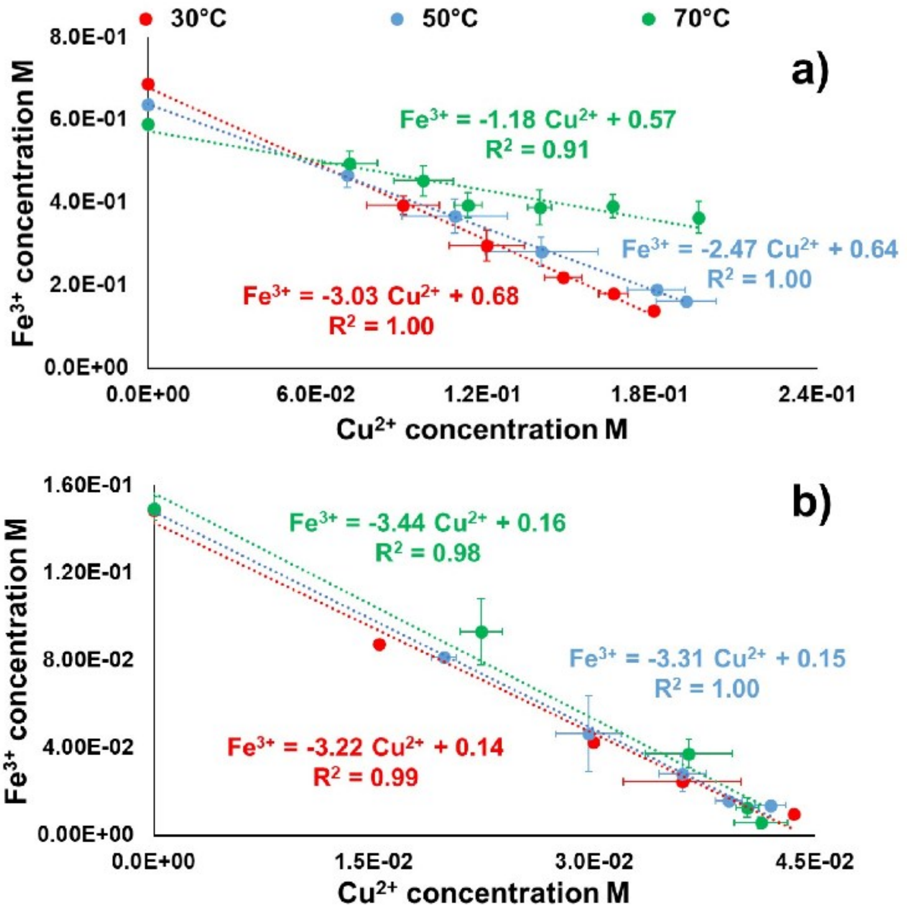


Figure 2.2. Correlation between $Cu^{2+}$ and $Fe^{3+}$ concentration in the chemical experiment with 35 g/L (a) and 9 g/L (b) of $Fe^{3+}$ at three different temperature (30°, 50° and 70°).

Figure 2.2 shows a molar ratio between $Fe^{3+}$ and $Cu^{2+}$ different from 2, as reported the Eq. 2.1, for two main reasons: 1) the $Cu$ also reacted with sulfuric acid (Hong and Valix, 2014; Wang *et al.*, 2009); 2) and $Fe^{3+}$ reacted with other metals of PCBs, such as *Zn*, *Ni*, *Al*, *Pb* and *Sn* (Table 2.3) (Brandl, Bosshard, and Wegmann, 2001; Wang *et al.*, 2009, 2018; Yang *et al.*, 2014, Chen *et al.*, 2015b).The low concentrations of the further metals in the used samples (between 10 and 100 times lower than Cu) does not affect the whole model. Nevertheless, considering the variability of PCB composition the highest contents of these elements needs to be integrated within the proposed models.

Table 2.3. Metal concentration at the end of each experiments.

| Metal concentration (g/L) | 9 g/L of $Fe^{3+}$ | | | 35 g/L of $Fe^{3+}$ | | |
|---|---|---|---|---|---|---|
| | 30° C | 50° C | 70° C | 30° C | 50° C | 70° C |
| Zn | 0.48 | 0.47 | 0.40 | 1.8 | 1.7 | 1.4 |
| Al | 0.02 | 0.01 | 0.02 | 0.1 | 0.2 | 0.3 |
| Ni | - | - | - | 0.01 | 0.01 | 0.01 |
| Pb | 0.01 | 0.01 | 0.01 | 0.04 | 0.05 | 0.04 |
| Sn | 0.01 | 0.01 | 0.03 | 0.03 | 0.05 | 0.38 |

Using the pseudo-first order model (Eq. 2.4) and pseudo-second order model (Eq. 2.5) the rate constants ($k_1$ and $k_2$) of the chemical reactions between $Cu^{2+}$ and $Fe^{3+}$ were estimated for each experimental plan (Table 2.4). The activation energy ($E_A$) was determined by plotting the reaction rate constants, by the natural logarithm of Arrhenius equation (Eq. 2.6), rearranging as (Eq. 2.11):

$$\ln k = -\frac{E_A}{R} \cdot \left(\frac{1}{T}\right) + \ln A \qquad (2.11)$$

Where the slope of the straight line was the ratio between activation energy ($E_A$) and the universal gas constant ($R$) and the intercept with $y$ axis was the *ln A* (Figure 2.3).

Table 2.4. Parameter estimation of Eq. 2.4 (pseudo-first order model) and Eq. 2.5 (pseudo-second order model).

| $Fe^{3+}$ conc. (g/L) | Temp. (K) | Pseudo-first order model | | Pseudo-second order model | |
|---|---|---|---|---|---|
| | | $K_1$ | $E_A$ (kJ/mol) | $k_2$ | $E_A$ (kJ/mol) |
| 35 | 303 | 0.89 | 17.1 | 8.12 | 18.7 |
| | 323 | 1.59 | 17.1 | 14.5 | 18.7 |
| | 343 | 1.95 | 17.1 | 19.2 | 18.7 |
| 9 | 303 | 2.47 | 17.1 | 83.6 | 18.7 |
| | 323 | 4.49 | 17.1 | 158.2 | 18.7 |
| | 343 | 5.42 | 17.1 | 197.4 | 18.7 |

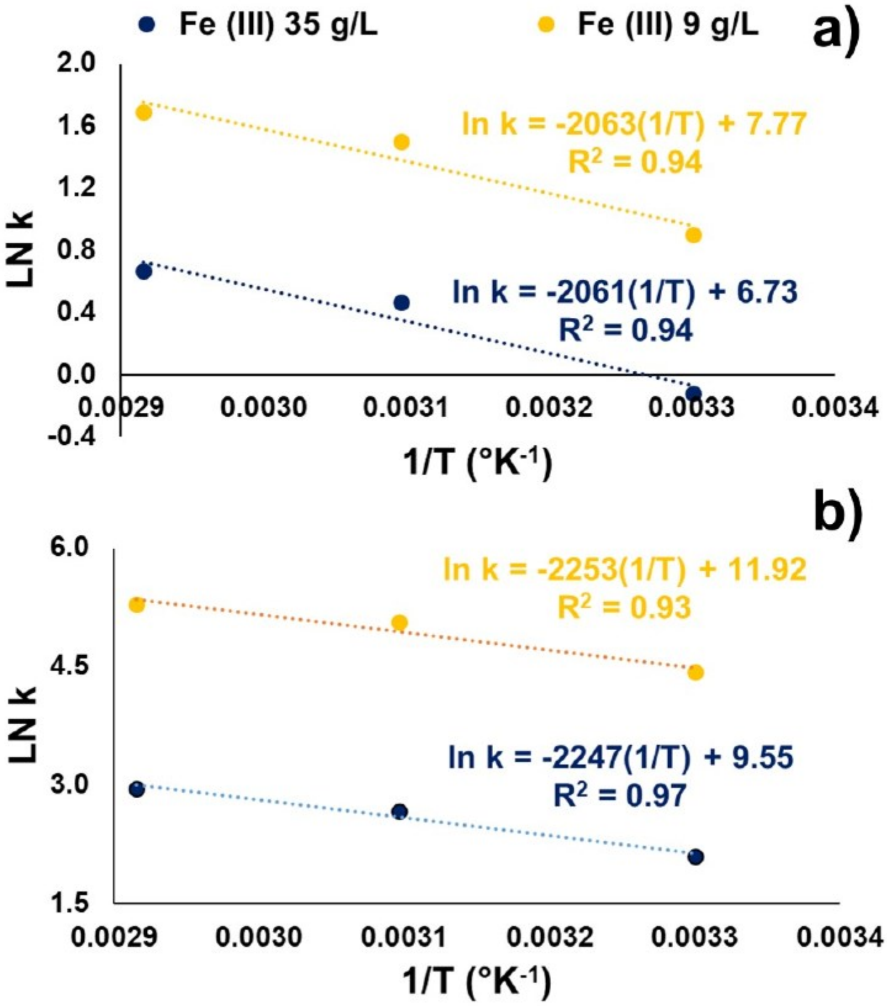


Figure 2.3. Linearization of Arrhenius equation with the rate constant (*k*) determined with pseudo-first order model (a) and with pseudo-second order model (b).

The activation energy of 17.1 and 18.7 kJ/mol were determined with pseudo-first order model and pseudo-second order model, respectively. As showed in Figure 2.4, the pseudo-second order model (Figure 2.4 b and d) represented the kinetic model of *Cu* chemical leaching better than pseudo-first order model (Figure 2.4 a and c), with a R-squared higher than 0.95. The same results ($E_A$ and $A$), obtained for the *Cu* leaching prevision by the pseudo-second order model, were used to describe the $Fe^{3+}$ trend using the following differential equation (Eq. 2.12):

$$\frac{dFe_t^{3+}}{dt} = -k_2\left(Fe_t^{3+} - Fe_f^{3+}\right)^2 \tag{2.12}$$

Integrating Eq. 2.12 with the boundary conditions of $Fe_t^{3+} = Fe_{tot}$ at $t = 0$, and $Fe_t^{3+} = Fe_t^{3+}$ at $t = t$, yields (Eq. 2.13):

$$Fe_t^{3+} = Fe_f^{3+} + \frac{Fe_{tot} - Fe_f^{3+}}{\left(Fe_{tot} - Fe_f^{3+}\right)k_2 t + 1} \tag{2.13}$$

Where $Fe_f^{3+}$ was calculated from the correlation between $Fe_t^{3+}$ and $Cu_t^{2+}$ graphic (Figure 2.2) for each temperature and initial $Fe^{3+}$ concentration.

The results (Figure 2.5) obtained with the pseudo-second order model, showed a R-squared between 0.85 and 0.94, lower than *Cu* kinetic model. The reasons were the same explained for the $[Fe^{3+}]/[Cu^{2+}]$ ratio different from 2 (Figure 2.2).

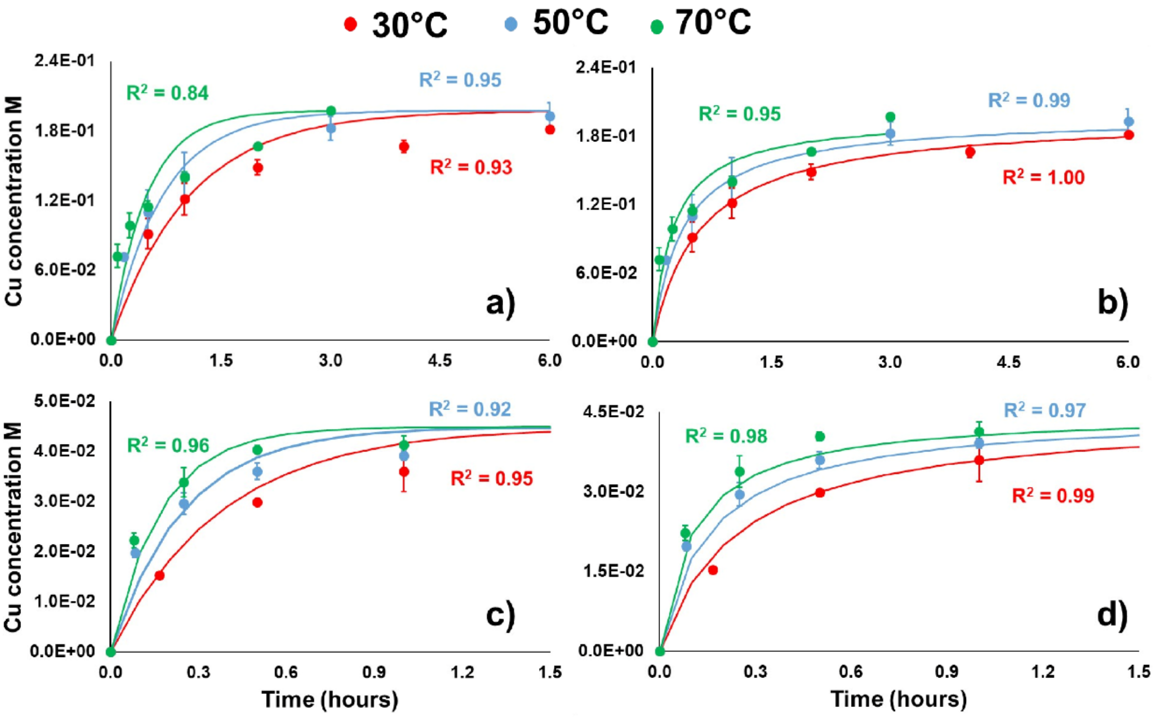


Figure 2.4. Experimental (point) and calculated (lines) by the pseudo-first order model (a and c) and by pseudo-second order model (b and d) with the activation energy and pre-exponential factor values with *Fe*$^{3+}$ concentration of 35 g/L (a and b) and 9 g/L (c and d).

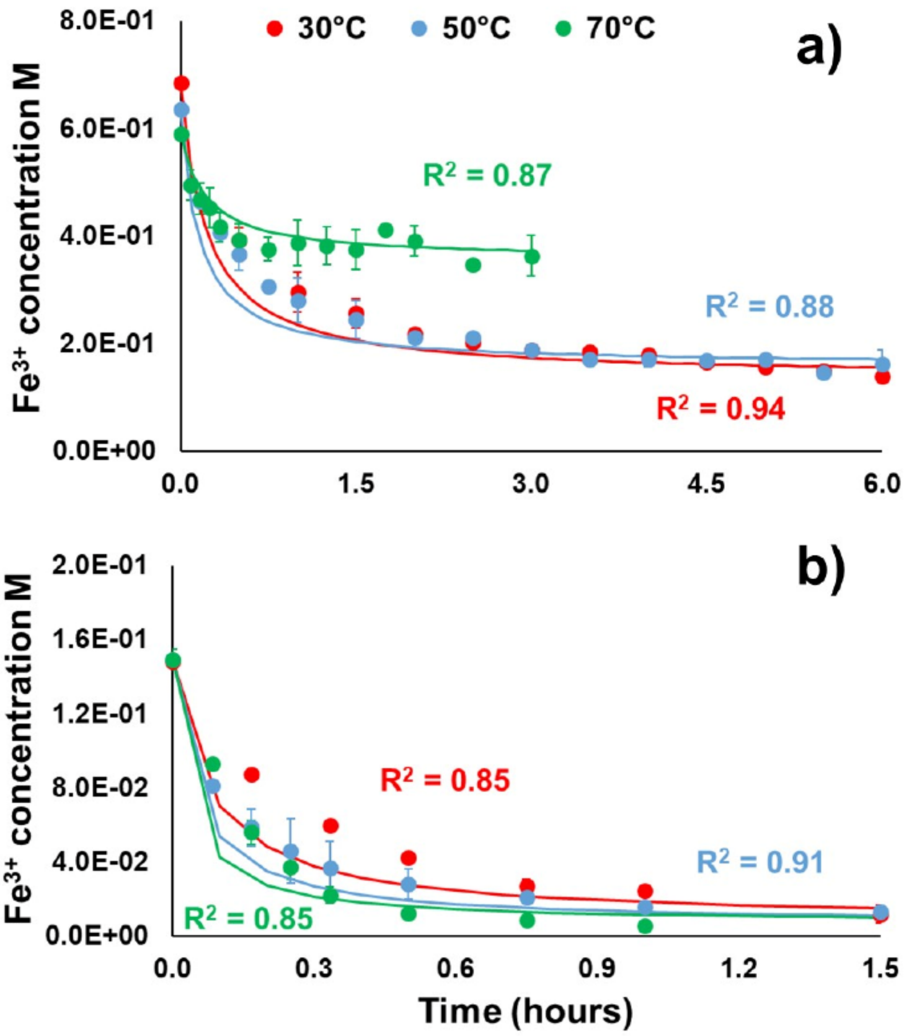


Figure 2.5. Experimental (point) and calculated (lines) by the pseudo-second order model with the activation energy and pre-exponential factor values determined for *Cu* model, with *Fe*$^{3+}$ concentration of 35 g/L (a) and 9 g/L (b).

The activation energy estimated by both pseudo-first order and pseudo second order model were lower than 75 kJ/mol. The activation energy greater than this value was representative of the chemical reaction control (Beolchini & Veglió, 1999; Peters, 1991). Therefore, to establish if the reaction kinetic was controlled by chemical reaction, by film or particle diffusion, the *Fe*$^{3+}$ diffusion through the liquid film or the PCB particle was studied by the mathematical model reported before (Eq. 2.7 and Eq. 2.9 for *Fe*$^{3+}$ liquid and particle diffusion model, respectively and Eq. 2.10 for $Cu^{2+}$ particle diffusion). The results (Table S2.1) confirmed that the kinetic reaction was controlled by the chemical reaction: a very bad fitting was achieved in all the studied cases: $Fe^{3+}$ liquid film (Figure S2.1) and particle diffusion (Figure S2.2) and $Cu^{2+}$ particle diffusion (Figure S2.3).From the results achieved by the pseudo-second order rate for $Cu_t^{2+}$and $Fe_t^{3+}$prevision could be explained by an equation where $Cu_t^{2+}$depended on $Fe_t^{3+}$concentration. Equalizing Eq. 2.3 and Eq. 2.12 can be found $Cu^{2+}_{(Fe_t^{3+})}$, and the resulting equation (Eq. 2.14):

$$Cu^{2+}_{(Fe_t^{3+})} = \frac{(Cu_0^0)^2 Fe_t^{3+} - (Cu_0^0)^2 Fe_{tot}}{Cu_0^0 Fe_t^{3+} - Cu_0^0 Fe_{tot} - Fe_t^{3+} Fe_{tot} + Fe_t^{3+} Fe_f^{3+} - \left(Fe_f^{3+}\right)^2 + Fe_f^{3+} Fe_{tot}} \tag{2.14}$$

Table 2.5 reports the $Cu^{2+}$ leached at the end of the experimental tests and the $Cu^{2+}$ predicted by the Eq. 2.14 using the measured *Fe* concentration. The predicted *Cu* concentration reported a percentage error lower than 10%.

Table 2.5. Experimental and predicted values of *Cu* leached with Eq. 2.14.

| $Fe^{3+}$ conc. (g/L) | Temperature (k) | Time (hours) | $Cu^{2+}$ M Experimental | $Cu^{2+}$ M Predicted | Percent error |
|---|---|---|---|---|---|
| 35 | 303 | 6 | $1.81 \cdot 10^{-1}$ | $1.87 \cdot 10^{-1}$ | 3% |
|  | 323 | 6 | $1.93 \cdot 10^{-1}$ | $1.90 \cdot 10^{-1}$ | 2% |
|  | 343 | 3 | $1.97 \cdot 10^{-1}$ | $1.97 \cdot 10^{-1}$ | 0% |
| 9 | 303 | 1 | $0.36 \cdot 10^{-1}$ | $0.35 \cdot 10^{-1}$ | 4% |
|  | 323 | 1 | $0.39 \cdot 10^{-1}$ | $0.43 \cdot 10^{-1}$ | 9% |
|  | 343 | 1 | $0.41 \cdot 10^{-1}$ | $0.44 \cdot 10^{-1}$ | 9% |

In the model presented before, the main identified problems were: the initial $Cu_0^0$ concentration, depending on the initial $Fe^{3+}$ concentration, and the worst $Fe_t^{3+}$prediction by the pseudo-second order than $Cu_t^{2+}$. Therefore, to solve the evidenced problems, a new mathematical model was proposed to predict the chemical reaction kinetic between $Fe^{3+}$ and $Cu^{0}$, following this differential equation for the *Cu* extraction (Eq. 2.15):

$$\frac{dCu_t^{2+}}{dt} = k_3(Cu_0^0 - Cu_t^{2+})(Fe_0^{3+} - mCu_t^{2+}) \tag{2.15}$$

Integrating Eq. 2.15, with the boundary conditions of $Cu_t^{2+}$ = 0 at *t* = 0 and $Cu_t^{2+} = Cu_t^{2+}$ at *t* = *t*, yield (Eq. 2.16):

$$\frac{\left(\ln\frac{Cu_0^0 - Cu_t^{2+}}{Cu_0^0} - \ln\frac{Fe_0^{3+} - mCu_t^{2+}}{Fe_0^{3+}}\right)}{\left(mCu_0^0 - Fe_0^{3+}\right)} = k_3 t \tag{2.16}$$

And for $Fe_t^{3+}$ trend (Eq. 2.17):

$$\frac{dFe_t^{3+}}{dt} = m\frac{dCu_t^{2+}}{dt} \tag{2.17}$$

Where $Cu_0^0$ is the initial *Cu* concentration in the PCB particle ($1.97 \cdot 10^{-1}$ mol), $Fe_0^{3+}$is the initial *Fe* concentration and *m* is the experimental *Fe*/*Cu* stoichiometric coefficient (Figure 2.2).

As showed in Figure 2.6, a good correlation higher than 0.92 for all the experiments, except for the test at 70°C and 35 g/L of *Fe*, is achieved by the proposed mathematical model (Figures 2.6 a and c). Nevertheless, considering the correlation between the experimental $Fe_t^{3+}$and $Cu_t^{2+}$concentrations and the calculated values by the model, a good fit was achieved with a R-squared of 0.99 (Figure 2.7 a). The activation energy obtained by this new model is around 25 kJ/mol (Figure 2.7 b).

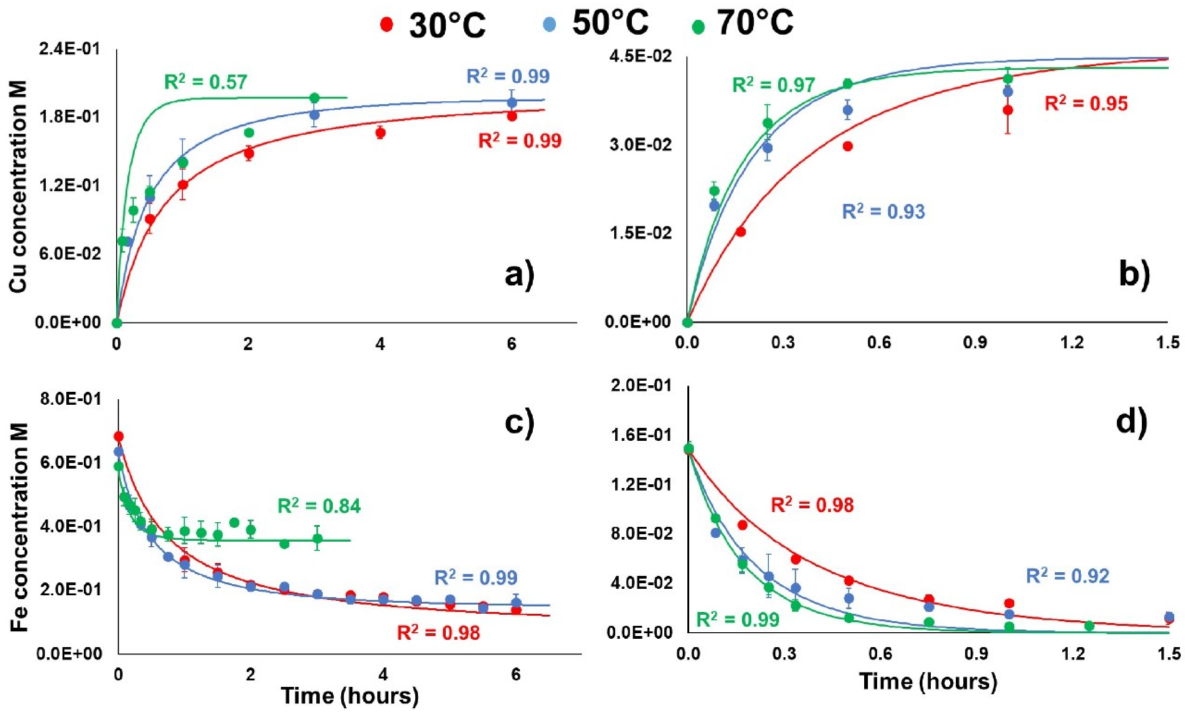


Figure 2.6. Experimental (point) and calculated (lines) by the new proposed model with the activation energy and pre-exponential factor values for *Cu* (a and b) and *Fe* (c and d) with initial $Fe^{3+}$ concentration of 35 g/L (a and c) and 9 g/L (b and d).

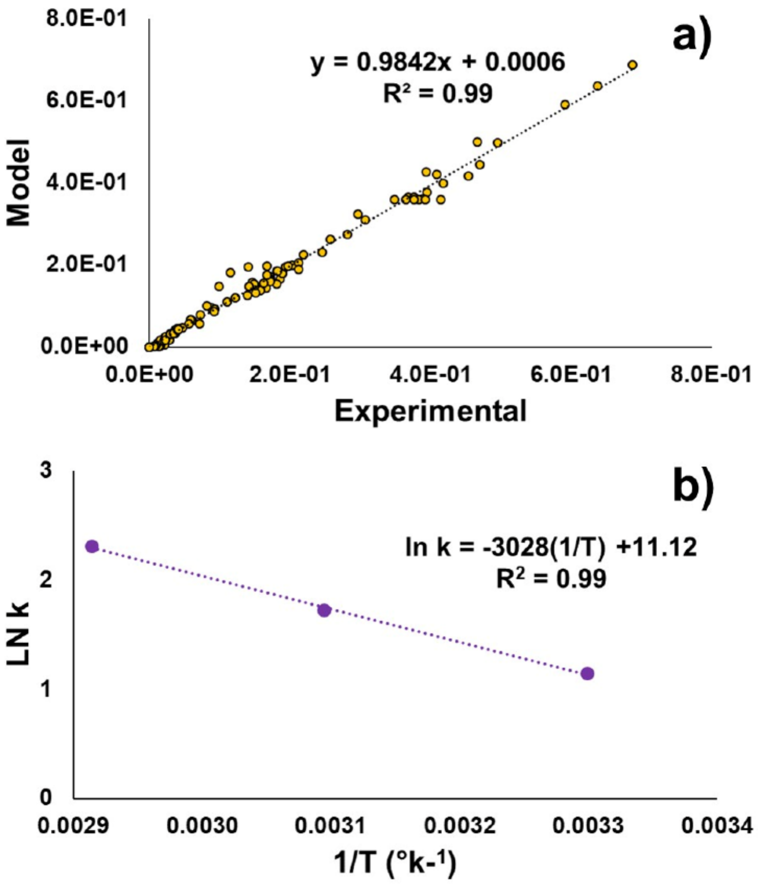


Figure 2.7. Correlation between the experimental data and the calculated value (a), and the linearization of Arrhenius equation with the rate constant determined with the proposed model (b).

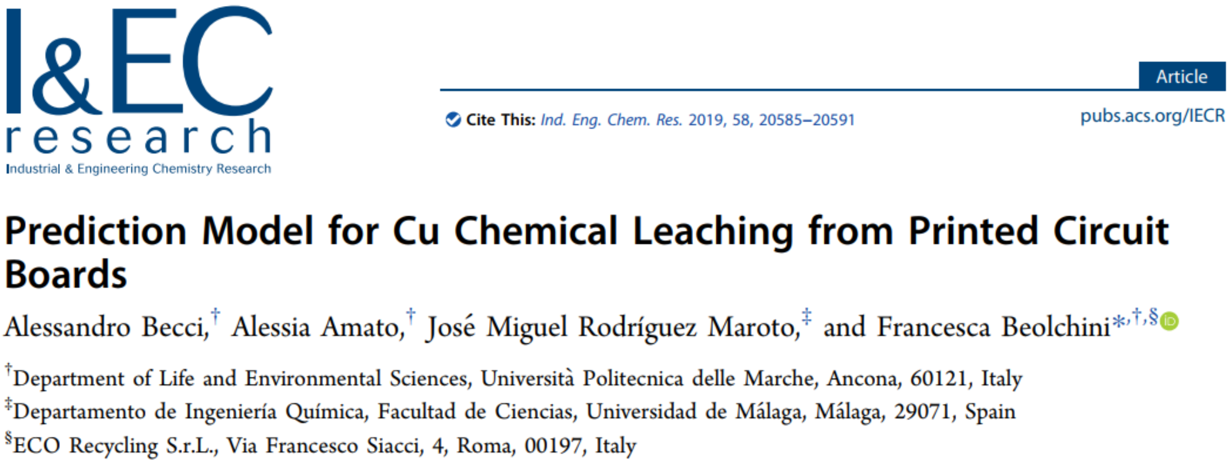
## 4. Conclusions

The study of the kinetic of the *Cu* chemical leaching from PCB, performed by $Fe^{3+}$ ions, allowed the identification of the chemical reaction, as the main step which controls the whole mechanism. This result was additionally confirmed by the bad linearization of both liquid film and particle diffusion for $Fe^{3+}$ and $Cu^{2+}$. Furthermore, the activation energy, around 18-25 kJ/mol, was calculated starting from the estimated kinetic constants. The whole mechanism was described by a pseudo-second order model, with a resulting R-squared higher than 0.95. A further mathematical equation (Eq. 2.14) was developed to predict the final leached *Cu*, starting from the preliminary measure of $Fe^{3+}$ concentration. Nevertheless, some criticalities, mainly due to the identification of the initial $Cu_0^0$ concentration (depending on $Fe_0^{3+}$) and the reducing rate of $Fe^{3+}$, were overcome by a new model (Eq. 2.16). This proposed equation was able to assess the recovered *Cu* considering its starting content in PCB (irrespective of the $Fe_0^{3+}$) and the $Fe_0^{3+}$concentration, at the specific reaction temperature.

The possibility to predict the *Cu* potentially recoverable from PCB is an essential information for the development of innovative processes of urban mining. Indeed, the variation of the relevant parameters allows the identification of the best operative conditions for the final optimization.

## 5. Products

Results illustrated and discussed in this chapter were used to support a peer reviewed article published on Industrial & Engineering Chemistry Research, ACS Publications (Figure 2.8).



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**Prediction Model for Cu Chemical Leaching from Printed Circuit Boards**

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Figure 2.8. Articles published on *Industrial & Engineering Chemistry Research*.

# 6. Supplementary material

Table S2.1: Parameter estimated by: Eq. 2.5 (pseudo-second order model), Eq. 2.7 (film diffusion model), Eq. 2.9 (particle diffusion model) and Eq. 2.10 (shrinking core model).

| $Fe^{3+}$ conc. (g/L) | Temp. (K) | $K_2$ (chemical constant rate) | $K_{F,D}$ (film diffusion constant rate) | $K_{P,D}$ ($Fe^{3+}$ particle diffusion constant rate) | $K_{P,D\,2}$ ($Cu^{2+}$ particle diffusion constant rate) |
|---|---|---|---|---|---|
| 35 | 303 | 8.12 | 0.69 | 0.28 | 0.0008 |
|  | 323 | 14.5 | 0.52 | 0.21 | 0.0009 |
|  | 343 | 19.2 | 0.20 | 0.03 | 0.0017 |
| 9 | 303 | 83.6 | 15.82 | 7.87 | 0.0002 |
|  | 323 | 158.2 | 24.89 | 13.14 | 0.0002 |
|  | 343 | 197.4 | 33.35 | 20.71 | 0.0002 |

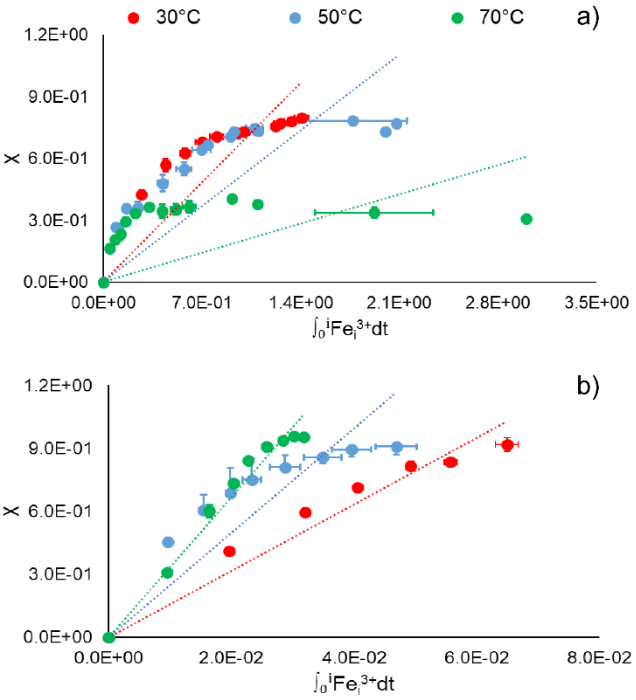

Figure S2.1: Linearization of liquid film diffusion model (Eq. 2.7) for $Fe^{3+}$ in the experiment with initial $Fe^{3+}$ concentration of 35 g/L (a) and 9 g/L (b).

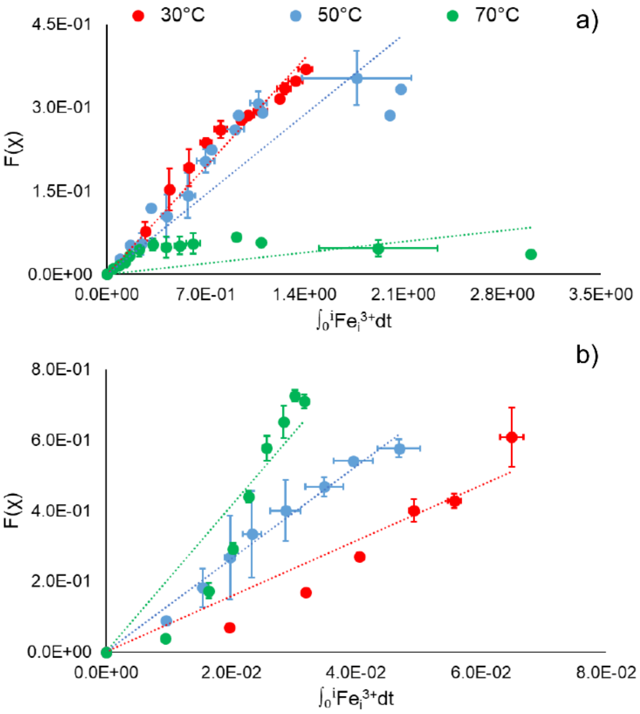

Figure S2.2: Linearization of particle diffusion model (Eq. 2.9) for $Fe^{3+}$ in the experiment with initial $Fe^{3+}$ concentration of 35 g/L (a) and 9 g/L (b).

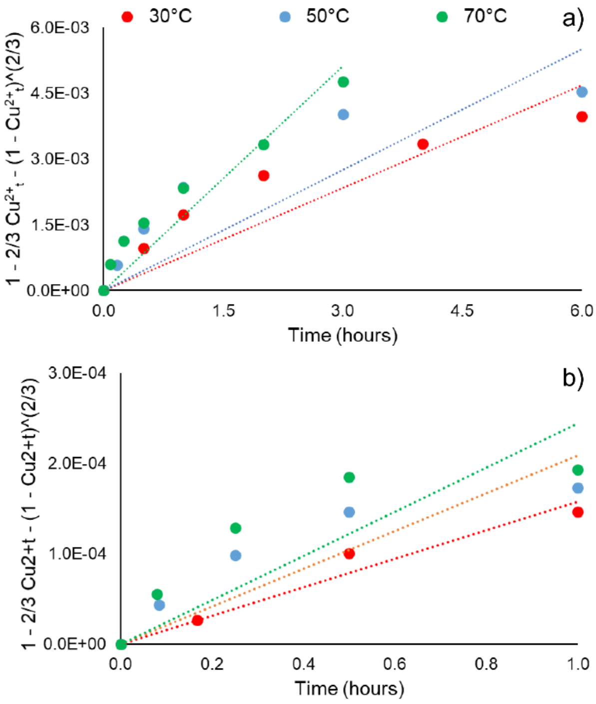

Figure S2.3: Linearization of particle diffusion model (Eq. 2.10) for $Cu^{2+}$ in the experiment with initial $Fe^{3+}$ concentration of 35 g/L (a) and 9 g/L (b).

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# Chapter 3: MATHEMATICAL MODELLING OF BIOLOGICAL EXTRACTION OF VALUABLE METALS FROM ELECTRONIC WASTE

## Abstract

This work reports a kinetic study of the bioleaching process for Cu extraction from waste printed circuit boards (WPCBs), using iron as oxidant agent. The kinetic study considered both the $Fe^{2+}$ oxidation by bacteria metabolism and the chemical reaction between $Fe^{3+}$ and Cu within WPCBs. The model was composed of a system of three differential equations characterized by three dependent variables and seven parameters. The first equation describes the bacteria abundance trend, the second one represents the variation of $Fe^{2+}$ concentration, and the third one focuses on the Cu extraction by the bio-generated $Fe^{3+}$. The developed model was consistent with a $R^2$ higher than 0.97. The results showed that the bacteria metabolism was 1.8-2.5 times faster than the chemical reaction. The determined mathematical model, suitable for the implementation at industrial scale, could be an important tool to predict the bioleaching mechanism.

## Content

## 1. Introduction

The modern society is becoming more and more dependent on electronic devices, that are characterized by short replacement cycle (Sodha et al., 2020). This tendency can be translated in a growing flow of new technologies to the market with a consequent increase of electronic waste amount (e-waste) (Z. Li et al., 2019; Moosakazemi et al., 2019). An amount of 45 million tons of e-waste was generated in 2016 with an estimated annual growth rate of 3-4% (Baldé et al., 2017; Evangelopoulos et al., 2019; X. Li et al., 2019; Schneider et al., 2019; Zhang et al., 2017). There are four main sources of e-waste: small/large home appliances, hospital medical equipment, office machines, and industrial equipment/machines (Kaya, 2019). Within the entire e-waste quantity, the WPCBs percentage is relevant (3-6%) (Awasthi and Zeng, 2019; Moyo et al., 2020; Pensupa et al., 2018; Tiuc, 2020). The WPCBs are composed for more than 40% (w/w) of metals (Pietrelli et al., 2019; Tiuc, 2020). Cu has an average concentration of 20% (w/w) of whole metals, and it is 10-40 time higher than that in copper rich minerals (Hsu et al., 2019; Qiu et al., 2019; Xiu and Zhang, 2009; Zhang et al., 2017). The annual Cu production is around 15 million tons and about 30% is used for the electric and electronic devices (Manjengwa, 2019). The Cu resulting from recycling is 36% of the total Cu production (Bonnin et al., 2015). Usually, to produce 1 ton of Cu from ores, around 1400-1700 kg of standard coal are used, and 20 tons of wastewater and 3 tons of hazardous material are generated (Qiu et al., 2019; Zeng et al., 2017). According to the Bureau of International Recycling ("BIR - Non-Ferrous Metals," n.d.) the Cu extracted from e-waste is able to save up to 85% of energy and reduce around 65% of $CO_2$ emissions, compare to the primary production (Bonnin et al., 2015). Therefore, the Cu recovery from WPCB recycling has a triple benefit: 1) the primary resource conservation, 2) the solid waste reduction, and 3) the prevention of the environmental pollution resulting from the Cu release (Akcil et al., 2015; D'Adamo et al., 2019; Moosakazemi et al., 2019). The most common techniques used for the e-waste recycling and metal recovery include three approaches: chemical, metallurgical and physical. The chemical processes include pyrolysis, gasification, combustion or the use of chemical agent (i.e. HCl, $H_2SO_4$, $HNO_3$, ammonium or ethylenediaminetetraacetic acid) (Kaya, 2019; Li et al., 2020; Pietrelli et al., 2019). However, the acid and the gases produced during the chemical processes generate a secondary pollution. For this reason, the biotechnologies have become more and more attractive in the last decades as eco-friendly and sustainable alternative (Fonti et al., 2015; Kaya, 2019). The Cu extraction from WPCBs by biotechnologies is studied by several works, mainly using *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) (Annamalai and Gurumurthy, 2019; Becci et al., 2020; Choi et al., 2004; Hubau et al., 2018; Ilyas et al., 2013; Joshi et al., 2017; Liang et al., 2013; Wu et al., 2018; Yang et al., 2014; Zhu et al., 2011). The mechanism for the Cu leaching from WPCBs follows the Eq. 3.1:

$$Cu^0 + 2\ Fe^{3+} \rightarrow Cu^{2+} + 2\ Fe^{2+} \tag{3.1}$$

The main advantage of the bioleaching processes is the $Fe^{2+}$ oxidation by bacteria metabolism (Eq. 3.2) with the possibility of the $Fe^{3+}$ regeneration for further extractions:

$$Fe^{2+} + \frac{1}{2} O_2 + 2\ H^+ \rightarrow Fe^{3+} + H_2O \tag{3.2}$$

This work aims at developing a mathematical model to combine the $Fe^{2+}$ oxidation by bacteria metabolism and the chemical reaction between $Fe^{3+}$ and Cu. The developed model is essential to describe in detail the main mechanisms of the bioleaching process. The resulting information can be relevant to: predict the possible toxicity of Cu and $Fe^{3+}$ on bacteria metabolism and understand how the bacteria oxidation influences the kinetic of the

Cu extraction. Considering both these aspects this model could be an important tool in the perspective of bioleaching scale-up.

## 2. Materials and Methods

### *2.1 Preparation of WPCBs*

The WPCB samples, mainly from personal computer, were prepared as follows: the material was shredded using stainless steel blades and pliers after manually removing the main parts of the electronic components (e.g., capacitors, batteries, and resistors). Thereafter, the refuse was crushed to obtain a granulometry of less than 0.5 mm, with an average Cu content of 25% (w/w), which was used for the bioleaching experiments.

### *2.2 Bioleaching process*

The microorganism used for the bioleaching process was *At. ferrooxidans* (DSMZ 14882T), obtained from the Deutche Sammlung von Mikroorganismenn und Zellkulturen GmbH, Germany. The bacteria culture grew in the DSMZ 882 medium, it was prepared as follows: 0.132 g of $(NH_4)_2SO_4$, 0.053 g of $MgCl_2{\cdot}6H_2O$, 0.027 g of $KH_2PO_4$, 0.147 g of $CaCl_2{\cdot}2H_2O$ and 49.8 g of $FeSO_4{\cdot}7H_2O$ dissolved in 1 L of MilliQ water and the pH adjusted to 1.6 with a 1M $H_2SO_4$ solution. The bioleaching approaches reproduced the condition reported by Becci et al. (2020). Therefore, the process was conducted in three phases; the first was the bacteria growth phase, where bacteria grew without WPCBs for 48 hours. At the end of this phase a known amount of WPCBs (50 g/L) were added and the second phase (first leaching step) started. After 48 hours, 80% of the leaching solution were replaced with new fresh medium and the third phase (second leaching step) beginning. The last step was conducted for 168 hours. The bioleaching experiment was carried out at the temperature of 30°C and at 120 rpm in an orbital incubator (S510).

### 2.3 Analytical determination

The total prokaryote abundance was determined by direct count on epifluorescence microscope following the procedure described by Danovaro, Manini, and Dell'Anno (2002). As described in the method, the bacteria were stained by acridine orange solution, then filtered through nucleopore 0.22 µm pore size filters. The total prokaryote count per mL of solution was calculated by Eq. 3.3:

$$\text{Total prokaryote count /mL} = \frac{N*C.O.*d}{mL} \tag{3.3}$$

Where $N$ is the mean bacteria number per optical section, $C.O.$ is the maximum optical section number (12,868), $d$ (100 times) is the sample dilution and $mL$ are the withdrew solution (0.5 mL).

The $Fe^{2+}$ and $Fe^{3+}$ concentrations were determined spectrophotometrically (Jasco model 7850) by the colorimetric thiocyanate method (Welcher and Hahn, 1964). On the other hand, the Cu concentration was measured by an atomic absorption spectrophotometer (Techcomp, AA6000).

## 3. Theoretical basis

### 3.1 Theory and background

The experimental results were used to fit the model and to determine the best parameter to describe the bacteria number, $Fe^{2+}$ and Cu temporal change. The parameters were estimated by the nonlinear least square procedure by a modification of the Levenberg-Marquardt algorithm (minpack.lm) (Marquardt, 1963; Moré, 1978). The bacteria growth rate (Eq. 3.5) and $Fe^{2+}$ oxidation (Eq. 3.6) was assumed to follow the Monod model (Eq. 3.4), in the first phase (bacteria growth phase) (Boon et al., 1999; Cabrera et al., 2005a; Hadi Shahcheraghi et al., 2012; Kumar and Gandhi, 1990; Nyavor et al., 1996; van Scherpenzeel et al., 1998). The cell number was limited by the product ($Fe^{3+}$) inhibition. The inhibition was assumed to be second order as these assumptions led to the best agreement between the observed and the predicted results (Kumar and Gandhi, 1990):

$$\mu_t = \frac{\mu_{max} \cdot Fe_t^{2+}}{k_S + Fe_t^{2+}} \tag{3.4}$$

$$\frac{dN_t}{dt} = \mu_t \cdot N_t - \mu_d \cdot (Fe_t^{3+})^2 \cdot N_t \tag{3.5}$$

$$\frac{dFe_t^{2+}}{dt} = -\frac{\mu_t}{Y_{NS}} \cdot N_t \tag{3.6}$$

Where N is the bacteria number (N°/mL), $Fe^{2+}$ and $Fe^{3+}$ are the iron concentration (g/L), $\mu_{max}$ (1/h) is the maximum specific growth rate, $k_S$ (g/L) is the substrate saturation constant, $\mu_d$ ($L^2/g^2 \cdot h$) is the inhibition parameter due to the $Fe^{3+}$ effects and $Y_{NS}$ (N°/g $Fe^{2+}$) is the bacteria yield on substrate ($Fe^{2+}$).

Whereas, in the second (first leaching step) and the third (second leaching step) phase, the Cu leaching rate was considered and Eq. 3.7 (Becci et al., 2019) was used to describe the chemical reaction between $Fe^{3+}$ and Cu:

$$\frac{dCu_t^{2+}}{dt} = [k \cdot (Cu_0^0 - Cu_t^{2+}) \cdot (Fe^{3+} - mCu_t^{2+})] \cdot A.W._{Cu} \tag{3.7}$$

Where k is the rate constant given by Arrhenius equation, $Cu_0^0$ (mol/L, 0.197) is the initial Cu concentration in WPCBs (calculated as the maximum Cu concentration in solution as a consequence of a complete extraction from WPCBs), m is the molar ratio between the consumed $Fe^{3+}$ and the dissolved $Cu^{2+}$ and $A.W._{Cu}$ is the Cu atomic weight. In Becci et al. (2019), $Fe^{3+}$ was the initial $Fe^{3+}$ concentration (constant), because that study focused on the chemical reaction between $Fe^{3+}$ and Cu. In our case, $Fe^{3+}$ concentration changes during time due to $Fe^{2+}$ oxidation by bacteria; as a consequence $Fe^{3+}$ cannot be constant and it depends on bacteria metabolism, as follows (Eq. 3.8):

$$Fe^{3+} = Fe_0^{3+} + \frac{1}{A.W._{Fe}} \cdot \int_0^t \frac{dFe^{3+}}{dt} dt = Fe_0^{3+} + \frac{1}{A.W._{Fe}} \cdot \int_0^t \frac{\mu_t}{Y_{NS}} \cdot N_t dt \qquad (3.8)$$

Where $Fe_0^{3+}$ (mol/L) is $Fe^{3+}$ concentration at the beginning of the step and the second term is $Fe^{3+}$ generated by bacteria metabolism, and $A.W._{Fe}$ is the Fe atomic weight. On the other hand, the differential equations, describing the bacteria abundance (Eq. 3.9) and $Fe^{2+}$ concentration (Eq. 3.10), have an additional term compared to Eqs. 3.5 and 3.6, due to Cu toxicity and $Fe^{2+}$ formation through the chemical reaction (Eq. 3.1), respectively:

$$\frac{dN_t}{dt} = \mu_t \cdot N_t - \mu_d \cdot (Fe_t^{3+})^2 \cdot N_t - \mu_{tox} \cdot (Cu_t^{2+})^2 \cdot N_t \qquad (3.9)$$

$$\frac{dFe_t^{2+}}{dt} = -\frac{\mu_t}{Y_{NS}} \cdot N_t + m \cdot \frac{A.W._{Fe}}{A.W._{Cu}} \cdot \frac{dCu_t^{2+}}{dt} \qquad (3.10)$$

Where $\mu_{tox}$ ($L^2/g^2 \cdot h$) is a constant parameter that represents the Cu toxic effect on bacteria growth.

The seven parameters ($\mu_{max}$, $k_S$, $\mu_d$, $\mu_{tox}$, $Y_{NS}$, k and m) are estimated through fitting Eqs. 3.7, 3.9 and 3.10 to the experimental temporal profile of Cu, bacteria abundance and $Fe^{2+}$, respectively.

### *3.2 Sensitivity analysis*

The sensitivity analysis on the estimated parameter from the mathematical model aims to identify the correlation between the parameters to fit the experimental data (Homma and Saltelli, 1996). The confidence limit at 95% through the parameters is estimated from the determined variance-covariance matrix (minpack.lm). The correlation matrix is calculated from the variance-covariance matrix (Beolchini and Vegliό, 1999). One thousand combinations through all the estimated parameters are elaborated by a random generation starting from the normal distribution with the best value and the standard error of each parameters (rnorm). The negative values are excluded from the simulations. The areas of the confidence limit at 95% for all variables are displayed. R software (version 3.6.2) is applied for all the calculations and estimates in the current study.

## 4. Results and Discussion

Table 3.1. The estimated parameters for the three phases with confidence limits of 95%.

| Param. | Bacteria growth phase | | First leaching step | | Second leaching step | |
|---|---|---|---|---|---|---|
| | Mean | St. error | Mean | St. error | Mean | St. error |
| $\mu_{max}$ (1/h) | $1.26 \cdot 10^{-1}$ | $0.10 \cdot 10^{-1}$ | $0.20 \cdot 10^{-1}$ | $0.15 \cdot 10^{-1}$ | $0.42 \cdot 10^{-1}$ | $0.02 \cdot 10^{-1}$ |
| $k_S$ (g/L) | 5.20 | 0.87 | 5.20 | 0.87 | 5.20 | 0.87 |
| $\mu_d$ ($L^2/g^2 \cdot h$) | $5.40 \cdot 10^{-5}$ | $1.01 \cdot 10^{-5}$ | - | - | - | - |
| $\mu_{tox}$ ($L^2/g^2 \cdot h$) | - | - | $7.69 \cdot 10^{-4}$ | $1.12 \cdot 10^{-4}$ | $1.47 \cdot 10^{-4}$ | $0.48 \cdot 10^{-4}$ |
| $Y_{NS}$ (N°/g $Fe^{2+}$) | $1.20 \cdot 10^{10}$ | $0.26 \cdot 10^{10}$ | $0.53 \cdot 10^{10}$ | $0.43 \cdot 10^{10}$ | $0.95 \cdot 10^{10}$ | $0.10 \cdot 10^{10}$ |
| k | - | - | 0.11 | 0.04 | 0.38 | 0.22 |
| m | - | - | 4.39 | 0.92 | 2.00 | - |

In the bacteria growth phase, the bacteria abundance and the $Fe^{2+}$ concentration showed the common trend for the pure batch culture (Figure 3.1) (Cabrera et al., 2005b; Chen et al., 2004; Kumar and Gandhi, 1990; Moreira et al., 2019). In detail, the microbial cells evidenced an exponential growth in the first 25 h of incubation, followed by a stationary phase due to the decreased $Fe^{2+}$ concentration. Table 3.1 reports the Eqs. 3.4 to 3.6 estimated parameters with confidence limits at 95%. The three parameters that describe the bacteria growth rate ($\mu_{max}$), the substrate saturation constant ($K_S$) and the bacteria yield on substrate ($Y_{NS}$) were in the range reported in the literature, between 0.04-0.16 1/h, 0.3-14 g/L and 0.25-5.9 $\cdot 10^{10}$ N°/g $Fe^{2+}$, respectively (Boon et al., 1999; Chavarie et al., 2009; Halfmeier et al., 1993b, 1993a; Molchanov et al., 2007; Nagpal, 1997; Nemati et al., 1998; Pagella et al., 1996; Rashidi et al., 2012; Zilouei et al., 2003). The maximum $Fe^{2+}$ oxidation rate (0.30 g

$Fe^{2+}$ oxidized/L·h) was achieved after 20 hours from the beginning, and it was reached at the end of the exponential growth phase (Figure 3.1).

A statistical analysis was performed to identify the correlation between the estimated parameters (Table 3.2).

Table 3.2. The correlation matrix for the 4 estimated parameters in the bacteria growth phase.

| | $\mu_{max}$ | $k_S$ | $\mu_d$ | $Y_{NS}$ |
|---|---|---|---|---|
| $\mu_{max}$ | 1 | 0.99 | 0.12 | 0.17 |
| $k_S$ | 0.99 | 1 | 0.10 | 0.15 |
| $\mu_d$ | 0.12 | 0.10 | 1 | 0.93 |
| $Y_{NS}$ | 0.17 | 0.15 | 0.93 | 1 |

From the results, a remarkable dependence between $\mu_{max}$-$k_S$ and $\mu_d$-$Y_{NS}$ was evident with a correlation coefficient of 0.99 and 0.93, respectively. The confidence helixes of the parameter were also showed to have more detail on the correlation dependence and on their standard error (Figure 3.2). These results confirmed the linear dependence between $Y_{NS}$ and $\mu_d$.

As concerns the fourth parameter, the inhibition parameter ($\mu_d$), the estimated value was $5.40 \cdot 10^{-5}$ $L^2/g^2 \cdot h$ and it was lower than reported in the literature ($6.32 \cdot 10^{-4}$ $L^2/g^2 \cdot h$) (Kumar and Gandhi, 1990), demonstrating that the inhibition on bacteria metabolism due to the product ($Fe^{3+}$) was negligible.

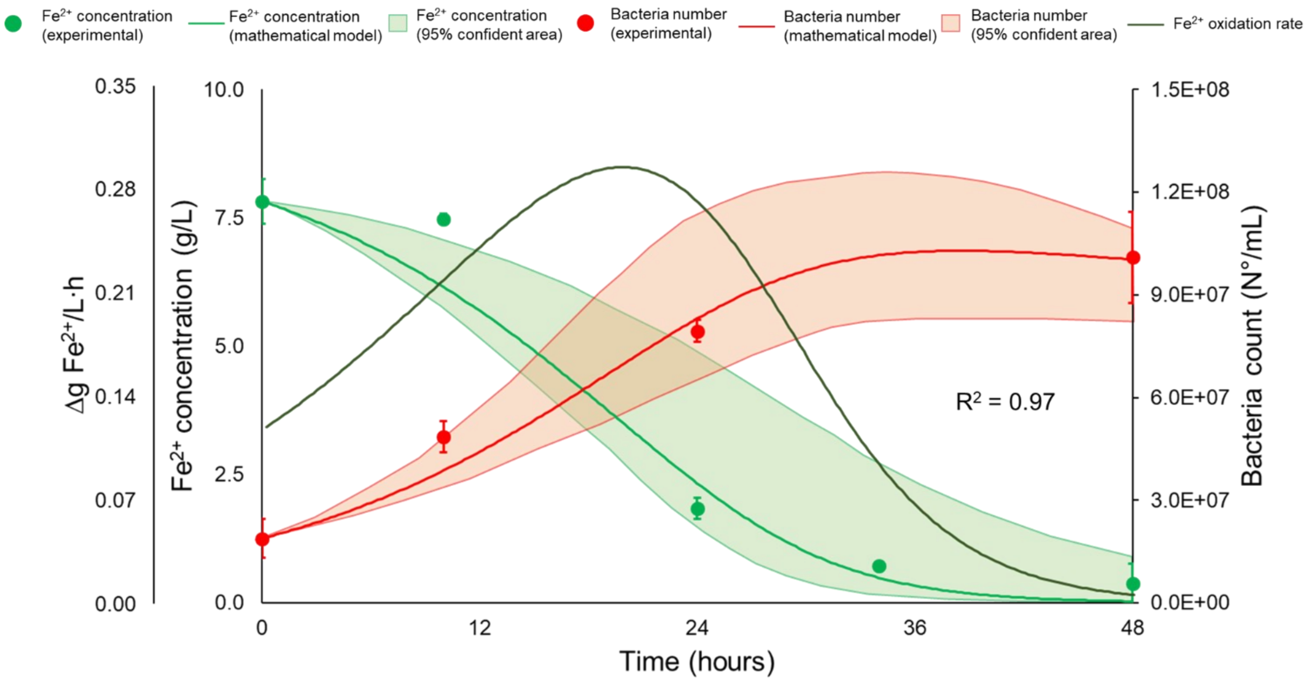


Figure 3.1. Bacteria count and Fe$^{2+}$ concentration trend during the bacteria growth phase.

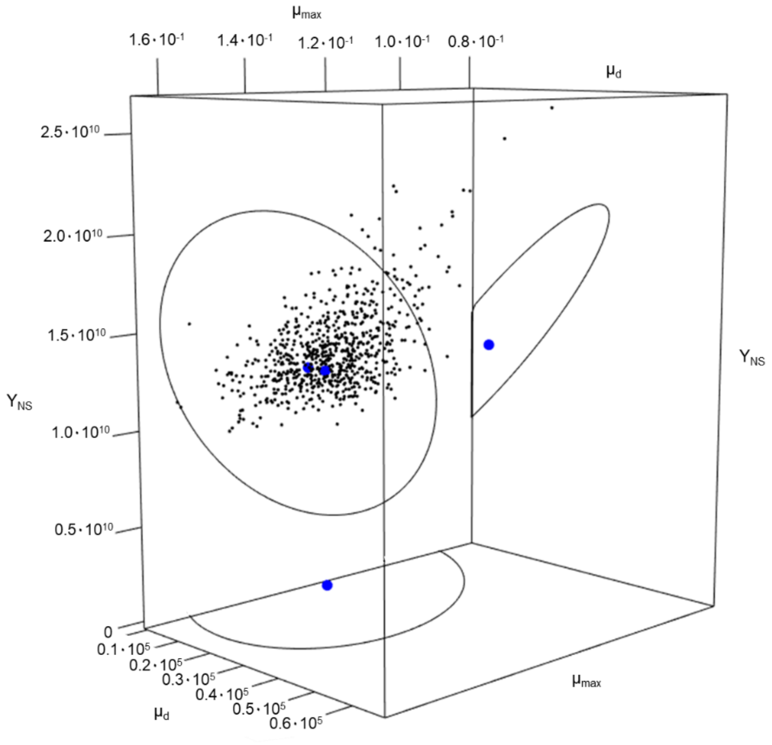


Figure 3.2. Confidence helixes at 95% and the random generated values to describe the bacteria growth phase.

In the second phase (first leaching step), a known amount of WPCBs (50 g/L) was added and the chemical reaction (Eq. 3.1) between $Fe^{3+}$ and $Cu^0$ started. At the beginning of this phase, the $Fe^{2+}$ and $Cu^{2+}$ concentration showed a rapid increase due to the chemical reaction. On the other hand, the bacteria cell showed a growth rate lower than the previous phase (Figure 3.3a). Table 3.1 reports Eqs. 3.7, 3.9 and 3.10 parameters. The estimated values for the maximum bacteria growth rate ($\mu_{max}$, $0.20 \cdot 10^{-1}$ 1/h) and the bacteria yield on substrate ($Y_{NS}$, $0.53 \cdot 10^{10}$ N°/g $Fe^{2+}$) were six and two times lower than the first phase, respectively. Such decrease was mainly due to the microorganism adaptation to the new environment. The Cu toxic effect ($\mu_{tox}$, $7.69 \cdot 10^{-4}$ $L^2/g^2 \cdot h$) on bacteria growth and metabolism was one order of magnitude higher than the $Fe^{3+}$ product inhibition ($\mu_d$) estimated in the bacteria growth phase. Moreover, such $Fe^{3+}$ product inhibition resulted to be irrelevant in this first leaching step, as demonstrated value of 0 estimated parameter $\mu_d$ (Table 3.1).

From the experimental and mathematical modelling data, an estimation of iron generation/consumption rates through the bacteria metabolism and the chemical reaction was carried out (Fig. 3.3b) to understand in detail the controlling steps in Cu bioleaching. Firstly, the $Fe^{2+}$ generation by the chemical reaction was faster than the $Fe^{2+}$ consumption by bacteria metabolism. Successively after 14 h, a change in the controlling step was observed: indeed, a mean $Fe^{2+}$ oxidation (bio) resulted to be 1.8 times faster than the $Fe^{2+}$ generation (chem). The maximum oxidation rate (0.24 g $Fe^{2+}$ oxidized/$L \cdot h$) was achieved around 20 h after the beginning, in accordance with observed in the bacteria growth phase.

Also in this case, a statistical analysis was performed to understand the dependence between the estimated parameters by the correlation matrix (Table 3.3).

Table 3.3. The correlation matrix for the 5 estimated parameters in the first leaching step.

| | $\mu_{max}$ | $\mu_{tox}$ | $Y_{NS}$ | k | m |
|---|---|---|---|---|---|
| $\mu_{max}$ | 1 | 0.95 | 0.98 | -0.04 | -0.02 |
| $\mu_{tox}$ | 0.95 | 1 | 0.92 | -0.17 | 0.22 |
| $Y_{NS}$ | 0.98 | 0.92 | 1 | -0.06 | -0.09 |
| k | -0.04 | -0.17 | -0.06 | 1 | -0.37 |
| m | -0.02 | 0.22 | -0.09 | -0.37 | 1 |

A remarkable correlation among $\mu_{max}$, $\mu_{tox}$ and $Y_{NS}$ is evident, while k and m are not significantly correlated with the other parameters. The estimated parameters k and m, that describe Cu leaching trend through bioleaching, were quite different from the parameter reported by Becci et al. (2019), 3.08 and 3.22, respectively, that concern Cu chemical leaching. As regards the stoichiometric ratio (m) between $Fe^{3+}$ and $Cu^{2+}$ the value of 3.22 was in the 95% confidence limit (Figure 3.4). The estimated rate constant (k) reported a value of one order of magnitude lower than reported by Becci et al. (2019); this record was mainly due to the lower agitation rate in the bioleaching experiment than the chemical leaching test and this determined a low frequency of collisions in the correct orientation between $Fe^{3+}$ and $Cu^0$.

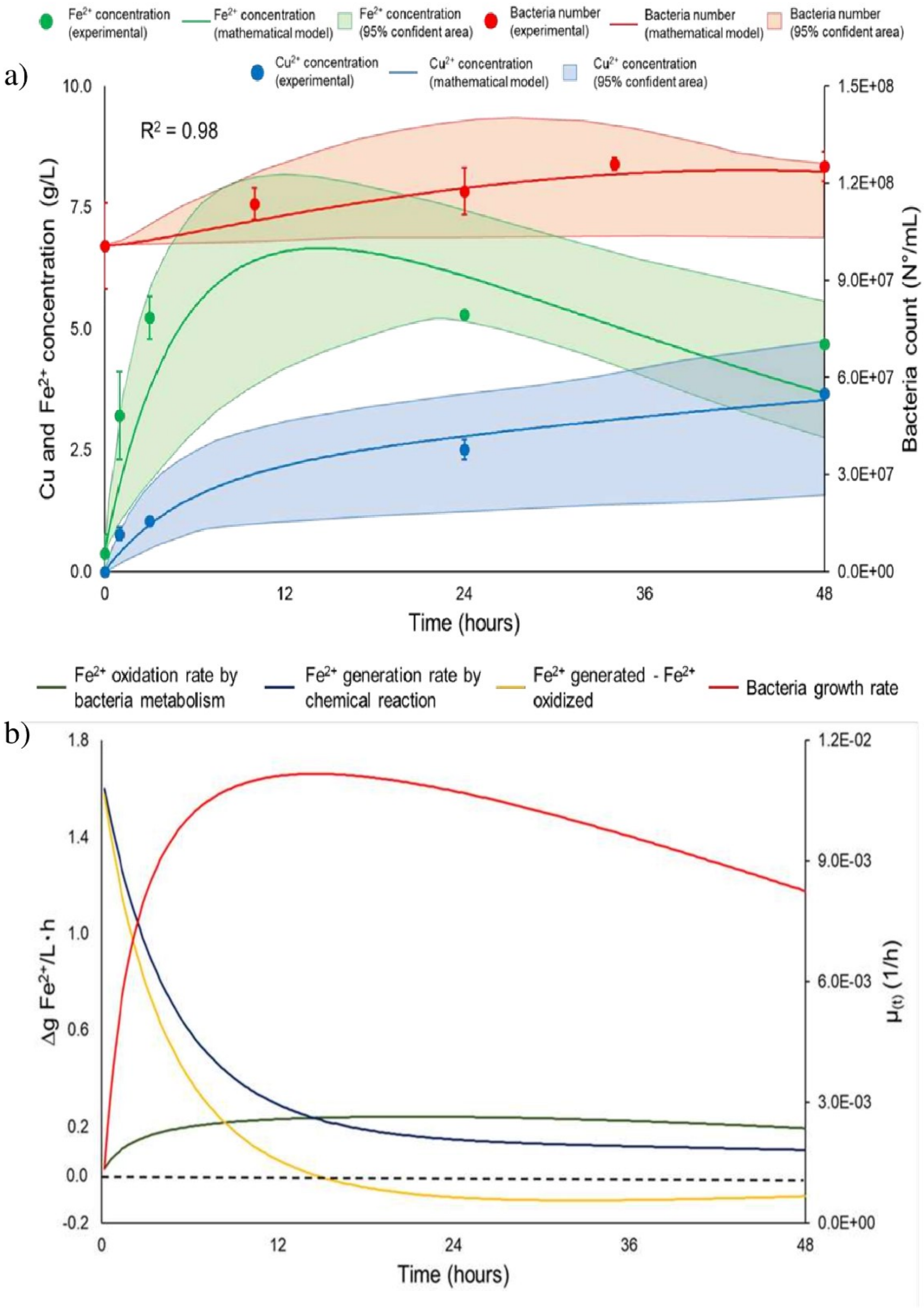


Figure 3.3. a) Bacteria count, $Fe^{2+}$ and $Cu^{2+}$ concentration trend in the first leaching step; b) the $Fe^{2+}$ oxidation and generation rate by bacteria metabolism and chemical reaction, and the time change of μ.

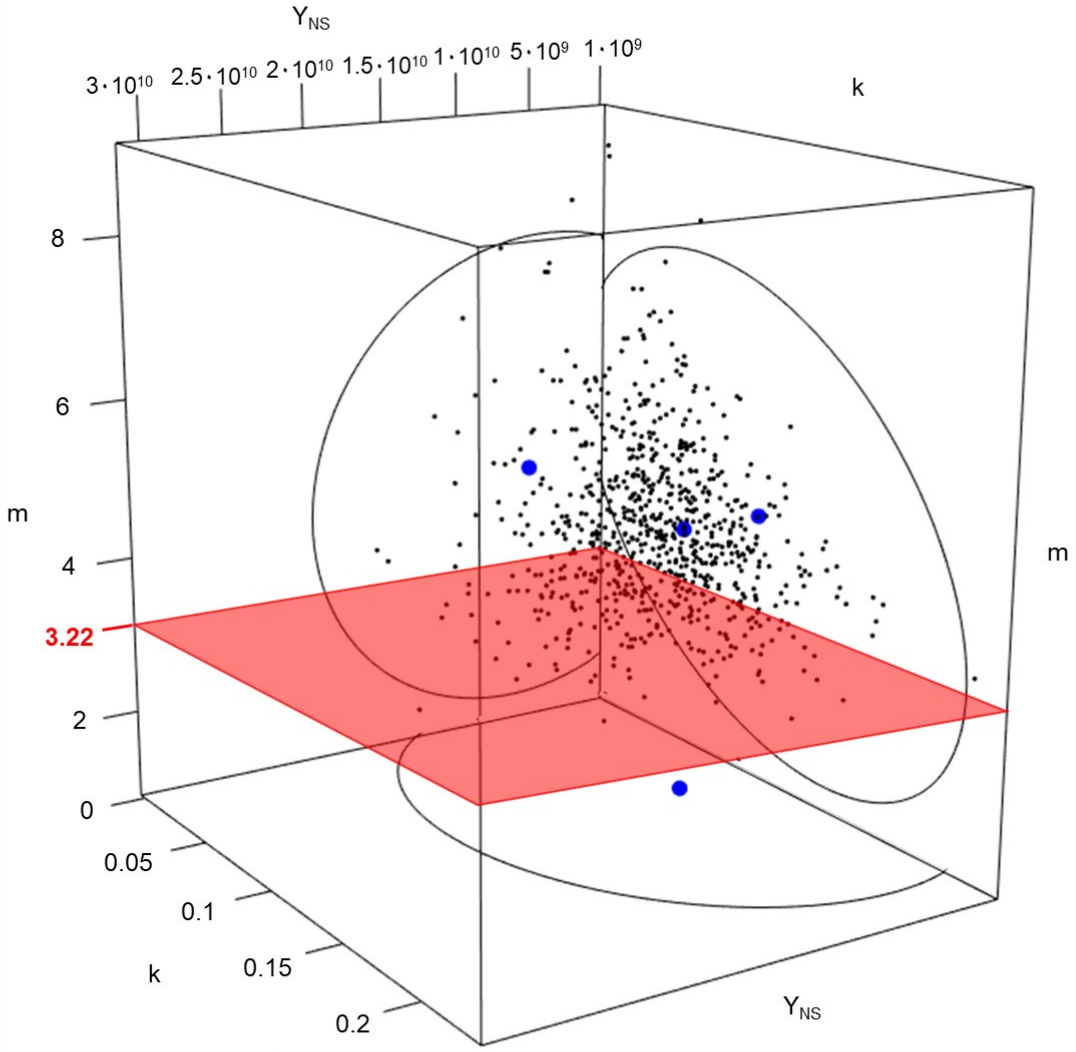


Figure 3.4. Confidence helixes at 95% and the estimated value to describe the first leaching step. The red plane represent the value of m reported by Becci et al. (2019).

In the third and last phase (second leaching step), the 80% of the leaching solution resulting from the previous step was removed and replaced with new fresh medium. For this reason, the microorganism abundance and $Cu^{2+}$ concentration decreased and $Fe^{2+}$ concentration increased than the first leaching step end. The bacteria count and $Fe^{2+}$ showed the same observed trend in the bacteria growth phase, with a slower kinetic (Figure 3.5a). Indeed, the microorganism exponential growth rate lasted 84 h and the $Fe^{2+}$ reached the concentration close to zero after 100 h from the beginning. The slowest kinetic trend for bacteria abundance and $Fe^{2+}$ oxidation was confirmed by the mathematical model. The estimated parameters $\mu_{max}$ and $Y_{NS}$ reported values lower than the bacteria growth phase (Table 3.1). The achieved value of $0.42 \cdot 10^{-1}$ 1/h for the maximum specific growth rate was comparable with that reported ($0.61 \cdot 10^{-1}$ 1/h) by Cabrera et al. (2005a) that study the influence of the $Cu^{2+}$ at the 10 g/L concentration on bacteria metabolism.

Furthermore, in this step, the bacteria abundance temporal profile showed the death phase after 120 h from the beginning. The decrease of cell number was mainly due to the lower $Fe^{2+}$ concentration as energy source for microorganism (Moreira et al., 2019). The estimated parameter for the Cu toxic effect ($\mu_{tox}$) reported a record 5 times lower than the previous phase. These results demonstrated that the leaching in more steps allowed an highest bacteria adaptation to the new substrates and an increasing leaching efficiency.

As concerns the assessment of iron production/consumption rates, the $Fe^{2+}$ oxidation by bacteria showed in the first 96 h a kinetic 2.5 times faster than the chemical reaction (Figure 3.5b). The two kinetics (bacteria metabolism and the chemical reaction) were comparable after 120 h from the beginning when the bacteria cells started to decrease, and Cu leaching was mostly completed. This result was another attestation of the highest microorganism adaptation.

Table 3.4. The correlation matrix for the 4 estimated parameters in the second leaching step.

| | $\mu_{max}$ | $\mu_{tox}$ | $Y_{NS}$ | k |
|---|---|---|---|---|
| $\mu_{max}$ | 1 | 0.11 | 0.15 | -0.33 |
| $\mu_{tox}$ | 0.11 | 1 | 0.89 | -0.43 |
| $Y_{NS}$ | 0.15 | 0.89 | 1 | -0.25 |
| k | -0.33 | -0.43 | -0.25 | 1 |

The correlation matrix (Table 3.4) shows a lower correlation between the parameters except for the $\mu_{tox}$-$Y_{NS}$ correlation (0.89). Another important results from the correlation matrix was the univocal results for the stoichiometric ratio (m = 2) between $Fe^{3+}$ and $Cu^{2+}$. This result demonstrated that the concentration of the other metals in WPCBs decreased due to the first leaching step, and so the stoichiometric ratio between $Cu^{2+}$ and $Fe^{3+}$ was equal to that reported in the Eq. 3.1 (Becci et al., 2019). On the other hand, the rate constant k reported a higher value than the first step leaching but all the possible value achieved in the previous step fell into the 95% confidence limit of the determined k in the second leaching step (Figure 3.6).

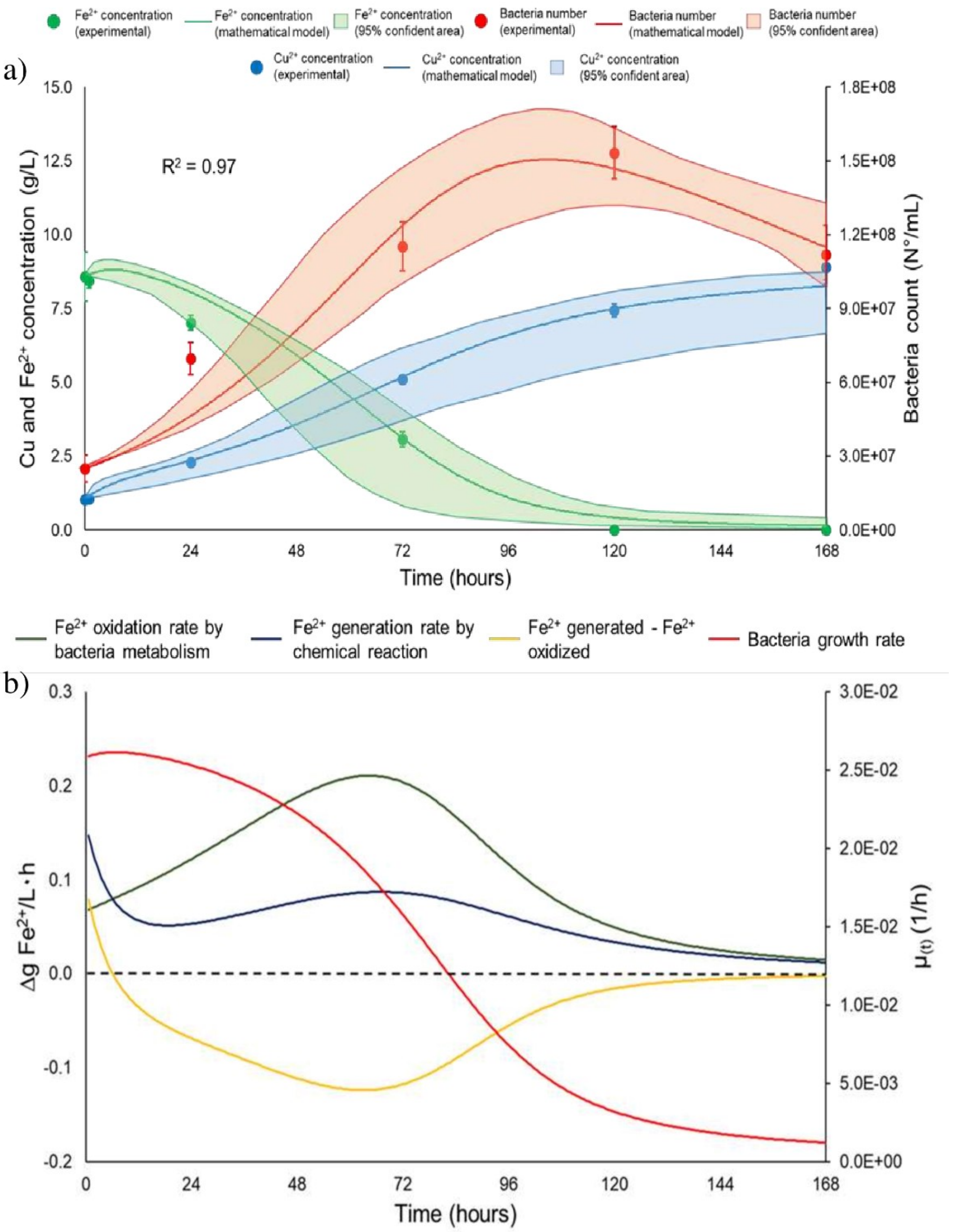


Figure 3.5. a) Bacteria count, $Fe^{2+}$ and $Cu^{2+}$ concentration trend in the second leaching step; b) the $Fe^{2+}$ oxidation and generation rate by bacteria metabolism and chemical reaction, and the time change of μ.

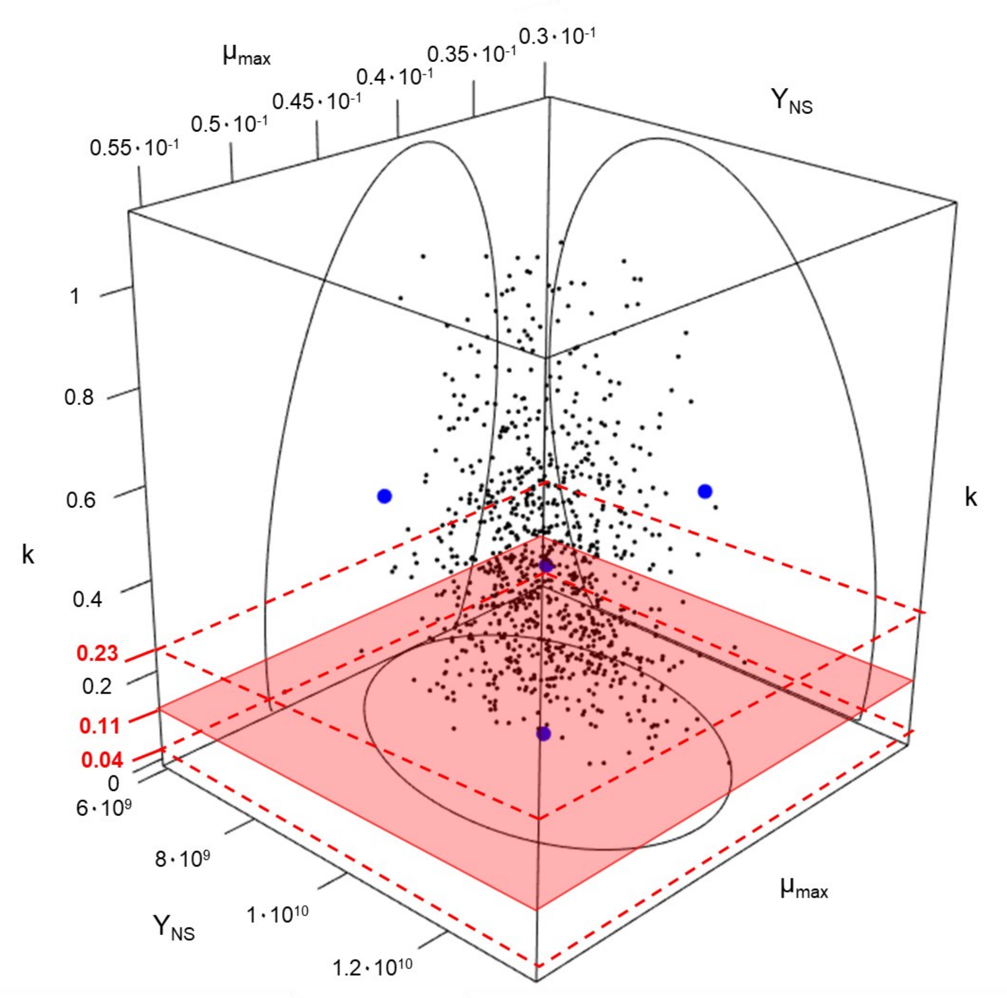


Figure 3.6. Confidence helixes at 95% and the estimated value to describe the second leaching step. The red plane represents the best, the maximum and the minimum value of k estimated for the first leaching step.

## 5. Conclusions

In this study, the kinetics and the mechanism of the Cu bioleaching from WPCBs by *At. ferrooxidans* was studied in detail. The following conclusions can be deduced from the present work:

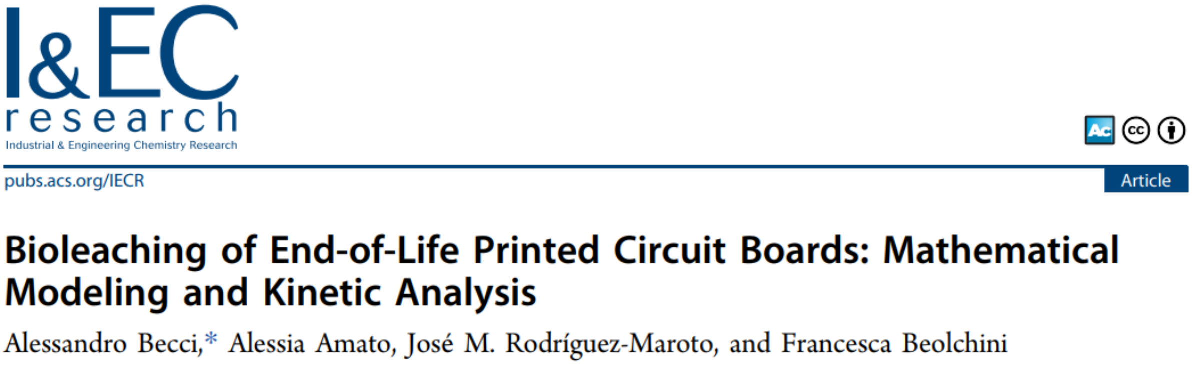
1. The developed mathematical model was in a very good agreement with the experimental data, as confirmed by a $R^2$ higher than 0.97 in all the three considered phases.

2. In the first phase of the first leaching the fastest step was the chemical reaction between the $Fe^{3+}$ produced in the bacteria growth phase and Cu within WPCBs. After 14 h from the beginning, bacteria metabolism resulted to be faster that Cu chemical extraction. The achieved results showed that the microorganism activity was 1.8-2.5 times faster than the chemical reaction.
3. A leaching carried oud in a multi-stages operation allowed to increase the microorganism adaptation to the substrates; the parameters showed as the maximum specific growth rate ($\mu_{max}$) and the bacteria yield on substrates ($Y_{NS}$) increased their value in the second leaching step than the first leaching step, and the Cu toxic effect ($\mu_{tox}$) decreased in the second one.
4. This work demonstrated that the WPCB (50 g/L) and the maximum Cu (8.91 g/L) concentrations did not have so significant toxic effects on bacteria metabolism. The $Fe^{2+}$ oxidation rate by microorganism decreased from a value of 0.30 g/L ·h, in the bacteria growth phase, to 0.21 g/L ·h in the last one.

The mathematical model for the Cu recoverable from WPCB was an essential information for the development of innovative bioleaching processes. Understanding in detail as the microbial metabolism influenced the chemical leaching could be useful for the implementation of the bioleaching approaches in the industrial scale.

## 6. Products

Results illustrated and discussed in this chapter were used to support a peer reviewed article published on Industrial & Engineering Chemistry Research, ACS Publications (Figure 3.7).



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Article

**Bioleaching of End-of-Life Printed Circuit Boards: Mathematical Modeling and Kinetic Analysis**

Alessandro Becci,* Alessia Amato, José M. Rodríguez-Maroto, and Francesca Beolchini

*Figure 3.7. Articles published on Industrial & Engineering Chemistry Research.*

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# Chapter 4: SUSTAINABLE METAL RECOVERY

## Abstract

The circularity represents the key strategy of the modern economy, where the waste becomes a raw material, ready to be reprocessed for a second life. In this context, the main aim of the present work was the development of a sustainable process for the recovery of *Cu* and *Zn* from end-of-life printed circuit boards (PCBs). The exploitation of this hazardous waste produces a double effect: the recovery of metals, suitable for the current metal market, and the avoided disposal of scraps produced worldwide. The process allowed to exceed the limits of several approaches reported in the literature, mainly due to the highest consumptions (both energy and raw materials) and the loss of *Zn* content. The best identified conditions, suitable for the treatment of both chemical and bio- leach liquor included: the Fe precipitation with sodium hydroxide, followed by the Cu cementation with Zn (Zn/Cu molar ratio of 1.1) and a final Zn precipitation with oxalic acid (O.A./Zn molar ratio of 1.5). The metals showed recovery efficiencies higher than 95% and purities up to 95%. An efficient recirculation system contributes to the sustainability growth, as confirmed by the carbon footprint assessment, towards the implementation of a zero-waste treatment. Future perspective should include the further exploitation of the residue, rich in precious metals.

## Content

## 1. Introduction

A constant technological development characterizes the modern society, involving the communication field, the energy production and the transportation system. The manufacture of many sophisticated devices can be translated into an increase of the raw material demand (metals, minerals and fuels), that was estimated around 1.4 million kg for individual, over his lifetime (Kyser et al., 2015). This value is bound to increase for the continuous growth of both the population and the living standards, mainly in the developing countries (Kyser et al., 2015). Nowadays, most of the resources required to support this growth come from mines, which represent less than one third of the earth surface. However, the identification of high-grade ore deposits is becoming more and more difficult and they are not able to sustain the raw material demand for a long time. Therefore, the mining industry is focusing on the low-grade ores, often located in remote areas or at great ocean depth, with significant environmental and economic impacts (Hein et al., 2013; Petersen et al., 2016). The potential effect of the deep-ocean mining have been studied within two major research programs, the EU-FP7 funded project “MIDAS” and the JPI-Oceans initiative “Ecological Aspects of Deep-Sea Mining” (Petersen et al., 2016). The main identified impacts are due to both the release of potential toxic metals and the decrease of oxygen content in the surface sediment with a consequent risk of specie decline (Hein et al., 2013; Koschinsky et al., 2003). Alternatively, European Commission has pushed the countries towards the development of urban mining activities, in which the waste (mainly waste of electrical and electronic equipment, WEEE) becomes a source of secondary elements, fulfilling the objectives of the circular economy (European Commission, 2018, 2017). The implementation of this strategy represents the solution of a double criticality: the identification of new mines and the reduction of the risk connected to the WEEE management (Leung et al., 2008; Musson et al., 2006; Shen et al., 2008). Waste from printed circuit boards (PCBs) is one of the most common WEEE (classified as hazardous waste) since this equipment represents the brain of many technologies (Xiang et al., 2010). The interest for the optimization of the recycling processes is due to the high concentration of *Cu* (around 25% *w/w*) and *Zn* (around 2% *w/w*), combined with precious metals (e.g. *Au*, *Ag* or *Pd*) (Arshadi and Mousavi, 2015; Becci et al., 2020; Birloaga and Vegliò, 2016; Faraji et al., 2018; Fonti et al., 2015; Hong and Valix, 2014; Jadhav et al., 2016; Oguchi et al., 2013; Zhang and Xu, 2016). Around 15 million tons of *Cu* are produced every year and 30% of this quantity is intended for the electric and electronic equipment production (Evangelopoulos et al., 2019). The relevance of the element is also connected to its availability because scientists had demonstrated that the global *Cu* ore deposits will be exhausted around 2030 (Wang et al., 2019). On the other hand, the Cu had a property that can be 100% recyclable and so the PCBs will be a fundamental Cu source (Bonnin et al., 2015; J. Chen et al., 2019; Xiu and Zhang, 2009). Nowadays, the five biggest companies which implement treatments for the recycling of PCBs are: Umicore (Belgium), Noranda (Canada), Boliden Ronnskar (Sweden), Dowa (Japan), Aurubis (Germany, Belgium, Bulgaria). These large-scale smelter facilities mainly use pyrometallurgical and hydrometallurgical techniques (Becci et al., 2020; Ma, 2019; Park and Kim, 2019; Rocchetti et al., 2018). Nevertheless, considering the limited number of the operating facilities, huge flows of waste are transported from all over the world, with significant impacts, from both the environmental and the economic point of view (Rocchetti et al., 2018). In this context, the development of innovative and sustainable treatments, suitable for the application in a real scale, is a necessity to build a more decentralized system of WEEE management (Amato et al., 2017a). Considering the availability of end-of-life PCBs and their high *Cu* content, literature is rich in processes for metal extraction by chemical and biotechnological approaches, using mainly the *At. ferrooxidans* bacteria (both in the presence of *Fe*, as oxidant agent). The extraction is followed by the recovery by different approaches and resulting efficiencies, summarized in Table 4.1. The included processes involve high agent/Cu ratio, irrespective of the selected approach. Furthermore, leaching solution shows significant *Zn* concentrations, often neglected by the literature, as confirmed by the Table 4.1. The interest for this metal is due to the rapid rise of its price, due to the criticality of ore depletion (De Michelis et al., 2007; Zhang et al., 2011). Established techniques for *Zn* recovery from different matrices include: electrolytic process (Porter, 1991; Winand, 1994), emulsion liquid membranes (Teresa et al., 1993), solvent liquid extraction with *D2EHPA* (Kongolo et al., 2003) and precipitation with *NaOH* or *KOH*, at pH around 6-7 (Sayilgan et al., 2010; Veloso et al., 2005).

In this context the present work aims at the development of a method for the metal recovery, going beyond the main weaknesses identified by the literature. High efficiency and purity achieved by a low concentration of the cementation agent represent the main targets of the process to ensure the environmental sustainability. The innovation of the proposed treatment is the combination and the optimization of consolidated treatments for the *Cu*, *Zn* and *Fe* recovery from PCBs leaching solutions, produced by the conditions reported by Becci et al. (2020). The authors describe both a chemical and a biological PCB enhancement for the identification of a possible effect on the process efficiency. Compared to the literature, the further possibility of a *Fe* recovery aims at its possible re-use for further leaching, avoiding the raw material consumption. Indeed, the process sustainability has a key role for the present research, as confirmed by the evaluation of the carbon footprint, addressed at both the identification of the best option and the main possible weaknesses to improve.

Table 4.1. State-of-art of Cu recovery. The treatments without energy consumption are carried out at room temperature, with an electricity request for agitation, comparable to that considered for our process.

| Treatment | Matrix | Process consumption | Efficiency % | Reference |
|---|---|---|---|---|
| Cementation | PCBs | 2 Zn/Cu molar ratio | 99 | Birloaga and Vegliò, 2016 |
| Cementation | PCBs | 2.4 Zn/Cu molar ratio | 95 | Birloaga et al., 2014 |
| Cementation | PCBs | N.A. (treat. at 60°C) | 73 | Chaurasia et al., 2013 |
| Electrochemical method | PCBs | 1.59 kWh/kg of Cu | 99 | Fogarasi et al., 2015 |
| Precipitation with oxalic acid | PCBs | 1M oxalic acid solution | 99 | Shah et al., 2018 |
| Solvent precipitation | Synthetic solution | 2 solvent/solution ratio | 97 | Aktas, 2011 |
| Solvent precipitation | Synthetic solution | 4 solvent/solution ratio | 99 | Moldoveanu and Demopoulos, 2015 |
| Cementation | Synthetic solution | 1-10 Zn/Cu molar ratio | 97 | Demirkıran et al., 2007 |
| Cementation | Synthetic solution | 15 Zn/Cu molar ratio | 99 | Karavasteva, 2005 |
| Solvent precipitation | Malachite | 1.75 solvent/solution ratio | 99 | Kokes et al., 2014 |

## 2. Materials and methods

### 2.1 *Preparation of the leaching solution*

Metal recovery tests were carried out using the end-of-life PCBs leaching solutions produced by the conditions described by Becci et al. (2020). The authors proposed two possible methods: a bio- and a chemical approach. More in detail, the characterization of the starting waste showed results comparable to the literature (Creamer et al., 2006; Ilyas and Lee, 2014; Wang et al., 2018; Zhang and Xu, 2016) (Table 4.2). Both the approaches were carried out at 30°C, at pH 1.6, producing the composition described in Table 4.2. The reactions involved were the following (Eqs. 4.1 and 4.2) (Ilyas and Lee, 2014; Yang et al., 2009):

$Cu^0 + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4$ ΔG = -82.90 kJ/mol (4.1)

$Zn^0 + Fe_2(SO_4)_3 \rightarrow ZnSO4 + 2FeSO_4$ ΔG = -295.40 kJ/mol (4.2)

The main bioleaching advantage, in spite of the longest reaction time, was the $Fe^{3+}$ regeneration thanks to the bacteria metabolism (Eq. 4.3) (Becci et al., 2020; Nemati and Webb, 1997; Rawlings et al., 1999; Yu et al., 2001):

$2FeSO_4 + 1/2O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O$ ΔG = -39.73 kJ/mol (4.3)

Table 4.2. Average metal concentrations in the PCB samples and in leaching solutions used for the recovery.

| Metals | PCBs composition (% *w/w*) | Bioleaching solution (g/L) | Chemical leaching solution (g/L) |
|---|---|---|---|
| $Fe^{2+}$ | - | - | 5.30 |
| $Fe^{3+}$ | - | 9.8 | 30.0 |
| Cu | 24.7 | 6.58 | 11.8 |
| Zn | 3.7 | 0.77 | 1.3 |
| Al | 0.4 | 0.08 | 0.2 |
| Pb | 1.3 | 0.03 | 0.04 |
| Sn | 2.1 | 0.06 | 0.1 |
| Ni | 0.03 | 0.005 | 0.01 |
| Co | 0.005 | 0.001 | 0.003 |

### 2.2 *The metal recovery treatments*

The choice of the metal targets (*Fe*, *Cu* and *Zn*) was due to their relevant content in the leach liquor, irrespective of the extraction approach. *Pb* and *Sn* precipitated during the leaching step, as oxide (Brandl et al., 2001; Ilyas et al., 2013; Liang et al., 2010) and the low concentration of further elements (i.e. *Al, Ni* and *Co*, Table 2) did not affect the recovery effectiveness. Overall, the techniques developed in the present work involved: *Fe* precipitation by *NaOH*, *Cu* cementation with *Zn* powder and *Zn* precipitation with oxalic acid (*O.A.*). As an alternative to these treatments, the precipitation by a solvent was carried out using *EtOH* or *MeOH*. The processes were repeated for both leach liquors (from bio- and chemical treatments, separately treated), at the operative conditions described in Table 4.3. Starting from the preliminary results (treatments 1-8), further experiments (treatments 9-22) included a pre-treatment for the *Fe* recovery, to decrease the co-precipitation phenomena which reduce the purity of the final product. The *Cu* cementation, included two variables: the *Zn* powder concentration (*Zn*/*Cu* moles ratio of: 1.5, 1.3 and 1.1) and the temperature (room temperature and 55°C). The *Zn* precipitation with *O.A.*, performed after the *Cu* recovery, aimed at the recovery of both the *Zn* from PCBs and that added as cementation agent. As concern the *Fe* precipitation with *NaOH*, different temperatures (40°, 60° and 80°C) were tested with and without a preliminary *Fe* oxidation by $H_2O_2$, in the chemical leaching solution case (with a $Fe^{2+}$ content of 85% of the total *Fe*). This additional step was carried out at 40° and 80°C, at three different $H_2O_2$ concentrations (0.2, 0.4 and 0.6 M). During each experiment, samples were collected at different interval to verify the time effect.

Alternatively, the precipitation by a solvent was carried out to determine the optimal parameters for a selective precipitation of the metal targets, as reported in Table 4.4. For each experiment, 25 mL of the starting volume were mixed with a variable quantity of *EtOH* or *MeOH*. The resulting solutions were mixed at 300 rpm, for 15 minutes, at room temperature.

### 2.3 *Analysis*

The solid characterization was carried out after microwave-assisted acid digestion of the samples by means of inductively coupled plasma (ICP) analysis (using Agilent ICP-MS 7500, Agilent technologies). The concentration of metals in residual solutions was determined by the atomic absorption spectrophotometer technique *(Techcomp, AA6000)*, after a preliminary filtration of the samples for the solid fraction removal. On the other hand, the $Fe^{3+}$ detection was performed by an UV/VIS spectrophotometer (Jasco Model 7850). The further addition of $KMnO_4$ to the samples allowed the complete $Fe^{2+}$ oxidation for the quantification of the total $Fe$ (at the absorption wavelength of 480 nm). The difference of the two measurements provided the concentration of the $Fe^{2+}$.

As concern the recovery efficiencies, they were calculated following the equation (Eq. 4.4):

$$Efficiency\ (\%) = [(C_0 - C_t)/C_0] \times 100 \tag{4.4}$$

Where $C_0$ (g/L) was the initial metal concentration and $C_t$ (g/L) was the metal concentration at $t$ time, during each experiment. In the precipitation by a solvent, the $C_0$ was calculated considering the dilution due to the additional volume of the organic solvent.

Table 4.3. Operating conditions of different metal recovery treatments. Including *Cu* cementation, *Zn* precipitation with *O.A.*, *Fe* precipitation with *NaOH*. (the 22 treatments are duplicated using both the bio- and chemical (chem.) leach liquors, except for 9,10 and 12-14). The efficiencies reported for Cu and Zn recovery represent the average of the comparable results from the bio and chem. leach liquor treatments.

*Cu* recovery from leaching solution (cementation)

| Treatment | *Zn*/*Cu* ratio | Temperature (°C) | Initial pH | Time (min.) | Recovery efficiency % |
|---|---|---|---|---|---|
| 1 | 1.5 | 55 | 1.6 | 60 | 95 ± 1 |
| 2 | 1.3 | 55 | 1.6 | 60 | 93 ± 1 |
| 3 | 1.1 | 55 | 1.6 | 60 | 91 ± 2 |
| 4 | 1.5 | 25 | 1.6 | 60 | 93 ± 1 |
| 5 | 1.3 | 25 | 1.6 | 60 | 92 ± 2 |
| 6 | 1.1 | 25 | 1.6 | 60 | 89 ± 1 |

*Zn* recovery after *Cu* cementation (oxalate precipitation)

| Treatment | *O.A.*/*Zn* ratio | Temperature (°C) | Initial pH | Time (min.) | Recovery efficiency % |
|---|---|---|---|---|---|
| 7 | 12 | 40 | 3.7 | 60 | 99 ± 1 |
| 8 | 3 | 40 | 3.7 | 60 | 67 ± 3 |

Fe recovery from leaching solution (sodium hydroxide precipitation)

| Treatment | $H_2O_2$ concentration (M) | Temperature (°C) | Working pH | Time (min.) | Recovery efficiency % |
|---|---|---|---|---|---|

| | | | | | Chem. | Bio. |
|---|---|---|---|---|---|---|
| 9 | - | 80 | 3.0 | 30 | 26 ± 2 | - |
| 10 | - | 60 | 3.0 | 30 | 28 ± 2 | - |
| 11 | - | 40 | 3.0 | 30 | 22 ± 2 | 94 ± 1 |
| 12 | 0.6 | 80 | 3.0 | 30 | 93 ± 1 | - |
| 13 | 0.6 | 60 | 3.0 | 30 | 88 ± 5 | - |
| 14 | 0.6 | 40 | 3.0 | 30 | 91 ± 1 | - |

*Cu* recovery after *Fe* precipitation (cementation)

| Treatment | *Zn*/*Cu* ratio | Temperature (°C) | Initial pH | Time (min.) | Recovery efficiency % |
|---|---|---|---|---|---|
| 15 | 1.1 | 25 | 3.0 | 60 | 99 ± 1 |
| 16 | 1.0 | 25 | 3.0 | 60 | 97 ± 1 |

*Zn* recovery after *Cu* and *Fe* recovery (oxalate precipitation)

| Treatment | *O.A.*/*Zn* ratio | Temperature (°C) | Initial pH | Time (min.) | Recovery efficiency % |
|---|---|---|---|---|---|
| 17 | 4.5 | 40 | 4.5 | 60 | 99 ± 1 |
| 18 | 3.0 | 40 | 4.5 | 60 | 99 ± 1 |
| 19 | 2.5 | 40 | 4.5 | 60 | 99 ± 1 |
| 20 | 1.5 | 40 | 4.5 | 60 | 99 ± 1 |
| 21 | 1.0 | 40 | 4.5 | 60 | 94 ± 1 |
| 22 | 0.75 | 40 | 4.5 | 60 | 82 ± 1 |

Table 4.4. Operating conditions of different metal recovery treatments by precipitation by a solvent (solv.). (The 24 treatments are duplicated using both the bio- and chemical leach liquor).

Leaching solution

| Solvent | Treat. | Solv./sol. ratio | pH | Recovery efficiency % | | |
|---|---|---|---|---|---|---|
| | | | | *Fe* | *Cu* | *Zn* |
| *EtOH* | 23 | 0.5 | 1.6 | 54 ± 1 | 25 ± 1 | 24 ± 1 |
| | 24 | 1 | 1.6 | 78 ± 1 | 67 ± 1 | 82 ± 1 |
| | 25 | 2 | 1.6 | 89 ± 1 | 93 ± 2 | 97 ± 1 |
| | 26 | 3 | 1.6 | 88 ± 1 | 89 ± 2 | 99 ± 1 |
| *MeOH* | 27 | 0.5 | 1.6 | 0 ± 0 | 16 ± 1 | 1 ± 1 |
| | 28 | 1 | 1.6 | 32 ± 3 | 31 ± 1 | 10 ± 3 |
| | 29 | 2 | 1.6 | 62 ± 5 | 72 ± 1 | 36 ± 7 |
| | 30 | 3 | 1.6 | 52 ± 5 | 74 ± 1 | 22 ± 7 |

Leaching solution after *Fe* recovery

| Solvent | Treat. | Solv./sol. ratio | pH | Recovery efficiency % | |
|---|---|---|---|---|---|
| | | | | *Cu* | *Zn* |
| *EtOH* | 31 | 0.5 | 3.0 | 75 ± 2 | 6 ± 1 |
| | 32 | 1 | 3.0 | 87 ± 2 | 87 ± 1 |
| | 33 | 2 | 3.0 | 88 ± 2 | 97 ± 1 |
| | 34 | 3 | 3.0 | 81 ± 2 | 99 ± 1 |
| *MeOH* | 35 | 0.5 | 3.0 | 71 ± 2 | 9 ± 1 |

| | | | | | |
|---|---|---|---|---|---|
| | 36 | 1 | 3.0 | 81 ± 2 | 21 ± 1 |
| | 37 | 2 | 3.0 | 85 ± 2 | 88 ± 2 |
| | 38 | 3 | 3.0 | 87 ± 2 | 93 ± 2 |
| Leaching solution after *Fe* and *Cu* recovery | | | | | |
| Solvent | Treat. | Solv./sol. ratio | Ph | Recovery efficiency % | |
| | | | | *Zn* | |
| *EtOH* | 39 | 0.5 | 4.5 | 32 ± 1 | |
| | 40 | 1 | 4.5 | 94 ± 2 | |
| | 41 | 2 | 4.5 | 99 ± 2 | |
| | 42 | 3 | 4.5 | 98 ± 2 | |
| *MeOH* | 43 | 0.5 | 4.5 | 15 ± 1 | |
| | 44 | 1 | 4.5 | 91 ± 2 | |
| | 45 | 2 | 4.5 | 98 ± 2 | |
| | 46 | 3 | 4.5 | 97 ± 2 | |

### 2.4 *Assessment of the carbon footprint*

The assessment of the carbon footprint aimed at the comparison among 8 different scenarios for the metal recovery from end-of-life PCBs. The different options include the methods developed within the present work (scenarios 1-6) and a hydrometallurgical process, including a sequential precipitation, chosen as reference (scenarios 7-8). The main objective of this analysis is the quantification of the balance between process impact and the advantage of the recovered metals. This result is essential for the definition of: the most sustainable option, the main criticalities of each scenario and the possible comparison with further techniques. As reported in Figure 4.1, the steps of: PCB production, use, end-use, collection and the preliminary waste treatment (included the extraction) are excluded from the system boundaries since they are common to all options. The *Fe* precipitation is the first step, and it needs *NaOH* (scenarios 1-3 and 7 from bioleaching) with the further addition of the $H_2O_2$ (for the $Fe^{2+}$ ions oxidation) in the options 4-6 and 8 which treat the solution from the chemical leaching. The treatments of *Cu* cementation and *Zn* precipitation are included in the scenarios 1 and 4. On the other hand, the precipitation by *EtOH* allows the metal recovery in the options 2 and 5. This approach is also used, in the scenarios 3 and 6, for the

*Zn* recovery, after *Cu* cementation. As concern the sequential precipitation (scenarios 7 and 8), it consists of a selective metal recovery by *NaOH*, at different pH: 3, 4 and 8 for *Fe*, *Cu* and *Zn*, respectively. The analysis assessed the impact connected to energy (0.5 kW/m$^3$ (Krzeminski et al., 2012)) and raw material consumed for the treatment of 1 kg end-of-life PCBs (chosen as functional unit). Table 4.5 summarizes the considered flows, and it highlights the highest electricity demand for the agitation. The thinkstep GaBi software-System and the Database for Life Cycle Engineering (compilation 7.3.3.153; DB version 6.115) were employed for the production processes of energy and raw materials and the assessment of the carbon footprint related to the 8 scenarios. Furthermore, literature data related to the impact of the involved recovery agents: *EtOH* bio-production (Maga et al., 2019) and *O.A.* synthesis (Pell et al., 2019) were used to fill the database gaps. To enhance the benefit of metal recovery, the avoided impact for the element primary production was assumed as environmental credit, with a negative value (Amato et al., 2019, 2017b; Amato and Beolchini, 2019; Pintilie et al., 2016). Despite of the *Fe* recovery, no credit was associated to this metal since it is present within the solution as leaching agent and not as element from PCBs (Becci et al., 2020). As concern the waste streams from the different processes, a recirculation of 95% was assumed. In this regard: the solution from precipitation by *O.A.* can be used for further leaching (scenarios 1 and 4, (Becci et al., 2020)), the wastewater from precipitation by *EtOH* can be considered suitable for the bio-production of acetic acid (with zero impact), (Scenarios 2-3, 5-6) (Gullo and Giudici, 2008; Park et al., 1991; Raspor and Goranovič, 2008) and those from scenarios 7 and 8 can be employed as basic solution within the waste exploitation processes (Amato et al., 2019, 2016; Rocchetti et al., 2016, 2015). A solution cooling, at 0°C, allows the removal of the accumulated $Na_2SO_4$ by crystallization, before the recirculation (Gudim et al., 2010).

Table 4.5. Input, output and final product flows considered for the carbon footprint assessment of the 8 scenarios (Functional unit: 1kg of shredded PCBs).

| Scenarios | Input flows | Output flows (waste streams) | Output flows (products) |
|---|---|---|---|
| Scenario 1 | Leaching solution 36 Kg<br>*NaOH* 1.27 kg<br>*Zn* 0.24 kg<br>*O.A.* 0.56 kg<br>Energy 0.017 kWh | Wastewater 36 kg | *Fe* 0.33 kg<br>*Cu* 0.23 kg<br>*Zn* 0.27 kg |
| Scenario 2 | Leaching solution 36 kg<br>*NaOH* 1.27 kg<br>*EtOH* 28.4 kg<br>Energy 0.025 kWh | Wastewater 64.4 kg | *Fe* 0.33 kg<br>*Cu* 0.23 kg<br>*Zn* 0.02 kg |
| Scenario 3 | Leaching solution 36 kg<br>*NaOH* 1.27 kg<br>*Zn* 0.24 kg<br>*EtOH* 28.4 kg<br>Energy 0.021 kWh | Wastewater 64.4 kg | *Fe* 0.33 kg<br>*Cu* 0.23 kg<br>*Zn* 0.25 kg |
| Scenario 4 | Leaching solution 20 kg<br>*NaOH* 0.70 kg<br>*Zn* 0.24 kg<br>*O.A.* 0.55 kg<br>$H_2O_2$ 0.41 kg<br>Energy 0.029 kWh | Wastewater 20 kg | *Fe* 0.73 kg<br>*Cu* 0.23 kg<br>*Zn* 0.26 kg |
| Scenario 5 | Leaching solution 20 kg<br>*NaOH* 0.70 kg<br>$H_2O_2$ 0.41 kg<br>*EtOH* 15.8 kg<br>Energy 0.034 kWh | Wastewater 35.8 kg | *Fe* 0.73 kg<br>*Cu* 0.23 kg<br>*Zn* 0.02 kg |
| Scenario 6 | Leaching solution 20 kg<br>*NaOH* 0.70 kg<br>*Zn* 0.24 kg<br>$H_2O_2$ 0.41 kg<br>*EtOH* 15.8 L<br>Energy 0.032 kWh | Wastewater 35.8 L | *Fe* 0.73 kg<br>*Cu* 0.23 kg<br>*Zn* 0.25 kg |

| | | | |
|---|---|---|---|
| Scenario 7 | Leaching solution 36 kg<br>*NaOH* 2.19 kg<br>Energy 0.027 kWh | Wastewater 36 kg | *Fe* 0.33 kg<br>*Cu* 0.22 kg<br>*Zn* 0.028 kg |
| Scenario 8 | Leaching solution 20 kg<br>*NaOH* 1.22 kg<br>$H_2O_2$ 0.41 kg<br>Energy 0.035 kWh | Wastewater 20 kg | *Fe* 0.73 kg<br>*Cu* 0.22 kg<br>*Zn* 0.027 kg |

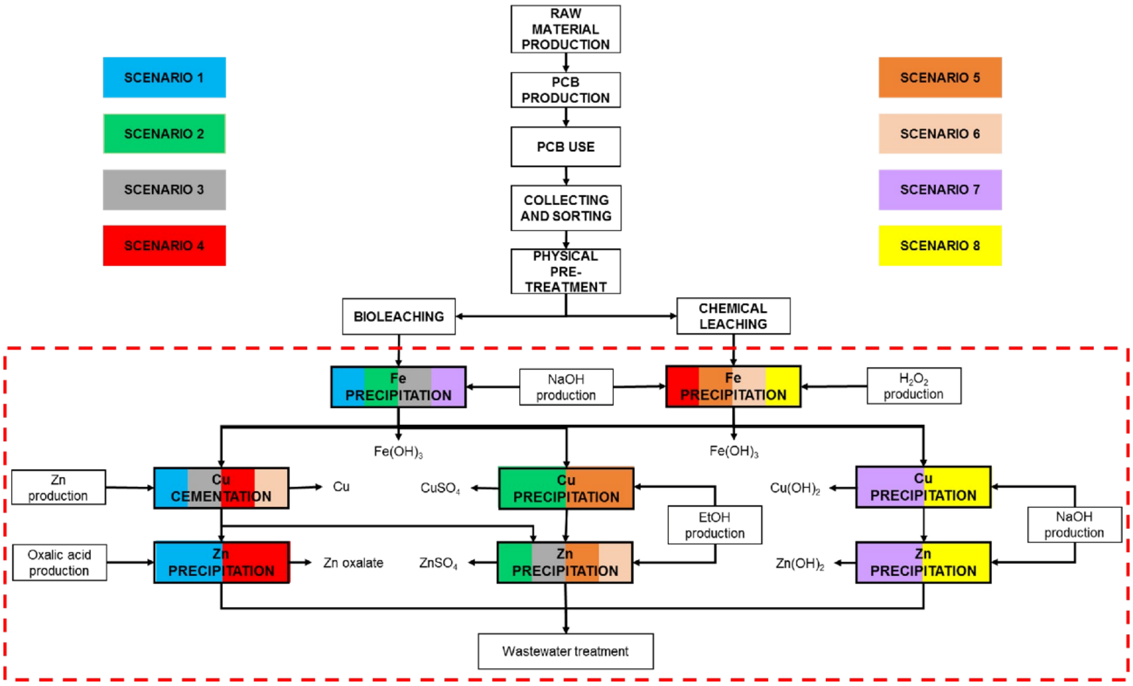

Figure 4.1. System boundaries considered for the carbon footprint assessment.

## 3. Results and discussion

### *3.1 The Cu recovery by cementation*

Figure 4.2 summarizes the results achieved at the different operative conditions considered for the *Cu* cementation. It is evident that there is neither a temperature nor a time effect, since the process was completed after 10 min, without significant differences after 30 and 60 min. Overall, the cementation yield obtained by a *Zn*/*Cu* molar ratio of 1.1 was 91 ± 2%, which increased of about 4% thanks to a $Zn/Cu$ molar ratio growth up to 1.5. The explanations of the highest $Cu$ recovery (Eq. 4.5), at the second condition are: the partial $Zn$ consumption by the $Fe^{3+}$ reduction (present in the leaching solution) to $Fe^{2+}$ (Eq. 4.7); the $Zn$ reaction with $H^+$ ions with the consequent pH increase from 1.6 to 3.5 (Eq. 4.6) (Annamalai and Murr, 1979; Demirkiran et al., 2007; Hsu et al., 1998; Ku and Chen, 1992).

$$Cu^{2+} + Zn^0 \rightarrow Zn^{2+} + Cu^0 \tag{4.5}$$

$$Zn^0 + 2\ H^+ \rightarrow Zn^{2+} + H_2 \tag{4.6}$$

$$Zn^0 + 2\ Fe^{3+} \rightarrow Zn^{2+} + 2\ Fe^{2+} \tag{4.7}$$

The first reason is confirmed by the $Fe^{3+}$ concentration trend, manly in the bioleaching case, where the total $Fe$ ion was present as trivalent form, which decreases up to 10% and 7% of the starting value, using a $Zn/Cu$ molar ratio of 1.1 and 1.5, respectively (Figure S4.1).

As concern the product purity, evaluated in a Cu-Fe-Zn system, differences were observed between the treatments of the solutions from the bio- (purity between 80 to 90%) and the chemical leaching (purity higher than 95%). In the first case, a $Fe$ presence was due to the precipitation of 16 ± 3% and 8 ± 2% of the initial concentration, using a $Zn/Cu$ molar ratio of 1.5 and 1.1, respectively. On the other hand, during the cementation of the chemical leach liquor, precipitation phenomena were negligible, with the growth of the $Cu$ purity. The sample analysis showed the further recovery of both $Ni$ and $Co$ during the cementation. Nevertheless, considering the low concentrations in the leach liquor, their content in the product is lower than 0.01%.

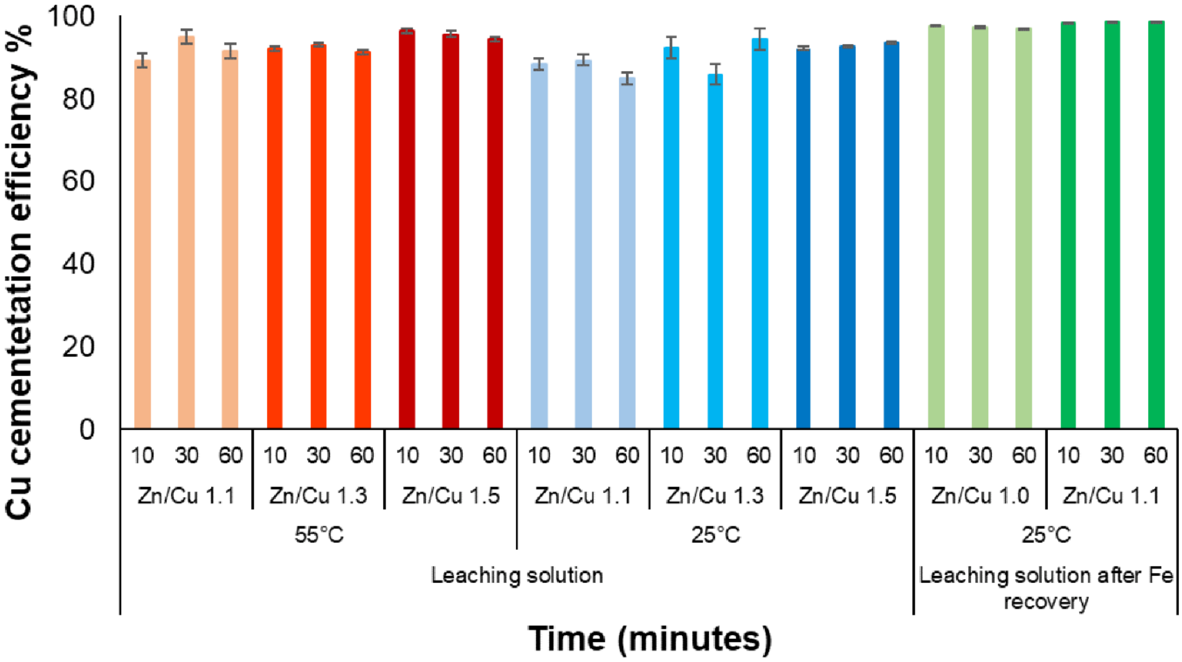

Figure 4.2. *Cu* cementation efficiency at the selected conditions of: temperature (55°C and 25°C), *Zn*/*Cu* molar ratio (1.5, 1.3, 1.1 and 1.0), before or after *Fe* recovery.

### *3.2 The Zn recovery by precipitation*

The second step of the process, after the *Cu* cementation, included the *Zn* recovery by precipitation with *O.A.* (Eq. 4.8) (Gudim et al., 2010; Horeh et al., 2016; Jr et al., 2018)

$$C_2O_4^{2-} + 2\,H^+ + ZnSO_4 \rightarrow ZnC_2O_4 + H_2SO_4 \qquad (4.8)$$

As reported in Figure 4.3, the best result was obtained with an *O.A.*/*Zn* ratio of 12, at 40°C, after 30 min, with a precipitation efficiency of 99 ± 1%, comparable to the sample at 60 min. Instead, an *O.A.*/*Zn* molar ratio of 3.0 decreased the *Zn* recovery yield up to 67 ± 3%. No differences were identified between the bio- and the chemical leach liquors since the previous *Cu* cementation produced comparable conditions in both the final solutions. Furthermore, a significant *Fe* precipitation (with efficiencies of 47 ± 8% and 96 ± 2% for 3 and 12 *O.A.*/*Zn* molar ratio, respectively, Figure S4.2) was observed at the end of the treatment. In agreement with that observed during the *Cu* cementation, the coprecipitation of *Fe* with the metal target produced a negative effect on the final product purity, around 55-

65% and 30-35% (in a Zn-Cu-Fe system) for Zn recovered from the bio- and chemical leaching solution, respectively.

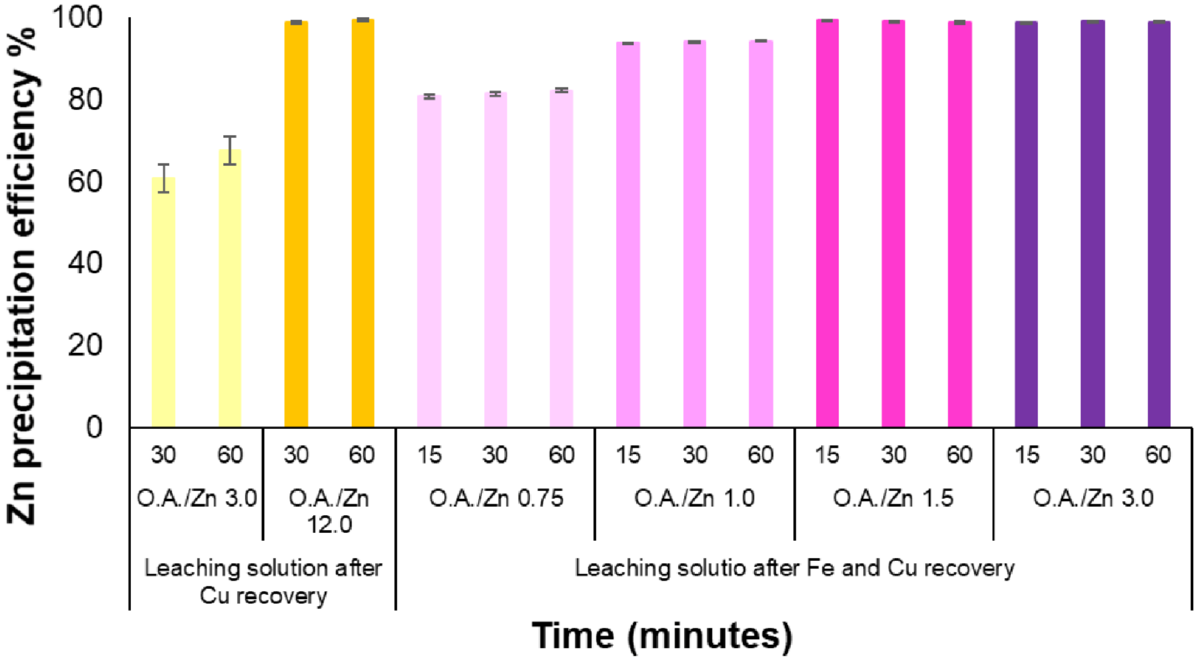

Figure 4.3. *Zn* precipitation efficiency at the selected conditions of: *O.A./Zn* molar ratio (0.75, 1.0, 1.5, 3.0, 12.0), after both *Cu* recovery and *Cu* and *Fe* recovery.

### *3.3 The Fe recovery by precipitation*

The *Fe* precipitation was carried out by precipitation with *NaOH*, at three different temperatures (40°, 60° and 80°C), increasing the initial pH from 1.6 to 3.0 (Eq. 4.9) (Gudim et al., 2010).

$$Fe_2(SO_4)_3 + 6NaOH \rightarrow 2Fe(OH)_3 + 3Na_2SO_4 \quad (4.9)$$

As showed in Figure 4.4, the treatments of the leaching solution from the chemical approach produced comparable results, irrespective of the temperature, with a minimum time effect, around 7%, between the samples at 10 min and 30 min. Comparing the three treatments, at different temperatures, the highest *Fe* recovery (28 ± 2%) was observed after 30 min, at 60°C. The metal speciation is the reason of the low achieved results, since the only $Fe^{3+}$

form (15% of the *Fe* content) can precipitate. The $H_2O_2$ addition, before the precipitation with *NaOH*, allowed to overcome this limit oxidizing the $Fe^{2+}$ with yields higher than 85%, irrespective of the selected temperature. More in detail, the best conditions identified for a complete *Fe* oxidation were: $H_2O_2$ concentration 0.6 M, temperature 40°C and 1 h (Figure S4.3). Thereafter, a selective *Fe* recovery was achieved at: pH 3.0, 40°C, 30 min of reaction time, with a final recovery efficiency of 94 ± 1%. The product showed the presence of about 5% of impurities, mainly due to the *Pb*, *Sn* and *Al* coprecipitation. On the other hand, the residual *Cu* and *Zn* in solution did not precipitate in the solid product. Considering this aspect, the possibility to carry out the *Fe* precipitation before the metal target recovery, allowed to solve the criticality of the coprecipitation phenomena, with a positive effect on both the efficiency and the purity results. As reported in Figure 4.2, this preliminary treatment increased the *Cu* recovery efficiency up to 97 ± 1%, using the lowest *Zn*/*Cu* molar ratio of 1.0, with a *Fe* absence in the product. Furthermore, the resulting metal, with a purity higher than 97% (*Cu-Zn-Fe* system), could be considered ready to be sent to second industry (Xiu and Zhang, 2009). A double reason explains the achieved result: the avoided *Zn* consumption by *Fe* (Eq. 4.7) and the highest pH (from 1.6 to 3.0, achieved by the $H_2O_2$ action and the *NaOH* addition), which decreases the $H^+$ reaction (Eq. 4.6). The positive effect of the previous *Fe* precipitation was evident also for the *Zn* recovery (Figure 4.3), where comparable efficiencies, between 82 and 99%, were achieved with a final purity around 100% (*Zn-Cu-Fe* system). Overall, the main advantage of the use of bioleaching solution is the avoided *Fe* pre-treatment with $H_2O_2$, since the whole *Fe* content is in the oxidized form thanks to the *At. ferrooxidans* action (Becci et al., 2020).

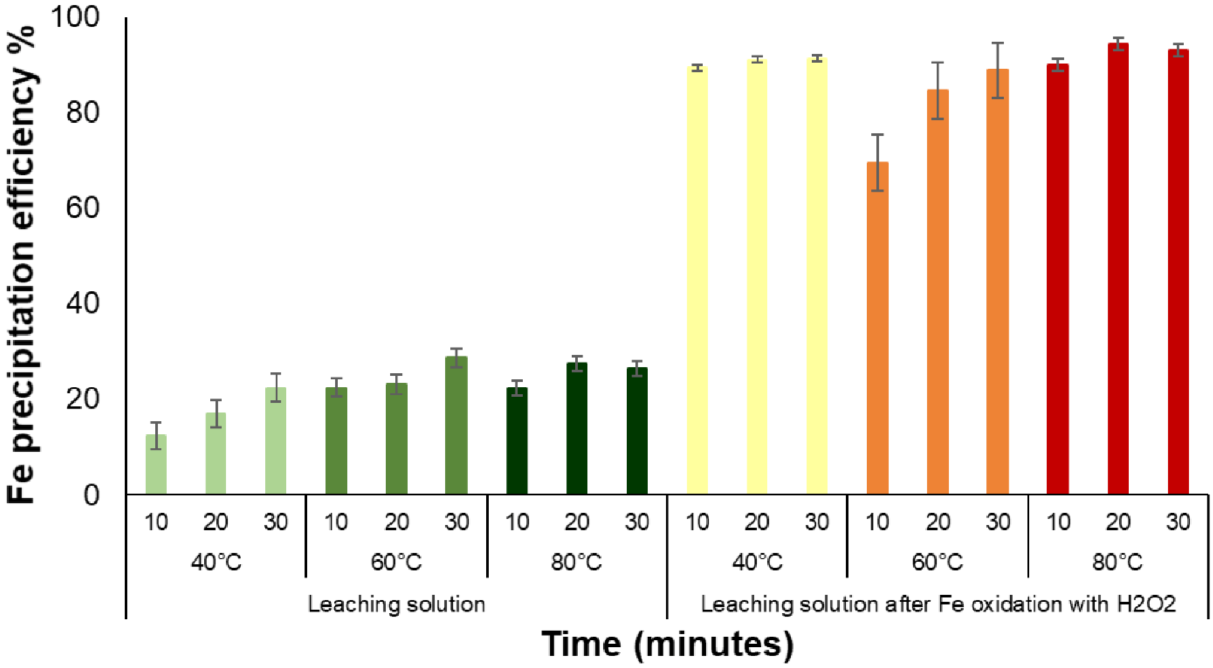

Figure 4.4. *Fe* precipitation efficiency at the three considered temperatures (40°, 60° and 80°C), before and after the *Fe* oxidation with $H_2O_2$.

### *3.4 The metal recovery by precipitation by a solvent*

A precipitation by a solvent (*EtOH* or *MeOH*) tested the possibility of a high selectivity precipitation of the metal target, in sulfate form, as an alternative to the three steps described in the previous paragraphs (Eqs. 4.10-4.13) (Kokes et al., 2014)

$$CuSO_{4(aq)} + xH_2O + C_2H_5OH \text{ (or } CH_3OH) \rightarrow CuSO_4{\cdot}5H_2O_{(s)} + (x-5)H_2O_{(\text{in EtOH or MeOH})} \quad (4.10)$$

$$ZnSO_{4(aq)} + xH_2O + C_2H_5OH \text{ (or } CH_3OH) \rightarrow ZnSO_4{\cdot}7H_2O_{(s)} + (x-7)H_2O_{(\text{in EtOH or MeOH})} \quad (4.11)$$

$$FeSO_{4(aq)} + xH_2O + C_2H_5OH \text{ (or } CH_3OH) \rightarrow FeSO_4{\cdot}7H_2O_{(s)} + (x-7)H_2O_{(\text{in EtOH or MeOH})} \quad (4.12)$$

$$Fe_2(SO_4)_{3(aq)} + xH_2O + C_2H_5OH \text{ (or } CH_3OH) \rightarrow Fe_2(SO_4)_3{\cdot}2H_2O_{(s)} + (x-2)H_2O_{(\text{in EtOH or MeOH})} \quad (4.13)$$

The evident difference between the two considered solvents (Figure 4.5) was due to the *Fe*, which showed the highest solubility in *MeOH*, with a consequent low precipitation. In addition, the *EtOH* was chosen as the best solvent for the lowest toxicity level (Figures 4.5

c and e). More in detail, its effectiveness was ensured at 2 *EtOH*/solution ratio, with metal recovery efficiencies higher than 90%, irrespective of the starting solution. Furthermore, the *EtOH*/solution ratio halving was possible after the previous *Fe* precipitation, with a double positive effect: the purity increases and the pH growth, which reduces the metal solubility. At these conditions, the precipitation efficiencies were maintained around 87% for both *Cu* and *Zn* (Figures 4.5 c and d). The main criticality of this approach is the low purity level, due to the *Cu* and *Zn* coprecipitation; the final product showed an average composition of 90% and 10% of Cu and Zn, respectively (*Cu-Zn* system). Therefore, a step sequence was proposed to increase the final $CuSO_4 \cdot 5H_2O$ percentage up to 99% (considering *Cu-Zn*). More in detail, the preliminary *Fe* recovery was followed by a treatment with a 0.5 *EtOH*/solution ratio with a recovery efficiency of 75 ± 2% and 6 ± 1% for *Cu* and *Zn*, respectively. A further phase with a 1 *EtOH*/solution ratio produced a solid with a mix of $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$ with an efficiency around 90%. The solid composition showed an average composition of 70% and 30% of *Cu* and *Zn*, respectively. To avoid the presence of both the metals in the second precipitation, a combination of the developed techniques was proposed including: *Fe* precipitation, *Cu* cementation (pure metal production) and the following pure $ZnSO_4 \cdot 7H_2O$ precipitation by *EtOH* (*EtOH*/solution ratio of 1). This option was able to combine the double advantage of the *Zn* recovery and a high purity for the three metal targets (higher than 95% for each metal in a Cu-Zn-Fe system).

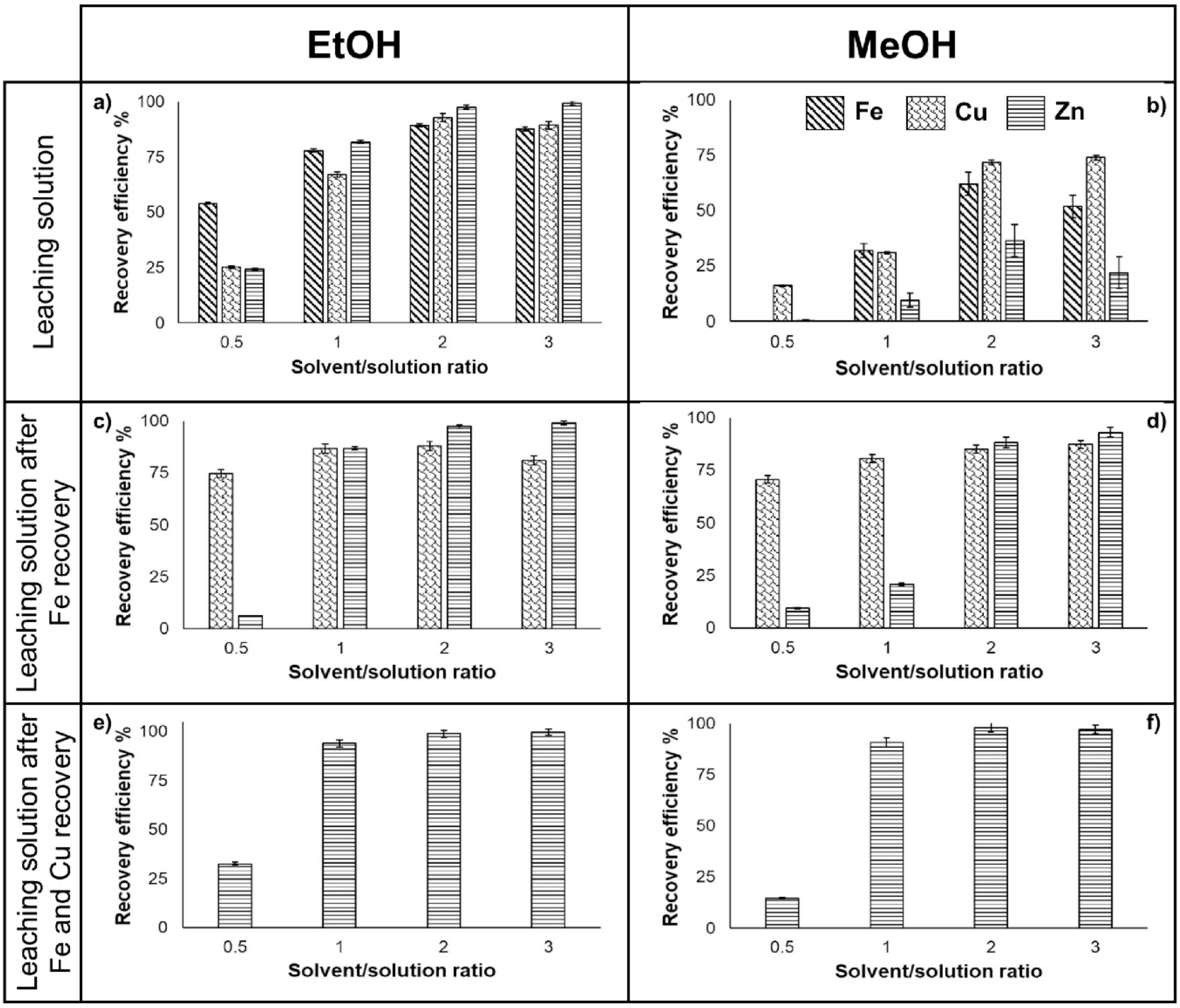

Figure 4.5. *Cu*, *Fe* and *Zn* precipitation as sulfate with solvent (*EtOH* and *MeOH*).

### *3.5 Assessment of the carbon footprint*

The described options produced satisfying results with efficiency greater than 95% for the three metal targets, combined with a high purity level (up to 100% in a *Cu*-*Fe*-*Zn* system). Considering this operative achievement, an environmental assessment was necessary to evaluate the most sustainable option. In this regard, Figure 4.6 identifies the scenario 1 (bio leach liquor, *Fe* precipitation, *Cu* cementation, *Zn* precipitation with *O.A.*) as the best choice with an overall impact of 0.3 kg$CO_2$-eq. The benefit achieved by a previous bio leaching is highlighted by the comparison with the scenario 4 (the same treatments using the chemical leach liquor); indeed, the bio extraction allows a complete $Fe^{2+}$ oxidation avoiding the $H_2O_2$ addition, which causes more than 50% of the impact of scenario 4.

On the other hand, the *EtOH* use characterizes the worst options, with a contribution of 80% and 60% on the whole $CO_2$ eq. emissions, in the scenarios 2-3 and 5-6, respectively. In this regard, the bio approach has a negative effect since it produces higher treated volume than the chemical one, with the consequent greatest *EtOH* consumption.

For the 8 considered options, the whole balance shows positive value, despite of the total metal recovery, because the environmental load due to the recovery processes cannot balance the credit related to the avoided metal primary production. Nevertheless, these results could be improved considering the high content of precious elements (e.g. *Au*, *Ag*, *Pd*) in the process residue, able to significant increase the whole environmental balance of the considered treatments.

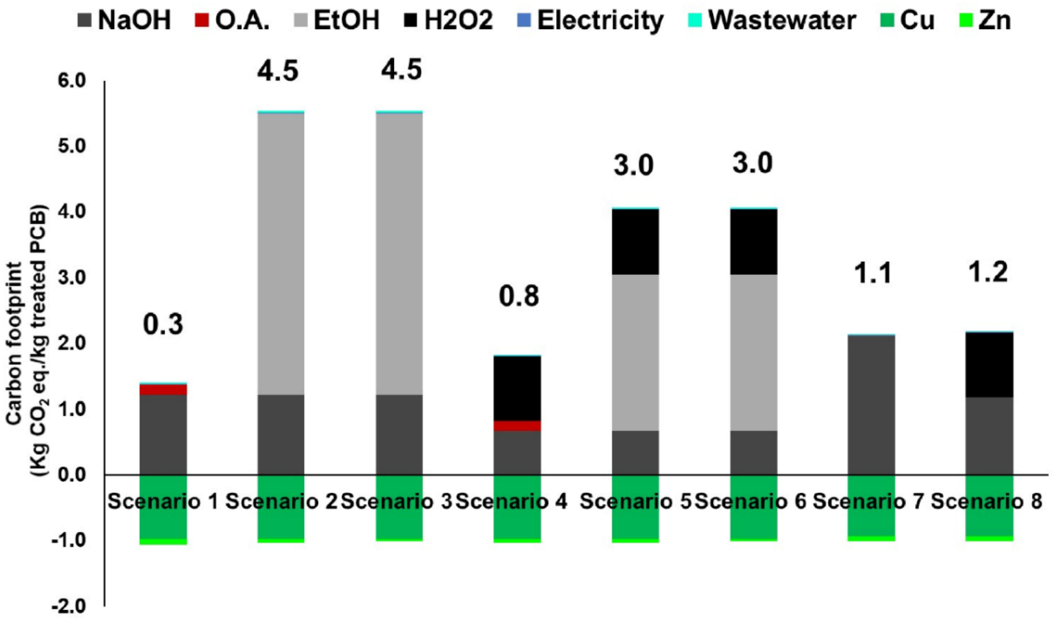

Figure 4.6. Evaluation of the carbon footprint of the 8 considered scenarios: 1) *Fe* precipitation, *Cu* cementation, *Zn* precipitation with *O.A.*, 2) *Fe* precipitation, *Cu* and *Zn* precipitation by *EtOH*, 3) *Fe* precipitation, *Cu* cementation, *Zn* precipitation by *EtOH*, repeated for chemical leach liquor (scenarios 4-6), reference scenarios of precipitation by *NaOH* for bio (7) and chemical (8) leach liquor.

### *3.6 Application and perspective*

In order to understand the potentiality of the developed treatment, the waste availability in European Union is an essential information. In this regard, 3.8 million tons of WEEE were collected in the 2015 (Evangelopoulos et al., 2019) and PCBs represent around 5% (w/w) of this quantity, with a metal content of about 40% (25 and 2% of Cu and Zn, respectively) (J. Chen et al., 2019; D’Adamo et al., 2019; Pietrelli et al., 2019). On the estimation basis, about 19 and 1.5 thousand ton of Cu and Zn, respectively, could be recovered from end-of-life PCBs. The relevance of the developed process is also connected to the metal forms, within the products, which affect their potential market. In this regard, the metal form of *Cu* from the cementation step ensures the presence of a receptor market which estimates a price around 3.6 $/kg (Awasthi and Zeng, 2019). On the other hand, the zinc, recovered as oxalate represents a product of interest, with a growing value thanks for its applications. Indeed, this agent is used as surface layer to increase the zinc corrosive resistance (Jr et al., 2018) or as precursor for the zinc oxide (*ZnO*) production (X. Chen et al., 2019). The ZnO use involves several field as photocatalysis, ultraviolet-screening and electric ceramic, nevertheless its current production causes higher impacts than the possible synthesis from oxalate, at 80°C (X. Chen et al., 2019; Guo et al., 2013; Kaur et al., 2013; Lilov et al., 2019; Patrinoiu et al., 2019; Zhuang et al., 2019). The satisfactory results, could be further improved by the recovery of precious metals from the process residue (e.g. Au and Pd) since the income from Cu and Zn recovery represents only the 10% of the total potential value from PCB exploitation (Awasthi and Zeng, 2019).

## 4. Conclusions

The modern society is experiencing a transition period from a linear to a circular economy, where an end-of-life product becomes a secondary resource, closing loops in industrial ecosystem and reducing the waste amount. Therefore, the final disposal should be the last option and the development of sustainable processes, able to improve the current recycling strategies, represents a priority. In this context, the present work focused on the exploitation of PCB waste, for the recovery of *Cu* and *Zn* content. Several approaches were presented, achieving efficiency higher than 95% for both the element target. Furthermore, the combination of the experimental activity, with an environmental assessment, identified the most sustainable strategy which includes the treatment of a solution from a bio-leaching, a preliminary *Fe* precipitation by *NaOH*, a *Cu* cementation by *Zn* powder, and a *Zn* recovery with *O.A*. The treatment produces an efficiency around the 100% for the three developed steps, with a product purity of about 100% (considering a *Cu*-*Zn*-*Fe* system) and a carbon footprint of 0.3 kg$CO_2$-equiv. The preliminary *Fe* precipitation has an essential role since it increases the purity of the metals and it produces a valuable solid useful for the recirculation in further PCB leaching. The process produced the best results thanks to the combination of established techniques, reducing the raw material consumptions. The useful sequence of treatments allowed the achievement of a simple and effective process designed for the scale-up in the perspective of urban mining strategies.

## 5. Products

Results illustrated and discussed in this chapter were used to support a peer reviewed article published on Resources, Conservation & Recycling, Elsevier (Figure 4.7).

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Sustainable recovery of Cu, Fe and Zn from end-of-life printed circuit boards

Alessia Amato*, Alessandro Becci, Francesca Beolchini

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Figure 4.7. Articles published on *Resources, Conservation & Recycling*.

## 6. Supplementary materials

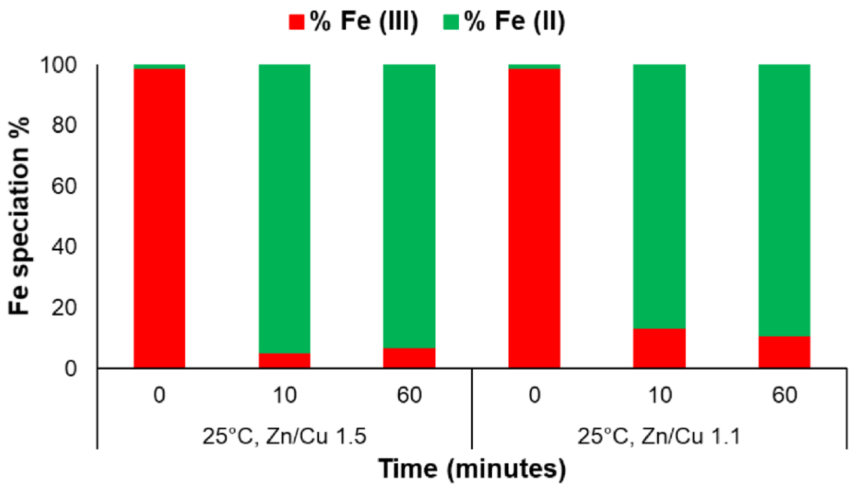

Figure S4.1. *Fe* speciation in the solution from bioleaching treatment during *Cu* cementation with *Zn.*

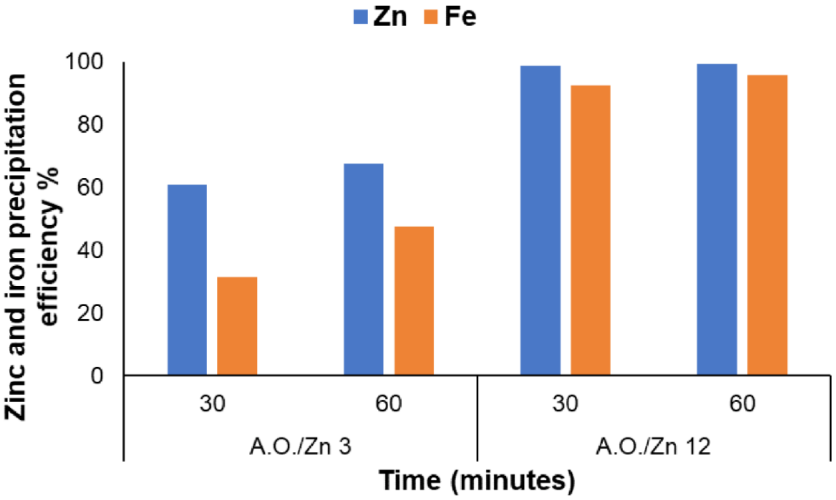

Figure S4.2. *Fe* and *Zn* precipitation with *O.A.*.

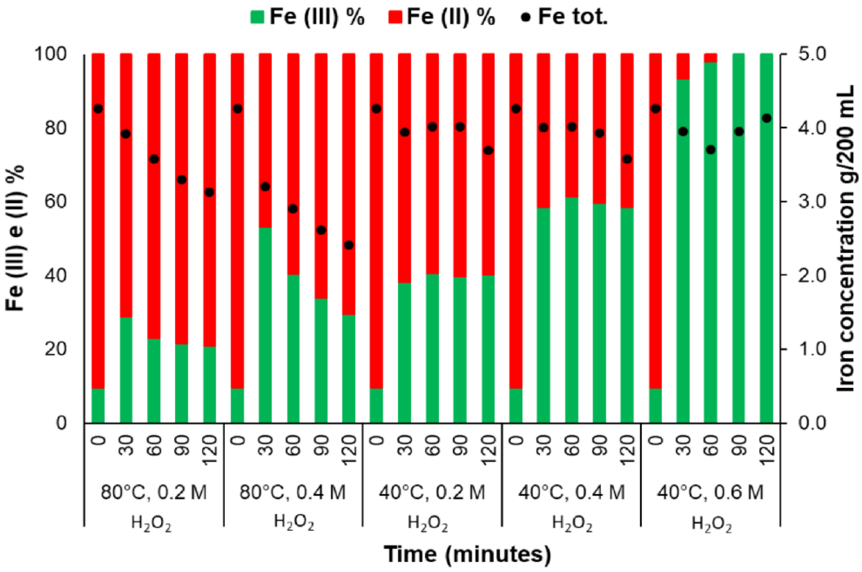

Figure A.3. *Fe* oxidation with $H_2O_2$ at different concentration (0.2, 0.4 and 0.6 M) and temperature (40° and 80° C).

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# Chapter 5: SUSTAINABILITY ANALYSIS OF THE BIOTECHNOLOGICAL EXTRACTION OF VALUABLE METALS FROM ELECTRONIC WASTE

## Abstract

The growing production and use of electric and electronic components have caused high rates of metal consumption and waste generation. The increase of these kinds of waste has pushed the research towards the development of high sustainability treatments for their exploitation. The circularity represents the key strategy of the modern economy, where the waste becomes a raw material, ready to be reprocessed for a second life. In this context, the main aim of this work is to identify the best conditions for a bioleaching process for Cu and Zn recovery from printed circuit boards (PCBs) able to make the treatment actually sustainable. Overall, the environmental assessment identified, as main criticalities to solve: the high energy demand of bioleaching process and the raw materials demand of the following recovery steps. These preliminary results have determined the choices in the further experiments. More in detail, the bioleaching process is improved by the increase of treated PCB concentration from the 5% reported in the literature to 10% (w/v) maintaining a leaching efficiency higher than 95% for Cu and Zn. This technical improvement is translated into the halved of energy demand. Furthermore, the environmental load of recovery can be reduced by a double strategy: avoiding Zn recovery or substituting the hydrometallurgical techniques by electrochemical approaches. This work is an example as the environmental impact assessment could drive the optimization and improvement of a developed process in laboratory scale.

Content

## 1. Introduction

In the last decades, the municipal waste generation has increased rapidly, with a particular contribution of waste from electrical and electronic equipment (WEEE). The WEEE constitutes the 8% of the total municipal waste, and it continues to increase by 10% every years (Kaya, 2016; Widmer et al., 2005). The reason of the fast growth of WEEE is the lifespan decrease of electric and electronic equipment, mainly due to the technological advancement and marketing strategies (Li et al., 2019; R. A. Mesquita et al., 2018; Moosakazemi et al., 2019). The average generated WEEE per inhabitant in the world is 5.9 kg, and the highest value per inhabitant is recorded in Europe (15.6 kg) (Baldé et al., 2017; Evangelopoulos et al., 2019). The objective of the European Directive 2002/96/EC on WEEE (WEEE-directive) has been the prevention of illegal WEEE landfill and incineration and to support the reuse and recycling, to preserve the primary resources, the energy and the environment (European Parliament, 2003; Hagelüken, 2005). The definition of WEEE reported in the European Directive is: "Electrical or electronic equipment which is waste... including all components, sub-assemblies and consumables, which are part of the product at the time of discarding." (European Parliament, 2012, 2003).

The Printed Circuit Boards (PCBs) are the brain part of EEE, and they constitute the 3-5% of the WEEE amount (Faraji et al., 2018; Vermeşan et al., 2020). The PCBs are constituted by three main layers: the structure (composed by fiberglass and Cu), and upper and lower compound units (composed by fiberglass, solder joints, conductive tracks, contacts, and solder mask) (Kaya, 2019). Typically, the average content of metals, ceramics and plastic are estimated of about 40%, 30% and 30% (w/w), respectively (Evangelopoulos et al., 2019; Jadhav, U. Su, 2015; Rocchetti et al., 2018; Tiuc, 2020; Zhang and Xu, 2016). As regard metals, the PCBs are composed of 20% Cu, 5% Al, 1% Ni, 1.5% Pb, 2% Zn, 3% Sn (w/w) with 250 Au, 1000 Ag and 110 Pd (ppm) (Baldé et al., 2017; Becci et al., 2019; Birloaga and Vegliò, 2016; Faraji et al., 2018; Kaya, 2019). The PCB "mineralogy" is completely different from mineral ores for metal refining. Natural ores are composed of oxidized or sulphured minerals. On the other hand, the PCBs contain pure metal or metal alloys in their structure (Kaya, 2019). The Cu and Au concentration in the PCBs are respectively 20-40 and 25-250 times higher than the concentration reported in the minerals (Hsu et al., 2019; Li et al., 2007; Qiu et al., 2019; Xiang et al., 2010). Therefore, the potential value of metal recovery from PCBs is estimated between 1000 and 25000 Euros per ton. The Cu is the further valuable metal which can be recovered from PCBs, (the third one after Au and Pd) (Awasthi and Zeng, 2019; Kaya, 2019; R A Mesquita et al., 2018).

Considering the different metal speciation, the technologies for metal extraction from PCBs are totally different from the mineral leaching (Weeden et al., 2015; Zhang and Xu, 2016). Indeed, after the collection and the presorting, the PCBs are pre-treated (e.g. dismantling and size reduction), pre-processed (e.g. separation and metal extraction), and finally end-processing (e.g. purification and refining) (Kaya, 2019; Rocchetti et al., 2018). Currently, various industries treat and recover metals from PCBs, including Umicore (Belgium), Noranda (Canada), Rönnskär (Sweden), Dowa (Japan) and Aurubis (Germany) (Kaya, 2019; Ma, 2019; Park and Kim, 2019). The main processes used in these facilities for metal extraction include pyro- and hydrometallurgical techniques. The pyrometallurgical processes need temperature above 2000°C. On the other hand, the hydrometallurgical approaches use chemicals for acid leaching (such as HCl, H2SO4, HNO3, HClO4, H2O2) and lower temperature (between 90°-200°C) (Kaya, 2019; Rocchetti et al., 2018). The main problems of these techniques are connected to the toxic gas emissions (such as dioxins) and the acid wastewater generation, damaging human health and environment (Zhang and

Xu, 2016). In the last decades, the interest for bioleaching/biotechnology was increased since they are considered green technology and more sustainable methods than chemical approaches, thanks to the low operative costs, energy demand and waste production (Beolchini et al., 2012; Fonti et al., 2015; Ilyas et al., 2013).

In the recent years, many researchers have studied the bioleaching approaches for metal recovery (mainly Cu, Zn, Al, Ni, Co) from PCBs using iron oxidizing bacteria (mainly *At. ferrooxidans*) (Bas et al., 2013; Becci et al., 2020; Hubau et al., 2018; Joshi et al., 2017; Liang et al., 2016, 2013; Nie et al., 2015a, 2015b; Priya and Hait, 2018; Rodrigues et al., 2015; Wang et al., 2018; Wu et al., 2018; Yang et al., 2014). The significant limits underlined by the literature are the long time required to complete the metal leaching and the low substrate amount treated with bioleaching treatment. In this context, the aim of this work is to identify the best operative conditions to make biotechnologies more ecofriendly than pyro- and hydrometallurgy approaches to make them more attractive for stakeholders involved in process scale-up. The challenge of sustainable development at the beginning of the twenty-first century has become a systematic one with environmental, social, and economic dimensions on an equal footing.

## 2. Material and methods

### *2.1 Preparation of the printed circuit boards*

The PCBs used in this study are recovered from personal computers. After manually removing the main parts of electronic components (the components of the upper and lower compound units, e.g. capacitors, batteries and resistors), the material that composed the structure layer is shredded using stainless steel blades and pliers to obtain a granulometry less than 0.5 mm. The plastic and the fiberglass are removed from pulverized PCBs for density, washing twice with saturated water with NaCl (Becci et al., 2020; Ruan et al., 2018). The metal composition of the resulting pulverized PCBs is the following (w/w): Cu 20 ± 5%, Zn 2.5 ± 1.0%, Ni 0.6 ± 0.2%, Al 1.2± 0.7%.

### *2.2 Assessment of the carbon footprint*

The main aim of the environmental impact assessment of the process is the identification of the PCB concentration at which the bioleaching process results the most sustainable extraction choice compared to pyro- and hydrometallurgy approaches. The assessment is referred to 1 L bioreactor. The bioleaching approach considered for the analysis is the process reported by Becci et al. (2020), where the PCB leaching by bacteria *At. ferrooxidans* is carried out in three phases: the first, where bacteria growth without PCBs; the second, when a known amount of the substrate is added to the medium; and the third, when the 80% of the solution is replaced with fresh medium. The mass and energy balances used for the environmental evaluation impact (input and output) are reported by Becci et al. (2020) as concerns biotechnological treatment (Scenario 1). The following step is the metal (Fe, Cu and Zn) recovery process (Amato et al. 2020). In detail, Fe is recovered by precipitation with NaOH, Cu by cementation and Zn by precipitation with oxalic acid (Scenario 1 Amato et al., 2020). The positive effects of the recovery of secondary raw materials (Cu and Zn) are quantified as avoided impacts for the primary production (Amato et al., 2017). The thinkstep GaBi software-System and the Database for Life Cycle Engineering (compilation 7.3.3.153; DB version 6.115) are used for the production processes of energy and raw materials and the quantification of the carbon footprint.

### 2.3 Bioleaching experiment

The bioleaching experiments are carried out to verify the possibility to achieve the environmental impact results. The tests are performed to verify the possible increase of the PCB concentration from 5% (the condition reported in Becci et al. (2020) work) to 15% (w/v). The other conditions are the same reported by Becci et al. (2020): 3 step leaching, reaction time of 11 days, 30°C, pH 1.6 and 120 rpm mixing.

### 2.4 Analytical determination

The leaching solutions are periodically analyzed for the determination of both Zn and Cu concentration, carried out by an atomic absorption spectrophotometer (AAS) (Varian spectrometer SpectrAA 200). On the other hand, the $Fe^{3+}$ detection is performed by an UV/VIS spectrophotometer (Jasco Model 7850) (Welcher and Hahn, 1964). At the end of the bioleaching experiment, the residual substrate is analysed, and the characterization is carried out after microwave-assisted acid digestion of the samples by means of inductively coupled plasma (ICP) analysis (using Agilent ICP-MS 7500, Agilent technologies). As concern the recovery efficiencies, they were calculated following the equation (Eq. 5.1):

$$\text{Efficiency (\%)}=[C_t/(C_{(E.S.S.)} + C_{(E.F.S.)} + C_S - C_{(B.S.S.)})]\times 100 \tag{5.1}$$

Where $C_t$ is the metal concentration (g/L) in the leaching solution at t time, $C_{E.S.S.}$ and $C_{E.F.S.}$ are the metal concentrations (g/L) at the end of the II and the I leaching steps, respectively, $C_s$ is the metal concentration (g/kg) in the residual PCB powder and $C_{B.S.S.}$ is the metal concentration (g/L) at the beginning of the II leaching step.

The total prokaryote abundance was determined by direct count on epifluorescence microscope following the procedure described by Danovaro et al. (2002). As described in the method, the bacteria were stained by acridine orange solution, then filtered through nucleopore 0.22 µm pore size filters. The total prokaryote count per mL of solution was calculated by equation (Eq. 5.2):

Total prokaryote count /mL = (N*C.O.*d)/mL (5.2)

Where N is the mean bacteria number per optical section, C.O. is the maximum optical section number (12,868), d (100 times) is the sample dilution and mL are the withdrew solution (0.5 mL).

## 3. Results and discussion

### *3.1 Environmental impact assessment*

The first target of the present study is to identify the best operative conditions in correspondence of which bioleaching for metal extraction is more environmental advantageous than the primary production of the same elements. A Monte Carlo simulation (around 6000 simulation), considering an uncertainty of the characterization factors from Gabi dataset (kg of $CO_2$-equivalent) of 10%, is carried out. The results show as, at the selected conditions, the bioleaching process has a higher environmental load than the primary production (Figure 5.1a), considering all the possible Cu and Zn concentrations reported in the literature (green points) (Bas et al., 2013; Becci et al., 2020; Hubau et al., 2018; Joshi et al., 2017; Liang et al., 2016, 2013; Nie et al., 2015a, 2015b; Priya and Hait, 2018; Rodrigues et al., 2015; Wang et al., 2018; Wu et al., 2018; Yang et al., 2014). Furthermore, the results show as the environmental impact in the global warming category decreases with the PCB concentration growth in the bioleaching process and using a PCB composed by high Cu concentration (Figure 5.1a). As reported in Figure 5.1b, the main critical step is the metal recovery phase (45% of the whole environmental load), mainly due to the Fe and Cu recovery. On the other hand, the results demonstrate as, in the biotechnological process, the main criticality is the energy demand due to the long time required. Therefore, the results suggest that a relevant environmental impact decrease could be achieved combining: the avoided Fe and Zn recovery and the PCB concentration increase. Indeed, the credit connected to Zn represents only the 5% of the environmental gain, not enough to balance the impact of its recovery. Moreover, in the bioleaching process, an energy from renewable sources is considered to decrease the impact due to the electricity production. Overall, as showed in Figure 5.2, a PCB concentration higher than 12% (w/v) makes the bioleaching the best choice. Nevertheless, the present paper focuses only on the carbon footprint and further assessments could take into account additional impact categories, able to quantify the effects connected to wastewater production and gas emission by pyro- and hydrometallurgical processes.

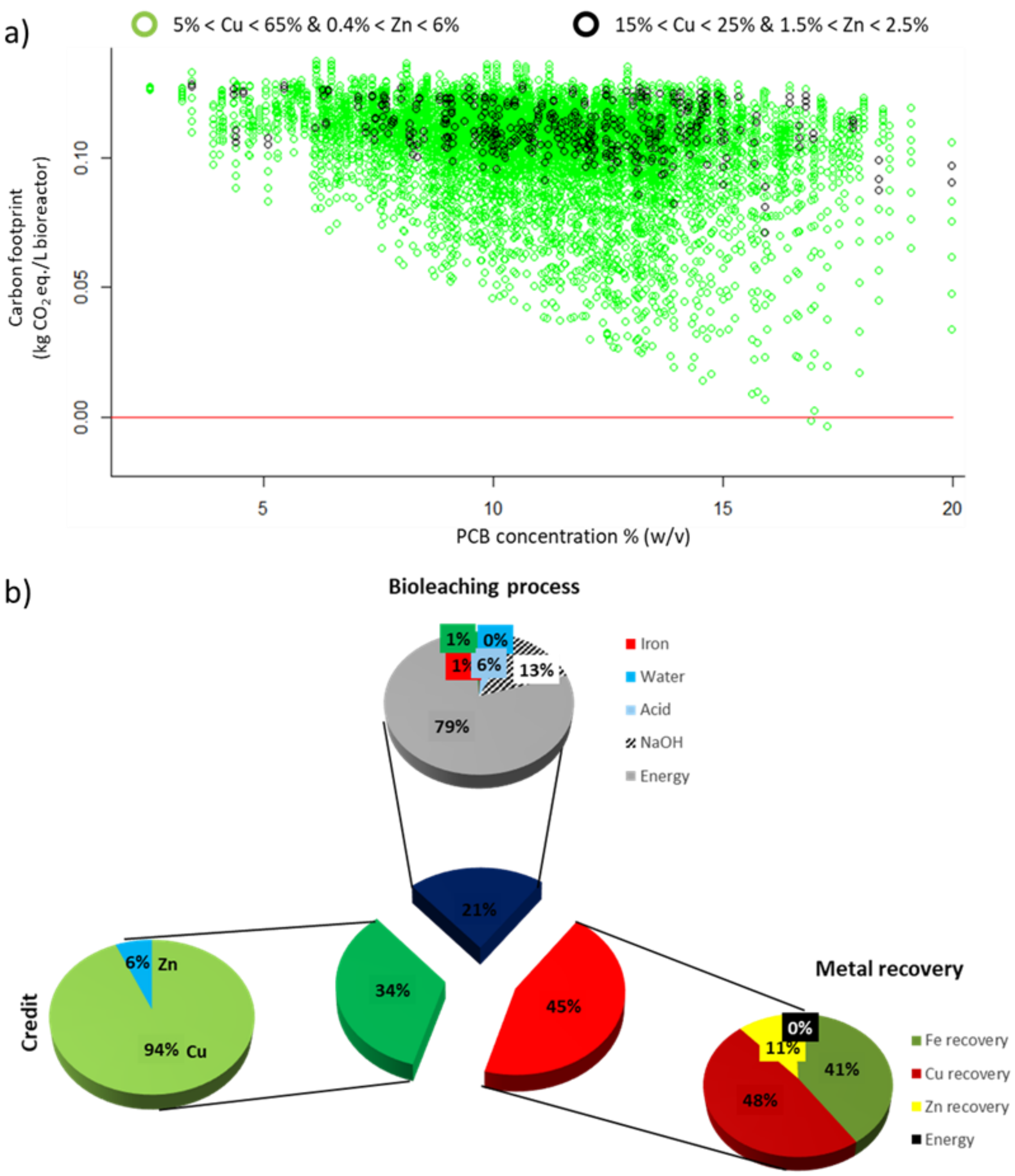

Figure 5.1. Carbon footprint of the metal extraction process from electronic waste as a function of PCB and Cu/Zn concentrations (output of the Monte Carlo simulation) (a). Partition of the environmental load in the different steps for metal extraction (b).

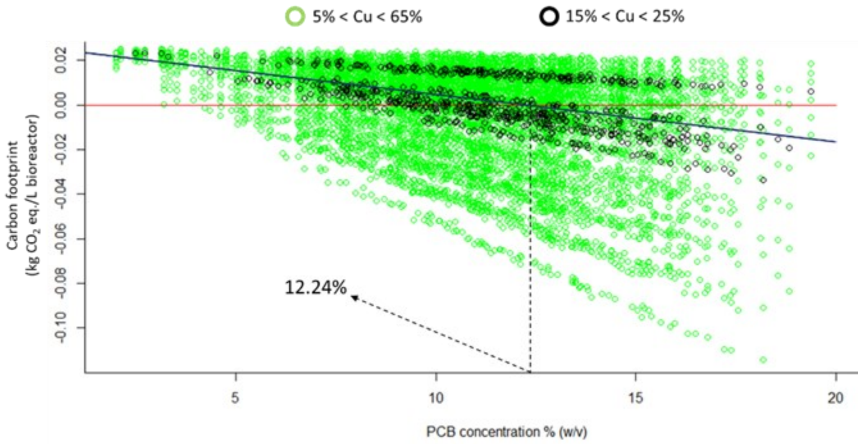

Figure 5.2. Carbon footprint of the metal extraction process from electronic waste as a function of PCB and Cu concentration considering only the Cu recovery and using energy from renewable sources (output of Monte Carlo simulation).

### *3.2 Bioleaching experiments*

The bioleaching experiments are carried out to verify the possibility to achieve the best conditions identified by the Monte Carlo analysis of the environmental assessment. The first test is carried out with the same conditions reported by Becci et al. (2020), increasing the PCB concentration from 5% to 10% (w/v) (Test 1). As Figure 5.3a shows, the bioleaching produces efficiencies around 40% and 80% for Cu and Zn, respectively, lower than the reported results by Becci et al. (2020) (higher than 95%). To increase the extraction yield, the following test (Test 2) is carried out increasing the bacteria inoculum from 20% to 50% to quicken the bacteria metabolism. The bacteria inoculum increase allows to decrease the bacteria growth phase time from 48 to 24 h (this time is enough to oxidize the whole Fe in solution). On the other hand, the I step time is extended from 48 to 72 h to increase the Cu and Zn leaching in this phase, decreasing the metal toxicity in the II step. However, the total time of the bioleaching time (11 days) remains the same. At these conditions, the final leaching efficiency is higher than 95% for both Cu and Zn (Figure 5.3a).

Both the Fe speciation and bacteria abundance results confirm the positive effect of the new tested conditions. At the end of the II leaching step the whole Fe is in the oxidized form ($Fe^{3+}$) and the bacteria growth rate shows a higher microorganism count than that recorded in the I leaching step (Figure 5.3b). These results demonstrate that the time increase in the I leaching step allows to enhance the Cu and Zn extraction, decreasing the metal toxicity on bacteria metabolism in the last one.

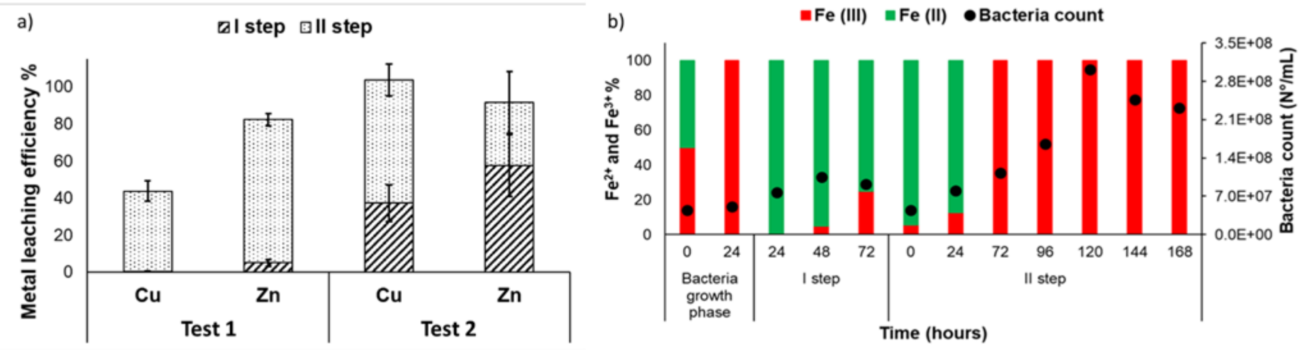

Figure 5.3. Leaching efficiency for Cu and Zn in the tests carried out with a PCB concentration of 10% (w/v) (a). Bacteria count and Fe speciation during the bioleaching processes in the Test 2 (b).

Nevertheless, the Monte Carlo simulation results demonstrate that the most relevant environmental gain is achieved for PCB concentration higher than 12% (w/v). Therefore, further tests are carried out with a PCB concentration of 15% (w/v) to find the maximum substrate suitable for the bioleaching process. The first test (Test 3) is carried out with the best conditions determined in the previous experiments. A leaching efficiency lower than 20% and 70% for Cu and Zn is achieved, respectively (Figure 5.4a). In this case to increase the leaching yield, the parameters, that are changed, are: the Fe concentration (from 10 to 15 g/L) in the Test 4, and the PCB concentration (Test 5), starting from a concentration of 10% (w/v) in the I leaching step and adding other 5% (w/v) at the beginning of the II step. The best results are achieved in the Test 4 with a leaching efficiency around 60% and 80% for Cu and Zn, respectively, both lower than the previous results (higher than 95%). The main problem observed in the Test 4 is the Fe precipitation on PCB substrates (around 35% of the Fe precipitated during the bioleaching process). Therefore, the kinetic and efficiency of the reaction between $Fe^{3+}$ and metals within PCBs decrease, for this reason the Fe at the end of the experiment is mainly in the oxidized form and not thanks to bacteria activity (Figure 5.4b). On the other hand, in the Test 5, with the Fe concentration of 10 g/L, the Fe precipitation is not verified. Nevertheless, the Fe is mainly in the reduced form ($Fe^{2+}$) due to the lowest bacteria abundance, comparable with the Test 4, but 4 times lower than the Test 2 (Figure 5.4c). The lowest bacteria count is mainly due to the PCB powder toxicity and not to metal toxicity since the Cu and Zn concentrations are lower than the concentrations obtained in the Test 2. Therefore, these results underline that the 10% (w/v) PCB concentration is the maximum substrates that could be treated with this bioleaching process.

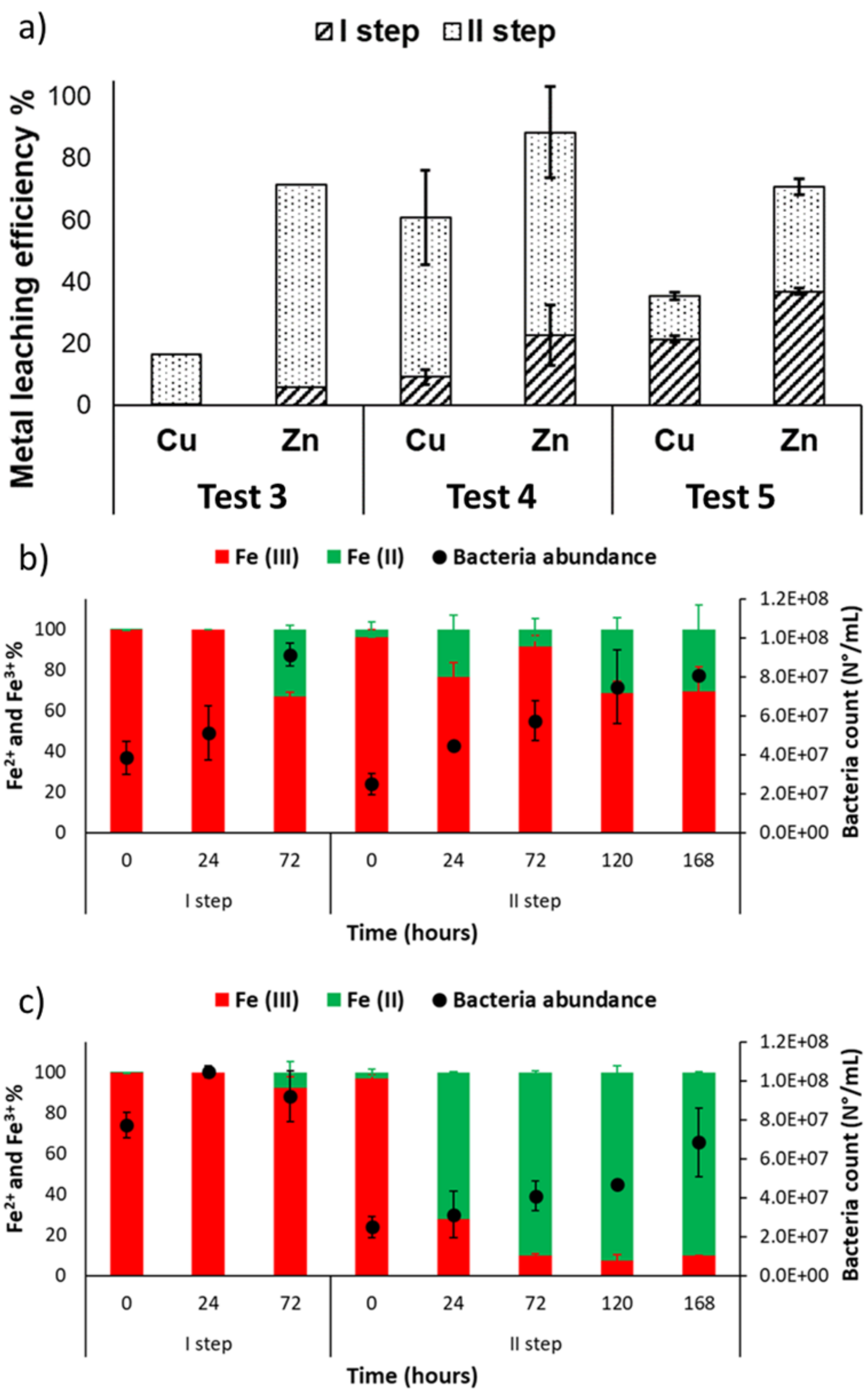

Figure 5.4. Leaching efficiencies for Cu and Zn in the test carried out with a PCB concentration of 15% (w/v) (a). Bacteria count and Fe speciation during the bioleaching process in the Test 4 (b) and in the Test 5 (c).

## 4. Conclusions

The modern society is changing the system from a linear to a circular economy, where an end-of-life product becomes a secondary resource of raw materials, closing loops in industrial ecosystem and reducing the waste amount. Nevertheless, the sustainability of the circular strategy implementation is not obvious and specific assessment should be always performed to verify the real advantage, driving towards an efficient reduction of the environmental impacts. In this context, the present work focused on the exploitation of PCB waste, for the recovery of Cu and Zn content. This work demonstrated the relevance of a preliminary LCA evaluation for three main reasons:

- *The identification of the critical steps that require to be improved;*
- *The identification of the main critical aspects within each phase (e.g. raw materials or energy);*
- *The creation of an effective experimental design.*

More in detail, the combination of the experiments and the LCA identified 10% (w/v) as maximum PCB concentration that could be treated by the bioleaching process, ensuring both sustainability and effectiveness. Furthermore, the environmental assessment highlights the high impact due to the further Zn recovery. This criticality can be overcome avoiding this step or substituting the proposed hydrometallurgical techniques by electrochemical approaches (the main process used in industrial scale).

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# Chapter 6: REVIEW

## Abstract

The citric acid is considered one of the most valuable weak organic acid on the market and its production by biotechnological approaches is a very interesting topic. Despite the related scientific research, the literature still lacks a state of art of the technological innovation change, necessary for the study of the inventions designed for the real scale implementation. In this context, the present review took into account more than 100 worldwide patents (1929-2018), necessary for the identification of the innovative markets and the most promising fields for the economic investments. The deepened study identified an increasing invention number, combined with the current worldwide citric acid export flows, with China as the leader (with an economic contribution of 75%, in 2017). In order to satisfy the requests of the market which has moved towards a circular economy, the possibility to use waste substrate represents one of the main options considered in the recent patents. The discussion highlights the sustainability improvement, achieved by the conversion from a submerged technology to a solid-state fermentation (*koji* process). The showed results are essential for both a scientific audience and the stakeholders citric acid production, in order to have a complete and updated overview about this topic.

## Content

## 1. Introduction

The citric acid (CA) is the most valuable weak organic acid, widely used on the market for many applications (Luo et al., 2017). It is a tricarboxylic acid with an essential role for the metabolism of aerobic organisms (Ciriminna et al., 2017). Studies about its production dates back many years when it was crystallized from lemon juice (18th century). Currently, the CA biotechnological production is mainly located in China and 2 million tonnes of product for year are expected to 2020 (Ciriminna et al., 2017). The use of this agent includes several fields, including food, textile, chemical and pharmaceutical industries (X. Sun et al., 2017). Nowadays, the experimental world production of CA mainly involves fungi and yeasts: *Aspergillus niger, A. wentii, A. clavatus, Penicillium luteum, P. citrinum, Mucor piriformis, Candida guilliermondii, Saccharomycopsis lipolytica, Trichoderma viride* and *Arthrobacter parafineus*. However, only *A. niger* and the closely related strain of *A. wentii* are chosen for the commercial production (Kuforiji et al., 2010). Overall, the processes include the previous fermentation followed by recovery and purification. The main critical parameters for the fermentation are: the high carbohydrate concentration, the maintenance of high dissolved oxygen, the constant agitation and the low pH value (Dhillon et al., 2011; Ferreira et al., 2016; Liu et al., 2016; Zhang and Roehr, 2002a, 2002b). As concern the further recovery and purification steps, the most common approaches are the $CaCO_3/H_2SO_4$ precipitation (Magnuson and Lasure, 2004; Pazouki and Panda, 1998; Singh Dhillon et al., 2011) and the liquid solvent extraction (by a solvent mixture) (Singh Dhillon et al., 2011; Wennersten, 2008). Alternatively, Ledakowicz et al. (2004) describe an anion-exchange method by tertiary amine resin.

Considering the relevance of the CA for the market, the recent literature has widely discussed its production by fungi, mainly *A. niger*, reporting several substrates and operative conditions (Auta et al., 2014; Liu et al., 2015; Maharani et al., 2014; Wang et al., 2017; Yu et al., 2017). Many reviews have deepened this topic, focusing on specific aspects as the fermentation variables (Hu et al., 2019; Show et al., 2015; Soccol et al., 2006; Tong et al., 2019) and the characteristics of the solid-state fermentation (Gowthaman et al., 2001; Letti et al., 2017; Soccol and Vandenberghe, 2003). Recent studies has focused on the possibility of a waste use as substrate (Hu et al., 2019; Sawant, 2018), following the circular economy principles. Nevertheless, the scientific literature should be integrated by the state of art of the technological innovation change, able to create an overview of the inventions designed for the application in real scale. This relevant aspect, represented by the registered patents, was already highlighted in other application fields (e.g. waste recycling or medical applications) to identify the most promising technologies for the creation of innovative markets (Amato and Beolchini, 2018; Garcia and Calantone, 2002; Li et al., 2019; Rocchetti et al., 2018; Zhang et al., 2019).

To fill the gap, the present work (updated to March 2019) analyzes 100 patents, between 1929 and 2018, showing the technological evolution of the last decades. The free access Espacenet platform was used as the main information source (European Office Patent, n.d.) using the keywords, for the patent search: “citric acid bioproduction”, “citric acid *Aspergillus niger*”, “citric acid *Candida*”, “citric acid *Penicillium*”, “citric acid *Aspergillus*”. This information source, created by the European patent office, ensures a worldwide invention overview (Amato and Beolchini, 2018).

## 2. *A. niger,* different methods to use raw material as carbon source

*A. niger* uses many substrates for its growth (i.e. maltose, mannose, galactose, fructose, sucrose and glucose). Nevertheless, glucose and sucrose produce the best results (both the greatest growth and the highest CA production) with a concentration of 10-14% (w/v) (Murad and Khalaf, 2003; Xu et al., 1989). Overall, the fungal fermentation can be carried out by three different fermentation techniques: submerged, surface (liquid surface culture) and solid-state (*koji* process) (Alam et al., 2010; Hang and Woodams, 1986; Karthikeyan and Sivakumar, 2010; Letti et al., 2017; Vandenberghe et al., 2004). Nevertheless, the highest patent number (Figure 6.1A) identifies the first method as the most common, as also confirmed by the literature which reports around 80% of the world production by this approach (Show et al., 2015; Vandenberghe et al., 1999).

### *2.1 Immobilization method*

The fungal immobilization method improves the CA production by *A. niger*. The fungal was embedded by calcium alginate to reduce the contact with the produced CA, decreasing the toxicity on the metabolism. This effect is due to the citrate, a strong inhibitor of the glycolytic enzymes 6-phosphofructokinase (Arts et al., 1987; Ates et al., 2002). The patent CN102864184 describes the use of an optimal medium composed of sucrose with a concentration of 120 g/L, at 30°C and 200 rpm (Jin, 2013a). A similar approach is reported in the patent CN107022541, which involves a pretreated fibrous material, as immobilization medium. An air sparging system improves the production, with a final yield of 93% (expressed as CA yield/glucose consumed), a CA concentration of 89 g/L and a fermentation rate of 1 g/L·h (Ying et al., 2017). The immobilization method allows multiple advantages: the production efficiency increase, the fermentation time reduction, the decrease of the biomass and the fermentation broth amount and a simplified separation between the product and the mycelia.

### 2.2 *Multiple step processes*

The fungal growths using a multiple step process is an alternative technique for the production increase. The invention CN107815421 describes a method where *A. niger* is cultivated in three different growth phases to prevent the criticality of the spore aggregation, allowing both the highest quantity and a size uniformity of the mycelium pellets. After this preliminary step, the matured seem liquor is transferred to the final fermentation tank. Consequently, the glucose is metabolized by the fungal and the conversion rate is improved, with lower fermentation time (Shi et al., 2018a). On the other hand, the inventions CN106868061, CN102181490 describe a method which includes a first step of *A. niger* growth by $CO_2$ gas sparging. When the fungal biomass is grown, it is transferred to a second section where fungi continue the CA production decreasing the reducing sugar content up to 1-3 g/L (Chen, 2017; Yong et al., 2011). These treatments increase the conversion rate and they reduce the residual sugar, compared to the traditional approaches. The pH shifting, during the acid production, is proposed in patents CN104277978, CN102851330. During the preliminary phase, the pH is 6.2-7.2, then its rise increases the production rate (Li et al., 2015; Xia et al., 2013). As explained in the invention CN102373242, the temperature modification improves the CA synthesis. More in detail, a 35-45°C range is selected during the rise phase of *A. niger* respiratory quotient and growth; thereafter, the value is reduced to 30-40°C, during the second step, when the biomass is constant and the respiratory quotient decreases. An additional increase (35-45°C) is necessary in the last step, when the sugar content is very low (Yongsheng et al., 2012a). The fungi age change affects the process efficiency, as reported in patents CN104099253, CN104087624. *A. niger* grows in a first medium to obtain a mature seed solution; part of the resulting dispersed mycelium is transferred to a fermentation medium (5-15% of inoculum concentration), when it reached the maximum growth rate. This second step finishes when the concentration of the reducing sugar in the fermentation medium is lower than 0.5%. The use of the dispersed mycelium seed solution from the second step avoids the repetition of the first step with a consequent continuous production of CA (Shi et al., 2014a, 2014b). A different method is described in the invention CN102443611, characterized by the addition (in the same fermentation tank) of a monosaccharide with 6 carbon atoms to the fermentation liquid, from 24 h after the *A. niger* inoculum to 5 h before the fermentation conclusion (Yongsheng et al., 2012b).

### *2.3 Metal use*

The metal ions have an essential role in the CA biosynthesis by *A. niger*. Nevertheless, the identification of the best concentration is necessary, since high concentrations, at uncorrected conditions, can be translated into a low efficiency production (Magnuson and Lasure, 2004; Schreferl et al., 1986). In this regard, the invention US5532148, includes the Mn (II) addition, with a concentration of 2.5-20 ppb, at pH 1.5-3.0, achieving the highest CA production between 4 and 7 days of fermentation. The further broth decolorization and ion-exchange, allow the production of an aqueous CA solution, with a purity around 98% (Datta and Bergemann, 1996). Alternatively, Mn, Zn (30-250 ppm), $[Fe(CN)_6]^{-4}$ (100-500 ppm) and Cu increase the acid production, at pH 1.5-2.0, in patents US5081025, US3936352, GB1392942, GB1342311 (Jungbunzlauer Spiritus, 1974; Jungbunzlbauer Spiritus UND CH, 1975; Kabil, 1976; Kirkovits and Edlauer, 1992).

### *2.4 Alternative techniques*

Additional strategies have been optimized to increase the CA production by A*. niger*. CN105586366, CN103497977 patents describe a stirring speed increase during the preliminary 6-20 h of incubation. This condition allows the hypha shape changes with the formation of small and compact pellets, able to increase the oxygen exchange capacity, with the consequent improvement of the acid synthesis (Luo et al., 2014; Shi et al., 2016). On the other hand, CN103695319 patent uses a sugar solution with neutralization wastewater to replace the traditional tap water (R. Li et al., 2014b). The possibility of a waste stream use as N and P source, combines a significant cost reduction with the decrease of the environmental load for the wastewater treatment.

Overall, a multi-step process represents the main option chosen for the improvement of CA production by *A. niger* with raw materials, as carbon sources. Around 50% of the patents in this section, referred to the last decade, confirmed an efficiency increase achieved by process suitable for the scale-up. On the other hand, the metal use is taken into account in old inventions, due to the high criticality of the element concentration which can decrease the process effectiveness (Anastassiadis et al., 2008; Soccol et al., 2006; Zhang and Roehr, 2002b).

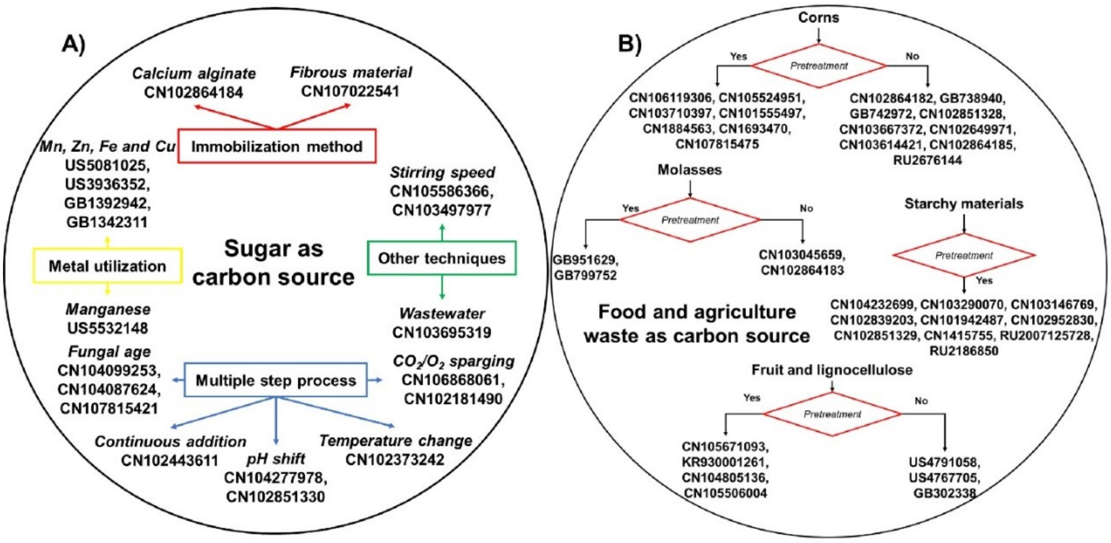


Figure 6.1. Patents related to the citric acid production using sugar (A) or agriculture and food waste as carbon source (B).

## 3. *A. niger*, food and Agriculture waste as carbon source

The possible substitution of the traditional glucose (or other sugars) by food and agriculture waste, as carbon source for the fungal metabolism, represents an interesting topic since 1920 (Figure 6.1B). The present paragraph focuses on the main streams considered for the technical innovation in this field.

### *3.1 Corns as carbon source*

Several kinds of corn have been used, as carbon source, to produce CA by *A. niger*, mainly: corn cobs, corn wheat, bran, soy, sorghum, corn sugar, wheat straw and rice. Usually, the substrates need a pretreatment to make the cellulose bio-available for the fungal metabolism. Patents CN106119306, CN105524951 describe a $H_2SO_4$ washing (for 1-3.5 h), followed by the addition of an enzyme (e.g. cellulase or cellobiase) to provide the hydrolysis and the conversion of cellulose into glucose. At the end of this pretreatment, *A. niger* is inoculated and the fermentation starts (Shen and Shen, 2016; Yin, 2016). On the other hand, the inventions CN103710397 and CN107815475 report a corn liquefied solution use, which needs the preliminary addition of β-cyclodextrin (pH 6.0-6.8 and 15-17% (w/v) of sugar concentration). Thereafter, the fungal is inoculated and the fermentation is carried out at 35-37°C, ensuring the oxygen supply and the necessary inorganic N amount (J. Li et al., 2014). The two-stages fermentation process, in the second invention, is conducted in a cylinder (16-24 h). The first step starts after the corn liquefied addition and it carries out for 2-8 h, at 35-39°C. In the second one a sucrose solution acts as supplementary carbon source and the citrate production continues until the sugar concentration is lower than 5 g/L. This multiply step design allows to increase both the pH and the nutrient concentration with a positive effect on the fungal growth (Shi et al., 2018b). The possibility of a cornstarch use avoids the pretreatment. More in detail, the medium described in the patent CN102864182, is prepared as following: cornstarch (20% w/v), $(NH_4)_2SO_4$ (0.2% w/v), $KH_2PO_4$ (0.2% w/v), $MgSO_4 \cdot 7H_2O$ (0.05% w/v) and MeOH (4% w/v). The fermentation is carried out at: pH 3.0, 30°C, 200 rpm, for 7 days (Jin, 2013b). Alternatively, the invention RU267614 combines the addition of the nutrients to the cornstarch, with the change of both the mixing speed and the aeration rate during the fermentation. During the growth phase, the two parameters increase from 120 rpm and 480 L/h of the first 6 h, to 250 rpm and 1920 L/h of the remaining 18 h (Sharova et al., 2018). Another waste carbon source which avoids the pretreatment is the corn sugar. The inventions GB738940, GB742972, CN102851328 describe the CA production by the N source addition combined with further salts (i.e. $KH_2PO_4$, $MgSO_4$, morpholine, $CaCl_2$, $MoO_3$, Zn), adjusting the pH at 2.6 to stimulate the acid synthesis (Jin, 2013c; Miles Lab, 1956, 1955). On the other hand, the dry corn powder needs a treatment before the fermentation, as described in the invention CN101555497. A water addition produces a paste, which is mixed with amylase and heated at 92-98°C, for a first step, and at 88-90°C, for a second one, maintaining the liquefaction for 1 h. About the 90% of the resulting solution is filtered and used for the inoculation (at 38°C), after the inorganic N addition (Yongsheng et al., 2009). Alternatively, *A. niger* is previously grown in a glucose solution for 24-48 h, at 35-38°C and 150-300 rpm. Thereafter, it is transferred in a seed tank with the bran medium and it is cultured at 36-38°C, for 4-6 days. After the adaptation to the new substrate, it is mixed with a bran solution for the fermentation, as described in the patents CN103667372, CN102649971 (Hu et al., 2012; Sun et al., 2014). Soybean molasses or sorghum powder are chosen as organic source in the inventions CN103614421, CN102864185, without a pretreatment. In both cases the selected conditions are: 26% (w/v) of sugar content, 35°C, pH 4.5, time 4 days and ventilating to supply oxygen (Cheng et al., 2014; Li, 2013). On the other hand, the wheat straw (patent

CN1884563) and the rice grain (patent CN1693470) require a preliminary enzyme addition (e.g. cellulase or α-amilase), at the concentration of 20 units/substrate gram. The further hydrolysis is conducted at the 40-45°C for 40-80 h, followed by a solution filtration and the fungal inoculation (Chen, 2006; Guo, 2005).

### *3.2 Fruit and lignocelluloses as carbon source*

The possibility of the kiwi use, as organic source is explained in the patent CN105671093, which produces CA by *A. niger* by a solid-state fermentation set-up. The fruit residues are washed and dried, at low temperature, to obtain a water content lower than 6%. Thereafter, the product is pulverized, and it is used for the fungal inoculation. The fermentation starts at 27-33°C and it carries on for 4-6 days (Tan and Shen, 2016). Further patents use grape pomace (US4791058), apple pomace (US4767705) or other kind of fruit (GB302338). In these cases, pretreatments are not required since the glucose is available for the metabolism (Hang, 1988a, 1988b; Montan UND Industrialwerke, 1929). On the other hand, the orange peel, in the invention KR930001261, is dried, pulverized and mixed with water, $NH_4NO_3$ and $MgSO_4$ to stimulate the fungal fermentation. After a pH adjustment at 4.5, the submerged fermentation starts at 10-30°C, for 3 days (Kang et al., 1993). With the same aim, a pretreatment is carried out on the lignocellulose described by CN104805136, thanks to the enzymatic hydrolysis by the enzyme cellulase (Bao et al., 2015). Patent CN105506004 uses pulverized konjak, obtaining a CA production of 150 g/L (Tang et al., 2016).

### *3.3 Starchy materials as carbon source*

Starchy materials need a preparation to be used as substrate. The invention CN104232699 suggests a preliminary solid fraction removal, followed by the addition of the *A. niger,* previously cultivated in a broth with untreated starchy material, for the fungal adaptation (Gao et al., 2014). Alternatively, the techniques in patents CN103290070, CN103146769 and CN102839203 requires an α-amylase supply and a high temperature to form a mixed slurry. After a solid/liquid separation, the resulting solution is used for the acid production, and the fermentation is curried out at 38-40°C. Thereafter, a N source is added to the medium and a ventilation is applied for 20-36 h, feeding the culture broth in batch, for 16-22 h. The fermentation finishes when the residual sugar is below 1% (w/v) (Liu and Liu, 2013; Luo et al., 2013; Zhou et al., 2012). Patent CN101942487 describes a method suitable for starchy material, also applied with specific substrates (cheap dried sweet potatoes CN102952830, puffed dried sweet potato CN102851329, unhusked CN1415755). It includes the addition of α-amylase enzyme for the reduction to amino acids and the increase of the bio-availability, avoiding a further liquid/solid separation (Guiyang et al., 2011; Guo and Zhu, 2003; Jin, 2013d, 2013e). The addition of a N source, in the patent RU2007125728, improves the conversion rate of sugar up to 87-93% (Шарова et al., 2009). Comparable results are achieved in the invention RU2186850 using metals (i.e. Zn, Fe or Cu) (Sharova et al., 2002).

### *3.4 Molasses as carbon source*

Same roots, commonly used for the commercial sugar production, are suitable for the CA synthesis. Among these substrates, cassava is chosen in patents CN103045659, CN102864183, without any pretreatments, carrying out the fermentation at 39°C, for 3-4 days and 300 rpm (Jin, 2013f; Li et al., 2013b). Satisfying results (up to 15% (w/v) of CA, oxalic acid free) are obtained by the molasses from beet sugar manufacturing, as described in the inventions GB951629 and GB799752. In the first one, 0.3% $K_4[Fe(CN)_6]$ and 0.2% $KH_2PO_4$ are added to the molasses, which shows a sugar amount of 6.0-7.5% (w/v) and a pH 5.7-5.9. This substrate is inoculated with *A. niger* in the presence of sparging air and the resulting fungal pellets are added to a similar medium, at pH 6.8 and a sugar content around 15% (w/v). The solution is agitated at 30°C for 40-48 h, the pH is adjusted to 3.0 by HCl and the air supply is replaced by the oxygen insufflation to stimulate the CA production (Miles Lab, 1958; Svenska Sockerfabriks AB, 1964) [102].

This paragraph encloses around 40% of the patents of the present review. This is due to the high price of glucose (or sucrose), as substrate from raw material, that increases the industrial production cost of the CA (Hu et al., 2019). The positive cost effect achieved by the waste use can be further enhance by the use the biomass from the fermentation for both the biogas production and the fertilizers preparation (Sawant, 2018). Overall the main materials selected for the inventions are the corns (around 45%) and the starchy (30%), because they combine the low cost with a great availability. The patents confirm the relevance of the multiple step set-up, where the first one is necessary for the fungal adaptation.

## 4. *A. niger,* mutagenic strain

Since the sixties, the researches have experimented innovative approaches which have involved the fungal genome mutations to increase the CA production by *A. niger* fermentation. The techniques of UV, γ-ray-induced and chemical mutagenesis are currently accepted as routine methods, although the patent number is lower than that related to the wild strain use (Gupta and Sharma, 1995; Ikram-ul-Haq et al., 2001) (Figure 6.2).

### *4.1 Sugar as carbon source – mutagenic strain*

The inventions CN106755138 and US2018195052 propose the modification of the succinic semialdehyde dehydrogenase (SSD) gene of the *A. niger* genome to obtain a recombinant

*A. niger* strain. The expression of the SSD gene is regulated by the low pH inducible Pgas promoter which initiates the expression of the SSD protein in *A. niger*. The final effect is the increase of CA production by enhancing the GABA pathway (Kumar and Punekar, 1997). The method, described in the inventions, utilizes the *A. niger* H915-1 as a host, with an efficiency increase of 10% and a time reduction of 10 hours, at 42°C (Jin et al., 2017; Liu et al., 2018). Alternatively, the patent CN106635847 integrates the *A. niger* low-affinity glucose transporter LGT1 gene into the *A. niger* genome to achieve the *A. niger* recombinant. The present method involves a low pH inducible promoter to initiate the expression of LGT1 protein in *A. niger,* increasing the uptake of glucose during the acidogenic phase. The result is an improvement of the CA production of 6.5%, at 42°C, with a time decrease of 10 h (F. Sun et al., 2017). On the other hand, the invention CN108018216 includes the modification of the glucosyltransferase genes to increase both the glucose assimilation and the conversion rate. The new genetic engineering strain allows a sugar concentration reduction of 10% and an increase of the CA yield around 10% (Li et al., 2018). The *A. niger* RCAM 02149 from the strain VKPM F-501 (patent RU2013151521), is produced by a genetic mutation by chemical mutagens (ether) and UV-radiation. This method allows about 90% of sugar conversion, at 32°C and a pH 1.7-7.0 (Выборнова and Шарова, 2015). In the patents CN103952318, CN103045487 the *A. niger* FY2013 and FYCA8561 strains is prepared from the FY2010 strain using a low-dose compound treatment by γ-ray treatment, at 90°C, for 5 minutes, adding the nitrosoguanidine (0.1 mg/ml). The resulting acid production is about 150 g/L (Li et al., 2013a; R. Li et al., 2014a). The patent CN103194398 is designed to obtain high-yield strains, named *A. niger* TN-A09, with high tolerance levels to both sugar and acid concentration and high-conversion rates. Overall, the production of CA is 18% (w/v), with a fermentation cycle of 60 h (Wang et al., 2013). On the other hand, the patent

WO2013082459 provides a genetic enhancement of a LaeA gene or an inactivation the Alg3 gene of *A. niger* to increase the acid production ability of the fungus (Dai and Baker, 2013). The *A. niger* CGMCC5342 and CGMCC5343 strains, in the inventions CN102533570 and CN102399702, respectively, are obtained by the genetic mutation of *A. niger* Co 827. The method allows a CA production of 15% (w/v), after 52 h at 30-40°C and a starting pH 4.0-5.0 (Junhua et al., 2012; Xiu et al., 2012). A portion of spore suspension is collected, after a grown of 5-6 days, in patent CN102352322 and mutated by N ions. The mutant slant is inoculated at 40°C, 220 rpm and cultured for 90-96 h. The achieved CA production is 10% higher than that of the original strain (He et al., 2012). Patent RU2428481 uses the fungal strain *A. niger* VKPM F-696 grown in a solution with sugar, in the presence of a water-soluble complex of C60 fullerene (0.5-0.7% w/v), with polyvinylchloride (0.7-1.2 mg/mL). The use of this technology increases the citrate synthesis activity of 30%, from the third day with an improvement of the product content around 11% and a final conversion efficiency of 98% (Nikiforova et al., 2011). The *A. niger* No CCM8210 strain (invention WO9710350) is developed from a starting variant obtained by protoplasts isolation from selected *A. niger.* The protoplasts are isolated from the hyphae in a stabilized solution (0.7 M NaCl + glucose), in the presence of $Ca^{2+}$. The resulting protoplasts, filtered and washed with water, are radiated by UV radiations, for 5 minutes. The further fusion is performed with wild strains to increase the growth and the production ability. This *A. niger* strain is subjected to a further mutation by the combination of UV-radiation and chemical mutagens (i.e. 5-bromouracide, 2-aminopurine, diethylsuiphate, ethylethane sulphonates and their combinations). The achieved CCM8210 strain is a high producing mutants, able to start fermentation at pH lower than 2.8, avoiding the production of undesirable organic acids. The strain has high conversion rate, about 1.3 g/L・h of CA. Overall, the variant produces up to 93 kg of CA from

100 kg of supplied sugar (Minarik et al., 1997). The *A. niger* R-3 strain is selected from the *A. niger*-119 strain using ethyleneimine, N-nitrosomethylurea and UV-radiation, in the patent US4380583. Its properties include the resistance to antagonist bacteria that may occur during the fermentation. Up to 99% of the whole synthesized acid consists of CA with a yield that reaches the 100% of sugar conversion (Karklin et al., 1983).

### *4.2 Waste as carbon source - mutagenic strain*

Molasses is the principal carbon source from food and agriculture wastes, used as substrate for the metabolism of *A. niger* mutagenic strain. The described patents show two main differences: the genome mutation kind and the production yield. The use of *A. niger* F-718 and molasses as substrate is reported in the invention RU2125607. The process includes a first stage at pH 4.0-5.0 and a fermentation, at 30°C. During the first 30-48 h of the process, a continuous feeding of both molasses and nutrient salts is requested. The second stage uses the microorganism suspension overflowing from the first stage, at 30°C and pH 2.0-3.0 (Avchieva and Berezhnoj, 1999). As an alternative, *A. niger* R-1 and P-1, are involved in the patents FR2361330, SU568677, GB1499093. Both fungi are produced by the combined activity of: UV-radiation, ethylene imine and N-nitrosomethyl urea on *A. niger* EU-119 (*A. niger* R-1) and *A. niger* FY-119 (*A. niger* P-1). The new strains increase the sugar conversion rate from 89% to 99% and the CA production yield from 94% to 97%. Furthermore, these variants show both a high resistance to antagonist bacteria and a low sensitive to the composition of the starting molasses, allowing the low quality substrate use (EX Z Biokhim Preparatov I MIKR, 1978; Rumba et al., 1978, 1977). The use of a mix of molasses medium and sugar solution, at different concentrations, is proposed in the patent RU2203322. More in detail, using the *A. niger* VKPM-817, the obtained sugar conversions to CA are: 87% with a 30 g/L sugar solution, 66% with 130 g/L and 96% with 150 g/L

(Shcherbakova et al., 2003). Sorghum condensed juice, molasses and sucrose are showed, as substrate, in the invention RU2192460 that uses *A. niger* VKPM-809 with final conversion rates of 60-90% (Krasikova et al., 2002). A high conversion of sugar to organic acid (9-41%) is described in the patent RU95113067 by the fungal VKPM-713 strain (from the *A. niger* F-326 strain genetic mutation) by UV, using several substrates (i.e. beet and sugar cane molasses sugar, food sugar, crude sugar, glucose and their mixtures, and fermentation conditions) (Ermakova et al., 1997). Alternatively, the microorganism strains 1015, 10577, 11414, 12846, 9142, 13794, 26036, with sugar beet or date molasses as carbon source, are used in patent FR2336477. In this case, the highest acid production rates (95% of CA) is achieved at these conditions: substrate pretreated by $K_4[Fe(CN)_6]$ (to remove metal traces), pH 2.7, 24 h (Fazeli Abbas, 1977). A pretreatment is required in the patent GB799752, for the impurity removal from molasses, corn starch hydrolyzed or beet molasses, adding $CaCO_3$. The nutrient salts are added to the broth: 0.15-0.2% of $(NH_4)_2CO_3$, 0.01-0.02% of $KH_2PO_4$, 0.08-0.15% $MgSO_4$ and 0.0002-0.0004% of Zn, at pH 2.5. The fermentation is carried out at 24-34°C by the *A. niger* mutant strain and the resulting CA production is about 10% (w/v), after 8-12 days (Miles Lab, 1958). The *A. niger* R-6 and R-5 strains are used with sugar beet molasses (LV11342) and sugar cane molasses (LV11340), as substrates, respectively. The fungal fermentation is carried out with a sugar amount of 15% (w/v), for 9-12 days. The resulting sugar conversion rate reaches the 100% in the first medium and the 85% in the second one, with a the CA content of 95-99%, in both cases (Roman Karklins and Skrastina, 1996; Romans Karklins and Skrastina, 1996). Alternative substrates are used with mutagenic strain, in patents CN106367359, CN108588133 (acorn) and RU2103346 (EtOH). In the first invention, *A. niger* AA120, obtained after mutagenic screening by the atmospheric and room temperature plasma (ARTP) technology, allows high CAand tannic acids production (Zhang et al., 2017). In the second one, acorns are pretreated with EtOH, to remove phenolic substances, and liquefied before the *A. niger* CICC 2716 inoculum. The acorn pulverized use improves the CA production (Zhang et al., 2018). In the last patent, *A. niger* VKPM-722 synthesizes the CA with a conversion rate higher than 80%, using EtOH or a mix of EtOH and sucrose (Vinarov et al., 1998).

Considering the deepened study carried out on the traditional strains, the best operative conditions have been identified and further development could mainly focus on the innovative substrates. Therefore, the possibility of mutagenic strains use has started to gain a foothold in the market, since they allow significant CA synthesis improvements. Currently, the inventions are equally divided between primary and waste substrates, but the trend of patents with residue use is destinated to grow in a circular economy perspective.

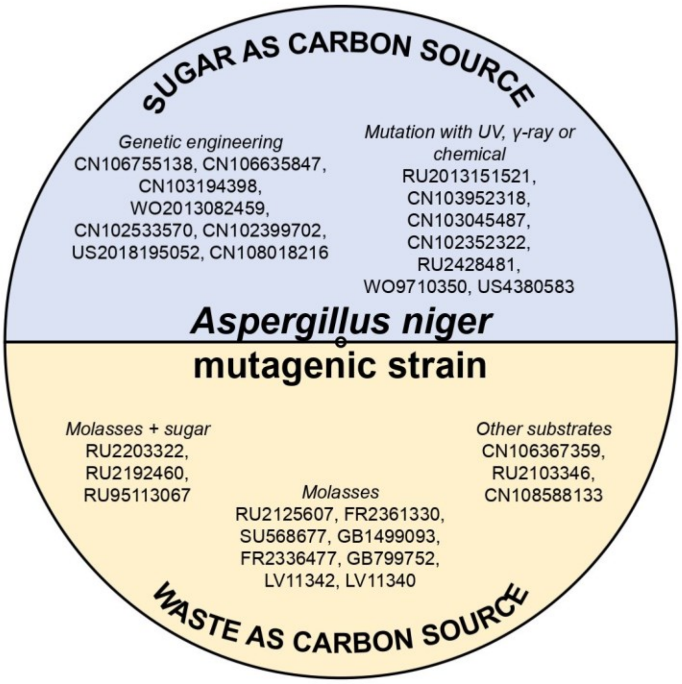


Figure 6.2. Patents related to the use of *A. niger* mutagenic strain with different substrates.

## 5. Discussion and perspectives

Figure 6.3 shows a whole CA production process which summarizes the treatments described in the present review, involving *A. niger*. As reported in the supporting materials (Table S6.1), the technological innovation literature describes the further use of other fungi (Anastassiadis et al., 1994; Company, 1975; Fujimaki et al., 1982; Hitachi Chemical CO LTD, 1975; INST Francais du Petrole Rech et Dacitivites Petroliere, 1976; Kinoshita et al., 1961; Leavitt, 1979; Matsumoto et al., 1984; Merck & CO INC, 1946; Okumura et al., 1970; Pfizer LTD, 1975; Rech et Dactivites Petrolieres, 1977; Tanaka and Kimura, 1972; Usines de Melie, 1958; “Werkwijze voor het langs microbiologische weg bereiden van citroenzuur,” 1972). Nevertheless, the related patents are mainly concentrated in the 70s and the interest has waned for the achieved low efficiencies; for this reason, these techniques has not been discussed in the present review.

The first identified variable in the scheme (Figure 6.3A) is the possible pre-treatment, if food or agriculture waste is used as substrate for the fungal growth. This step could be required to remove metals and/or to make carbon source bio-available for the fungal metabolism. Currently, the industrial production mainly uses sugars from hydrolysis of plant starch. The following step includes the fermentation, irrespective of the selected conditions. The average conditions which promote the fungal metabolism, with high sugar conversion rate (around 95%) and a high acid concentration (100-150 g/L) include: sugar concentration 10-15% (w/v), pH 1.5-4.5, stirring speed 120-400 rpm, temperature 27°-35°C, air supply and nutrient salts addition (mainly 0.2% w/v of $(NH_4)_2SO_4$ and of $KH_2PO_4$ and 0.1% of $MgSO_4$) and/or metal addition (Mn, Fe and Zn). A final phase of recovery and purification closes the treatment.

As concern the technological evolution overview, the sharp increase of the number of patents in the last 20 years, confirms the strong interest for the acid bioproduction, especially in China. As showed in Figure 6.3B, there is an evident change in the invention origin, from European and US to Chinese, starting from 2000. The reason can be found in a modification of the trade routes, which currently identify China as one of the most important export-oriented country. In this regard, 824,000 tons were exported from China, principally to India (9%), Turkey (5.2%), Japan (5%), Mexico (5%) and Indonesia (3.9%), in 2015.  In the same year, 1,007 tons of CA was imported to China mainly from Germany (30%), Canada (18%), Japan (17%) and Austria (12%), with a whole cost of USD 5 million ("Jiangsu Guoxin Union Energy CO LTD," 2016).

Furthermore, considering the increase of the CA demand, China aimed at the production process improvement with an increase of its economic exportation load of about 10%, from 2015 to 2017. In this regard, Figure 6.4a attributes to China the 75% of the whole trade value connected with the CA exportation, followed by Netherland, USA and Germany (each one with a contribution around 3%). On the other hand, USA (12%), Germany (8%), Mexico (5%) and India (5%) represent the main importers in the world (Figure 6.4b). Whereas, the Chinese production independence is confirmed by an importation trade lower than 1% (United Nations International Trade Statistics Database, 2018).

Additional information about the main critical aspects addressed in the patents can be deduced from the trends in Figure 6.3B. In this regard, the current technological innovation, has focused on the identification of innovative substrates, mainly agriculture waste, in agreement with the scientific literature which tends to fulfill the circular economy rudiments (Singh et al., 2011).

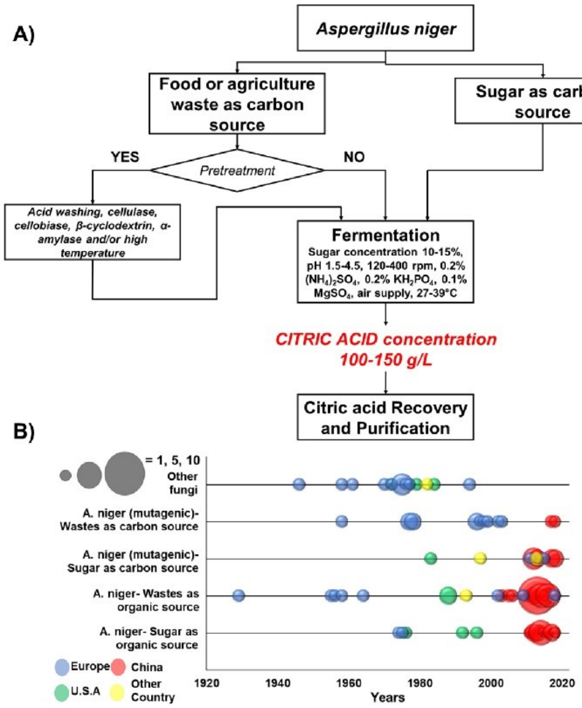


Figure 6.3. General scheme (A) and technological evolution (B) for citric acid production. The ball size is proportional to the available patents number.

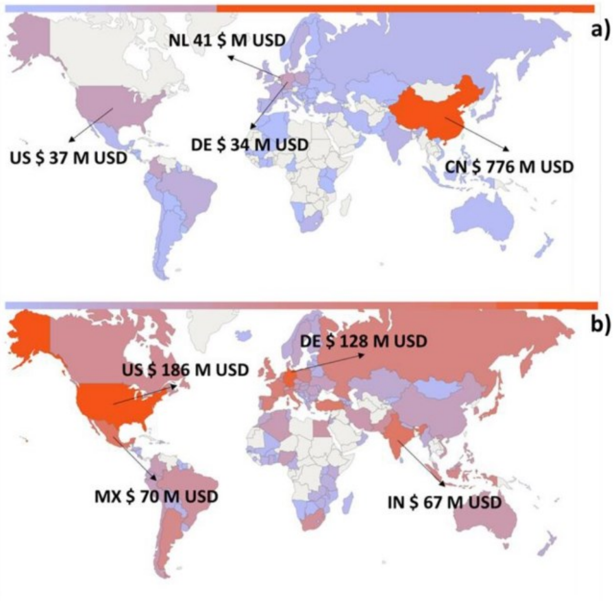


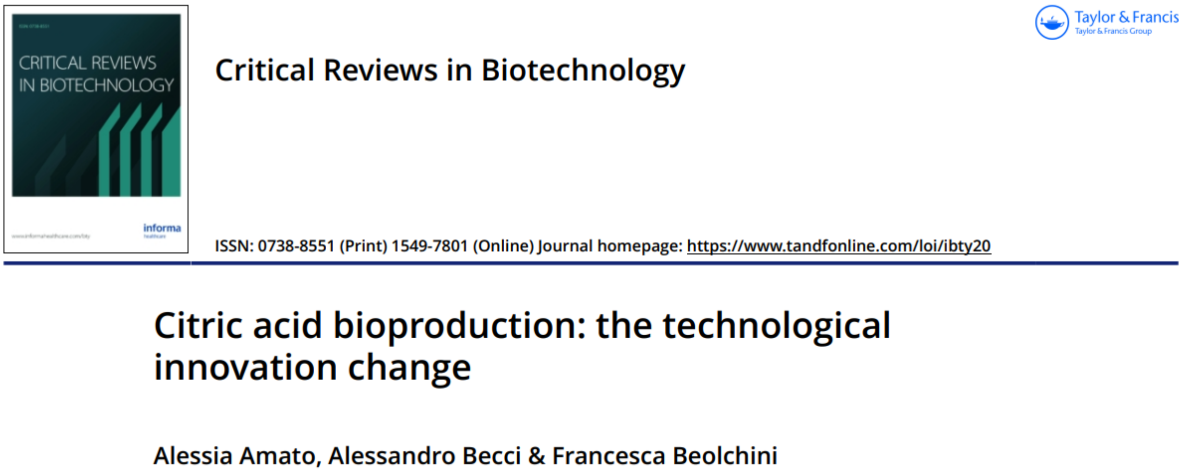
Figure 6.4. Worldwide economic flows related to the citric acid export (a) and import (b), in 2017 (evaluated by information of United Nations International Trade Statistics Database) (United Nations International Trade Statistics Database, 2018).

## 6. Conclusions

The present review proves the necessity of a technological innovation study to understand the real state-of-art in a specific field. A patented invention is designed for the implementation on a real scale and it should be commercially available. Furthermore, for the grant of a patent it is necessary to prove the actual novelty level, ensuring the innovation level of the review. Compared to the traditional literature, the invention overview helps with the market previsions thanks to the additional information related to the origin and the state of development. This focus on the patents about the CA production, over a long period of time, highlighted the growing interest for this topic and a technology progress, in agreement with the market demand. In this regard, a relevant increase of the waste substrate proves the development of the circular economy model. Further studies, with a similar approach, could deep the aspect of the fermentation design change. The scientific literature confirms as the research has moved towards the conversion of the most consolidated submerged process to the innovative solid-state fermentation (*koji* process). This simple and eco-sustainable technique combines the possibility of an agro-industrial waste use with a low energy demand and a minimum wastewater production. The choice of a limited in-depth of this topic is due to the low number of the related patents, probably connected with the current scale-up difficulties. This technique cannot be implemented in the bioreactors for submerged process and many criticalities are still due to the lack of standardized processes.

## 7. Products

Results illustrated and discussed in this chapter were used to support a peer reviewed article published on Critical Reviews in Biotechnology, Taylor & Francis (Figure 6.5).



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Citric acid bioproduction: the technological innovation change

Alessia Amato, Alessandro Becci & Francesca Beolchini

Figure 6.5. Articles published on *Critical Reviews in Biotechnology*.

## 8. Supplementary materials

Table S6.1: Patent list

| Cod. number | Title | Year |
|---|---|---|
| RU2676144 | Method for producing invertase and citric acid | 2018-12-27 |
| CN108588133 | Method for preparing citric acid through liquid state fermentation of acorns | 2018-09-28 |
| US2018195052 | Method for increasing citric acid production by *Aspergillus niger* fermentation | 2018-07-12 |
| CN108018216 | Method for increasing sugar utilization rate and yield of citric acid in citric acid fermentation and application | 2018-05-11 |
| CN107815475 | Method for producing citric acid fermentation liquor by two-stage fermentation | 2018-03-20 |
| CN107815421 | Method for seed culture of *Aspergillus niger* and preparing citric acid | 2018-03-20 |
| CN107022541 | Immobilization method of *Aspergillus niger* | 2017-08-08 |
| CN106868061 | Preparation method of citric acid | 2017-06-20 |
| CN106755138 | Method of increasing yield of citric acid produced by *Aspergillus niger* fermentation | 2017-05-31 |
| CN106635847 | Recombinant *Aspergillus niger* capable of improving yield of citric acid and preparation method of recombinant *Aspergillus niger* | 2017-05-10 |
| CN106367359 | *Aspergillus niger* and application thereof to preparing citric acid from fermented acorns | 2017-02-01 |

| | | |
|---|---|---|
| CN106119306 | Method for preparing citric acid from corncobs | 2016-11-16 |
| CN105671093 | Method for producing citric acid from kiwi fruit offcut | 2016-06-15 |
| CN105586366 | Method for improving fermentation performance of citric acid on basis of mycelium structure control | 2016-05-18 |
| CN105524951 | Method for preparing fermentation liquor of citric acid by utilizing extrudate obtained after low-temperature extrusion and enzymolysis | 2016-04-27 |
| CN105506004 | Method for producing citric acid by fermenting konjak powder residues | 2016-04-20 |
| CN104805136 | Method for producing citric acid by using lignocellulose raw material | 2015-07-29 |
| RU2013151521 | Strain *Aspergillus niger*-producer of citric acid | 2015-05-27 |
| CN104277978 | *Aspergillus niger* seed liquid preparation method and citric acid fermentation preparation method | 2015-01-14 |
| CN104232699 | Method for fermentation production of citric acid | 2014-12-24 |
| CN104099253 | Citric acid *Aspergillus niger* seed continuous culture method based on mycelium pellet dispersion technology | 2014-10-15 |
| CN104087624 | Method for producing citric acid by continuous fermentation of *Aspergillus niger* | 2014-10-08 |
| CN103952318 | Citric acid high-yielding *Aspergillus niger* FY2013 and application thereof | 2014-07-30 |
| CN103710397 | Technique for preparing citric acid by fermenting beta-cyclodextrin mother solution | 2014-04-09 |
| CN103695319 | Bacterial strain for producing citric acid and method for preparing citric acid by fermenting same | 2014-04-02 |
| CN103667372 | Method for preparing bran starter for citric acid by liquid inoculation | 2014-03-26 |
| CN103614421 | Method for producing citric acid by fermenting soybean molasses | 2014-03-05 |
| CN103497977 | Method for preparing citric acid by fermentation | 2014-01-08 |
| CN103290070 | Method for producing citric acid through continuous batch feeding fermentation | 2013-09-11 |
| CN103194398 | Citric-acid high-yield strain and screening method thereof | 2013-07-10 |
| CN103146769 | Method for preparating citric acid by fermentation | 2013-06-12 |
| WO2013082459 | Enhanced citric acid production in *Aspergillus* | 2013-06-06 |
| CN103045487 | Bacterial strain for producing citric acid and method for fermenting and producing critic acid through fermentation of bacterial strain | 2013-04-17 |
| CN103045659 | Method for detoxification in production of citric acid by utilizing cassava fermentation | 2013-04-17 |
| CN102952830 | Method for preparing citric acid through fermenting cheap dried sweet potatoes by utilizing *Aspergillus niger* | 2013-03-06 |
| CN102864183 | Method for preparing citric acid by fermenting cassava raw material residue-removed clear solution with *Aspergillus niger* | 2013-01-09 |
| CN102864185 | Method for producing citric acid by fermenting sorghum powder | 2013-01-09 |
| CN102864182 | Method for preparing citric acid by fermenting corn starch by using *Aspergillus niger* | 2013-01-09 |
| CN102864184 | Method for producing citric acid by utilizing immobilized *Aspergillus niger* | 2013-01-09 |

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|---|---|---|
| CN102851329 | Method for preparing citric acid through fermenting puffed dried sweet potato raw material by *Aspergillus niger* | 2013-01-02 |
| CN102851328 | Method for preparing citric acid through fermenting corn sugar solution by immobilized *Aspergillus niger* | 2013-01-02 |
| CN102851330 | Method for preparing citric acid through fermentation | 2013-01-02 |
| CN102839203 | Enzymolysis method of starch raw material and method for preparing citric acid by fermenting | 2012-12-26 |
| CN102649971 | Method for cultivating *Aspergillus niger* mouldy bran and method for preparing citric acid through fermentation | 2012-08-29 |
| CN102533570 | *Aspergillus niger*, application of *Aspergillus niger* and method for preparing citric acid by fermentation | 2012-07-04 |
| CN102443611 | Production method of citric acid | 2012-05-09 |
| CN102399702 | *Aspergillus niger* and application thereof as well as citric acid preparation method through fermentation | 2012-04-04 |
| CN102373242 | Citric acid preparation method | 2012-03-14 |
| CN102352322 | High-temperature-resistant citric acid producing strain | 2012-02-15 |
| CN102181490 | Method for producing citric acid | 2011-09-14 |
| RU2428481 | Citric acid production method | 2011-09-10 |
| CN101942487 | Method for fermenting and preparing citric acid by adding saccharifying enzyme | 2011-01-12 |
| CN101555497 | Preparation method of citric acid fermentation solution | 2009-10-14 |
| RU2007125728 | Method for preparation of citric acid, α-amylase and glucoamylase | 2009-01-20 |
| CN1884563 | Method for fermenting and producing citric acid using steam-explosion straw as raw material | 2006-12-27 |
| CN1693470 | New method of producing citric acid using paddy fermentation | 2005-11-09 |
| CN1415755 | Method for producing citric acid by fermenting paddy rice | 2003-05-07 |
| RU2203322 | Diploid strain of *Aspergillus niger* as producer of citric acid | 2003-04-27 |
| RU2192460 | Strain of fungus *Aspergillus niger* as producer of citric acid | 2002-11-10 |
| RU2186850 | Method of citric acid production | 2002-08-10 |
| RU2125607 | Continuous method of producing citric acid and its salts | 1999-01-27 |
| RU2103346 | Fungus strain *Aspergillus niger* - A producer of citric acid | 1998-01-27 |
| RU95113067 | Strain of fungus *Aspergillus niger* F-713 - A producer of citric acid | 1997-06-20 |
| WO9710350 | Process, apparatus and microorganism strain for the manufacture of citric acid | 1997-03-20 |
| LV11342 | A method for preparing of citric acid producer *Aspergillus niger* action of combustible materials especially of industrial and household waste | 1996-10-20 |
| LV11340 | Microscopical fungus *Aspergillus niger* R-5 as citric acid producer | 1996-10-20 |
| US5532148 | Process for producing of citric acid and monovalent citrate salts | 1996-07-02 |

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|---|---|---|
| DE4407441 | Fermentation process for the continuous production of citric acid | 1994-09-15 |
| KR930001261 | Process for preparing citric acid by tangerin peelings | 1993-02-22 |
| US5081025 | Process for the fermentative production of citric acid from carbohydrates | 1992-01-14 |
| US4791058 | Grape pomace as substrate for microbial production of citric acid | 1988-12-13 |
| US4767705 | Apple pomace as substrate for microbial production of citric acid | 1988-08-30 |
| US4424274 | Process for producing citric acid from hydrocarbons by fermentation | 1984-01-03 |
| US4380583 | Method of preparing seeding material for production of citric acid | 1983-04-19 |
| JPS5779890 | Method for suppressing production of isocitric acid in citric acid fermentation of petroleum hydrocarbon | 1982-05-19 |
| US4178211 | Process for producing citric acid | 1979-12-11 |
| FR2361330 | Citric acid preparation by fermentation - using *Aspergillus niger* R-1 strain on nutrient medium containing molasses | 1978-03-10 |
| GB1499093 | Method of preparing citric acid | 1978-01-25 |
| SU568677 | *Aspergillus niger* P-1 strain as producer of citric acid | 1977-08-15 |
| FR2336477 | Citric acid production by fermentation of sugar beet or date molasses - using *Aspergillus niger* of Mucor piriformis sp. organism | 1977-07-22 |
| GB1464334 | Process for the production of citric and isocitric acids by a culture of a diploid *Candida lipolytica* yeast | 1977-02-09 |
| GB1428440 | Fermentative production of citric acid | 1976-03-17 |
| US3936352 | Process for the production of citric acid | 1976-02-03 |
| GB1418561 | Citric acid production | 1975-12-24 |
| GB1418511 | Process for the production of citric acid and salts thereof | 1975-12-24 |
| GB1392942 | Process for the production of citric acid by submerged fermentation | 1975-05-07 |
| GB1380938 | Process for producing citric acid by fermentation | 1975-01-15 |
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# Chapter 7: METAL EXTRACTION FROM ELECTRONIC WASTE USING *A. niger* AND PRODUCTION OF ORGANIC ACIDS THROUGH FUNGI WASTE GROWTH

## Abstract

The growing production and use of electric and electronic components have led to higher rates of metal consumption and waste generation. To solve this double criticality, the old linear management method (in which a product becomes waste to dispose), has evolved towards a circular approach. Printed circuit boards (PCBs) are the brains of many electronic devices. At the end of their life, this equipment represents a valuable scrap for the content of base metals such as Cu and Zn (25 and 2% wt, respectively) and precious metals such as Au, Ag, and Pd (250, 1000, and 110 ppm, respectively). Recently, biotechnological approaches have gained increasing prominence in PCB exploitation since they can be more cost-efficient and environmentally friendly than the chemical techniques. In this context, the present paper describes a sustainable process which uses the fungal strain *Aspergillus niger* for Cu and Zn extraction from PCBs. The best conditions identified were PCB addition after 14 days, $Fe^{3+}$ as oxidant agent, and a pulp density of 2.5% (w/v). Extraction efficiencies of 60% and 40% for Cu and Zn, respectively, were achieved after 21 days of fermentation. The ecodesign of the process was further enhanced by using milk whey as substrate for the fungal growth and the consequent citric acid production, which was selected as a bioleaching agent.

## Content

## 1. Introduction

In recent years, the production of electrical and electronic equipment (EEE) has substantially increased with the development of science and technology (Arshadi and Mousavi, 2015a; Baldé et al., 2017). At the same time, the average lifetime of electronic products has also been drastically reduced (to around 2 years), resulting in a massive generation of waste from electrical and electronic equipment (WEEE) (around 44.7 million tons in 2016) (Jadhav, U. Su, 2015; Kaya, 2019). Printed circuit boards (PCBs) represent about 3–5% of the total WEEE collected every year (Jadhav et al., 2016). They are composed of metals (around 40 wt %), ceramics (around 30 wt %), and plastics (about 30 wt %) (Evangelopoulos et al., 2019; Jadhav, U. Su, 2015; Rocchetti et al., 2018; Vermeşan et al., 2020; Zhang and Xu, 2016). The metal fraction includes 20% Cu, 5% Al, 1% Ni, 1.5% Pb, 2% Zn, and 3% Sn (w/w) (Baldé et al., 2017; Birloaga and Vegliò, 2016; Faraji et al., 2018; Kaya, 2019). The presence of considerable amounts of metals represents a very critical issue for their possible release into the environment in the case of incorrect management (Xia et al., 2018). Nevertheless, this aspect also represents an opportunity for a transition towards a circular approach following the principle "resource–product–regenerated resource", where the waste is converted into a resource (urban mining) (D'Adamo et al., 2019; Ljunggren Söderman and André, 2019). The most conventional options used by industries to extract metals from PCBs are pyrometallurgy and hydrometallurgy (Kim et al., 2016; Rocchetti et al., 2018). Pyrometallurgical approaches produce pollutant emissions (dioxins and furans), and they usually involve high operation costs. Hydrometallurgy is a low energy-cost process which needs large amounts of chemical agents. Alternatively, biohydrometallurgy is often simple, environmentally friendly, and economical, responding to the sustainability principles essential for the development of a circular economy (Beolchini et al., 2012; Jadhav and Hocheng, 2014; Kim et al., 2016).

Several studies have described metal extraction from PCBs using bacteria, mainly *Leptospirillum ferrooxidans*, *Acidithiobacillus thiooxidans*, *A. ferrooxidans,* and *Sulfobacillus thermosulfidooxidans*, principally for the recovery of Cu and other metals such as Zn, Sn, Pb, and Ni (Arshadi and Mousavi, 2014; Becci et al., 2020; Brandl et al., 2001; Gu et al., 2014; Hong and Valix, 2014; Ilyas et al., 2013; Işildar Arda, Jack van de Vossenberg, Eldon R. Rene, 2016; Lee and Dhar, 2012; Liang et al., 2013; Priya and Hait, 2018; Rodrigues et al., 2015; Wang et al., 2009; Yang et al., 2009); *Chromobacterium violaceum*, *Pseudomonas fluorescens,* and *Bacillus megaterium* have also been used for Au recovery (Arshadi and Mousavi, 2015b; Chi et al., 2011; Pham and Ting, 2009; Pradhan and Kumar, 2012; Tay et al., 2013). On the other hand, fungal bioleaching has several advantages since fungi show a greater ability to tolerate toxic materials, a faster leaching action than bacteria, and the ability to grow in both alkaline and acidic mediums (Horeh et al., 2016). *Penicillium simplissimum*, *P. chrysogenum,* and *Aspergillus niger* are the most common eukaryotic microorganisms used for metal leaching from different solid residues such as electronic scraps (Brandl et al., 2001; Faraji et al., 2018; Horeh et al., 2016; Kim et al., 2016), contaminated soil (Ren et al., 2009), spent catalyst (Aung and Ting, 2005; Santhiya and Ting, 2006, 2005), flay ash (Rasoulnia et al., 2016; Rasoulnia and Mousavi, 2016; Xu et al., 2014), and red mud (Qu et al., 2013; Vakilchap et al., 2016). Citric, oxalic, and gluconic acids are the organic acids produced in the highest quantities by *A. niger* and are used for waste exploitation (Amato et al., 2020a; Magnuson and Lasure, 2004; Ren et al., 2009). In detail, bioleaching with *A. niger* uses PCBs (Brandl et al., 2001; Faraji et al., 2018) or batteries (Horeh et al., 2016; Kim et al., 2016) as substrate for metal extractions. High leaching efficiencies of 60% and 100% for Cu and Zn, respectively, were achieved after 21 days of fermentation with low pulp density of 0.1-0.5% (w/v).

Considering the current end-of-life PCB availability and the relevant content of Cu and Zn, the present paper aims to improve the sustainability of the process compared to the current state of the art. The possibility to increase the quantity of treated PCBs makes the treatment more attractive for stakeholders and suitable for industrial scale-up. Many conditions were investigated, including the possible inclusion of food industry waste as fungal growth substrate to improve the environmental sustainability of the treatment.

## 2. Materials and Methods

### *2.1. Preparation of Waste Printed Circuit Boards (PCBs)*

PCBs used in this paper were obtained from computer devices. They were shredded by stainless steel blades and pliers after manually removing the main parts of electronic components (e.g., capacitors, batteries, and resistors). Finally, the residue was crushed to obtain a granulometry smaller than 0.5 mm, suitable for the bioleaching experiments. The metal fraction was separated from the plastic and flame retardants by density and the PCB powder was washed with NaCl-saturated water. The resulting PCBs had mean metal concentrations of 25% Cu and 2% Zn.

### *2.2. Microorganisms and Inoculum*

Fungal microorganisms, classified as *A. niger*, were isolated in the laboratory from environmental samples. The inoculation of fungi was carried out inside sterile Petri dishes with a diameter of 100 mm in YPD broth (10 g/L yeast extract (Y), 20 g/L peptone (P), and 20 g/L D-glucose (D)), where 1.5% agar was added. The medium, before being used, was stirred and heated to 60 °C to achieve a homogeneous amber color and subsequently autoclaved. Finally, 100 mg/L of antibiotic (rifampicin) was added. The inoculated plates were incubated at room temperature for about 7-10 days. One-milliliter aliquots of the prepared inoculums were inoculated to 100 mL of the glucose medium. The glucose medium was prepared with the following composition: solution A was composed of 2.5 g of $(NH_4)_2SO_4$, 0.25 g of $MgSO_4 \cdot 7H_2O$, and 0.025 g of $KH_2PO_4$ dissolved in 450 mL of distilled water; solution B was composed of 1 g of yeast in 50 mL of distilled water; solution C was composed of 150 g of D-glucose dissolved in 500 mL of distilled water; and solution D was composed of 1 g/L $ZnSO_4 \cdot 7H_2O$, 0.05 g/L $MnSO_4 \cdot H_2O$, and 0.1 g/L $FeSO_4 \cdot 7H_2O$. The solutions A, B, and C were mixed and autoclaved, and 1 mL of the solution D was added to the resulting solution. The pH of solution was adjusted to 6.5 in the first day and readjusted to pH 3 during the bioleaching experiments. The bioleaching tests were carried out in 250 mL Erlenmeyer flasks which were incubated at 30 °C and shaken at 120 rpm. Each treatment was performed in duplicate. The pH was recorded by a pH meter inoLab Multi 720 (WTW).

### 2.3. *Bioleaching Experiments*

The bioleaching processes were conducted to verify the effect of two factors: the PCB addition at different fermentation times and the addition of two oxidant agents ($Fe^{3+}$ or $Mn^{7+}$). The first factor was monitored by adding PCBs at three different times: at the beginning, after 7 days, or after 14 days of fermentation. The bioleaching process was carried out for 7 days after the PCB addition. In the case of PCB addition at the beginning, the longest time of 14 days allowed the fungal growth and acid production. The pH of medium was continuously monitored and readjusted to 3 using a 2M NaOH solution. The second factor (the oxidant agent) was tested by adding $Fe^{3+}$ (40.67 g/L of $Fe_2(SO_4)_3$) or $Mn^{7+}$ (6.14 g/L of $KMnO_4$) simultaneously with the PCB addition at all tested conditions. The $Fe^{3+}$ and $Mn^{7+}$

amounts were determined by stoichiometric ratio with Cu, following Esq. 7.1 and 7.2 (Becci et al., 2019; Cui and Zhang, 2008; Zhang and Xu, 2016):

$$Cu^0 + 2\ Fe^{3+} \rightarrow Cu^{2+} + 2\ Fe^{2+} \quad (7.1)$$

$$5\ Cu^0 + 2\ Mn^{7+} \rightarrow 5\ Cu^{2+} + 2\ Mn^{2+} \quad (7.2)$$

The PCB concentration in bioleaching experiments was 2.5% (w/v). At regular time intervals, both citric acid production (2, 7, 14, and 21 days) and metal concentration (2, 4, 7, 10, 14, 16, 18, and 21 days) were monitored.

A chemical control test was carried out at the same bioleaching conditions to confirm the effect of the citric acid with or without oxidant agents. The operative conditions were PCB concentration 2.5% (w/v), pH 3.0, 30 °C, and 7 days. The citric acid concentration chosen was the same as that of the organic acid produced by *A. niger* in the bioleaching experiments (15 g/L), and the same amounts of $Fe^{3+}$ and $Mn^{7+}$ were used.

Additional tests were carried out to test the possibility of producing citric acid using an alternative carbon source to reduce the environmental load due to the glucose consumption for the fungal growth. Two kinds of agriculture and food residues were used, olive wastewater and milk whey (a cheese production residue). Both kinds of waste were used without or with an ozonation pretreatment (30 min and a flux of 7 $gO_3/L\cdot h$). The choice of these residues was due to their high COD content (around 150 g COD/L). Furthermore, these waste flows represent a relevant management problem in the Mediterranean area (Boukhoubza et al., 2009; Hande Gursoy-Haksevenler and Arslan-Alaton, 2014). Their use allows for the solution of a double problem by decreasing the consumption of raw materials and the reducing the amount of food waste disposed, in agreement with the circular economy pillars.

### *2.4. Analytical Determination*

The concentrations of Fe ($Fe^{3+}$ and $Fe^{2+}$), $Mn^{2+}$, $Zn^{2+}$, and $Cu^{2+}$ were periodically analyzed in the leaching solutions. The concentrations of Mn, Zn, and Cu were measured by an atomic absorption spectrophotometer (Techcomp, AA6000). On the other hand, the quantification of the Fe content was performed by a UV/VIS spectrophotometer by the colorimetric thiocyanate method (Jasco Model 7850). The total Fe concentration was determined by oxidizing $Fe^{2+}$ to $Fe^{3+}$ with potassium permanganate, and consequently the $Fe^{2+}$ concentration was calculated as the difference between total Fe and $Fe^{3+}$ concentrations. The concentration of citric acid produced by *A. niger* in the medium was quantified by the Water HPLC instrument.

### *2.5. Statistical Analysis*

In order to verify the effect of oxidant agents ($Fe^{3+}$ or $Mn^{7+}$) and the best time for both PCB and oxidant agent addition (at the beginning, after 7 days of fermentation, or after 14 days of fermentation), a two-way analysis of variance (ANOVA) was carried out. When significant differences were observed, an SNK post hoc comparison test ($\alpha = 0.05$) was also performed. An additional statistical analysis was also conducted aimed at confirming the leaching role of both citric acid and oxidant agent.

## 3. Results

### *3.1. Bioleaching Experiments*

Figure 7.1 shows the Cu and Zn leaching profiles in the bioleaching tests with PCBs. These results demonstrated that the leaching efficiency for both Cu and Zn increased when the PCBs and oxidant agent were added 14 days after the beginning of fermentation. This was achieved thanks to the highest citric acid production by *A. niger* when PCBs/oxidant agent were added at the end of fungal metabolism (Figure 7.2). In detail, the citric acid reached the concentration of 13.8 ± 4.5 g/L at these conditions, even though 0.033 ± 0.002 and 1.6 ± 0.7 g/L of citric acid were produced when PCBs and oxidant agent were added at the beginning of fermentation and after 7 days after the start of fermentation starting, respectively. The lowest citric acid production by *A. niger* was due to both the metal toxicity and the PCB inhibition on fungal metabolism due to the high substrate concentration (Brandl et al., 2001; Faraji et al., 2018; Jadhav, U. Su, 2016).

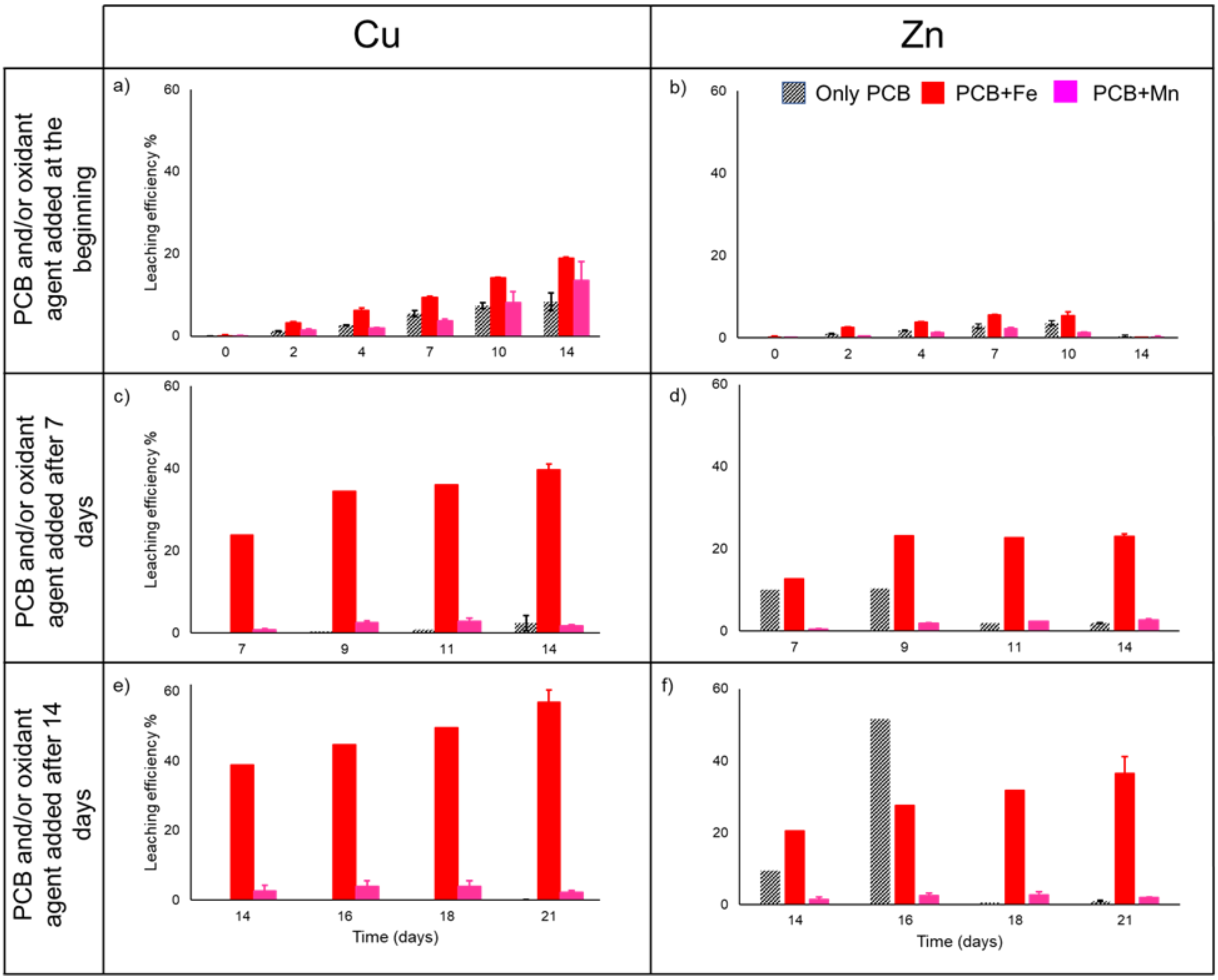


Figure 7.1. Cu (a, c, e) and Zn (b, d, f) leaching efficiency time profile in the bioleaching tests with *A. niger* and with the printed circuit boards (PCBs) and/or oxidant agent (ferric iron or potassium permanganate) added at the beginning (a, b), after 7 days (c, d), or after 14 days (e, f) of the fermentation period.

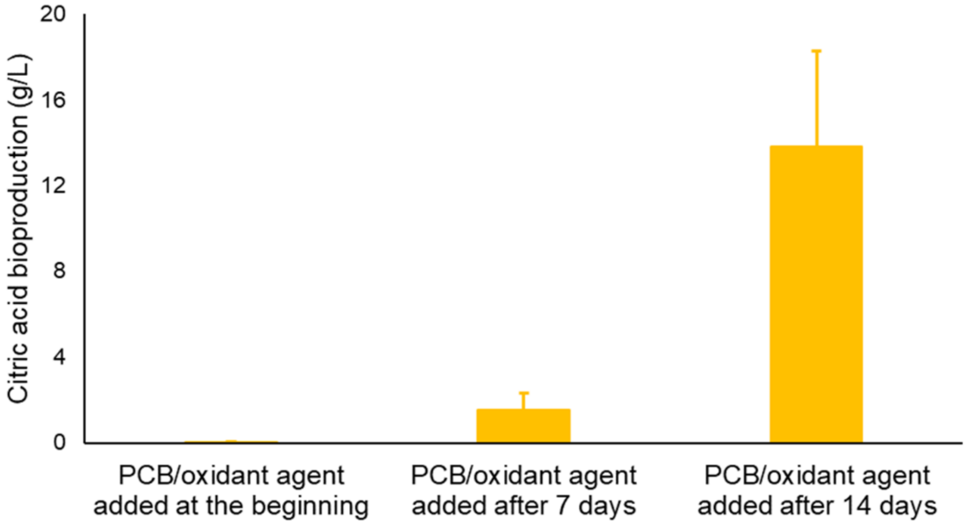


Figure 7.2. Citric acid concentration in the bioleaching experiments with *A. niger*.

The results proved the positive effect of $Fe^{3+}$ as an oxidant agent, with an increase of both Cu and Zn leaching (Figure 7.1). On the other hand, the $Mn^{7+}$ were not relevant at all to the tested conditions. In further detail, Cu leaching rose from 19.0 ± 0.2% when $Fe^{3+}$ was added at the fermentation beginning to 57.0 ± 3.0% when it was added after 14 days. The same trend was observed for Zn leaching efficiency, from 5.6 ± 0.1% to 36.5 ± 4.8%. The highest recovery efficiency of PCBs and $Fe^{3+}$ added after 14 days was explained by the highest citric acid concentration, which increased the Fe dissolution from 10.6 ± 2.6% to 60.5 ± 5.3% (Figure 7.3). Moreover, the Fe speciation demonstrated that the total dissolved Fe reacted with PCB powder at the highest citric acid concentration (Choi et al., 2004). Therefore, at the end of the experiment, Fe was completely in the reduced form ($Fe^{2+}$) due to the reaction with Cu and Zn (Eq. 7.1). In the other tested conditions, around 50% of the dissolved Fe reacted with PCBs to leach metals. The statistical analysis (ANOVA) confirmed the positive effects of both the PCB and the oxidative agent ($Fe^{3+}$) addition after 14 days with a P value lower than 0.05. The $Fe^{3+}$ use produced an additional advantage. In the tests without $Fe^{3+}$, the Zn leaching efficiency after 2 days decreased from 2.8 ± 0.5%, 10.3 ± 0.7%, and 51.5 ±

0.5% to around 0% when PCBs were added at the beginning, after 7 days, or after 14 days of the fermentation period, respectively. Fe created a stable complex able to prevent the Zn precipitation in oxalate form (Amato et al., 2020b; De Michelis et al., 2007); this was due to the oxalic acid present as a by-product of citric acid synthesis by *A. niger* metabolism (Horeh et al., 2016; Magnuson and Lasure, 2004).

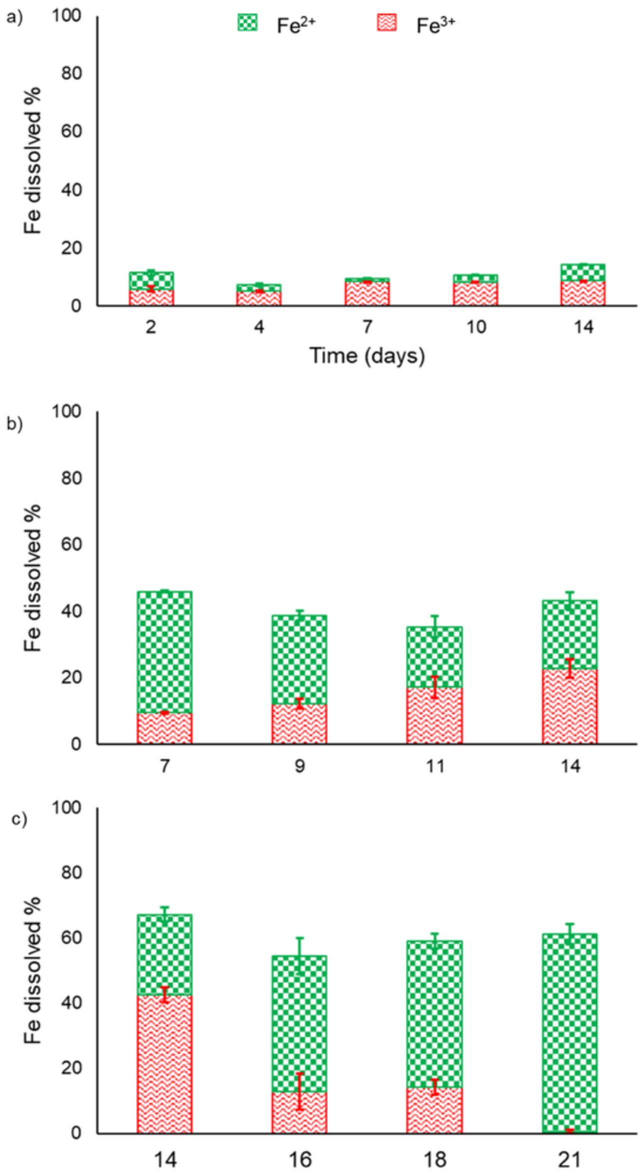


Figure 7.3. Fe dissolution and speciation in the bioleaching experiments: PCBs and $Fe^{3+}$ added at the beginning (a), after 7 days (b), or after 14 days (c) of the fermentation period.

### 3.2. Chemical Controls and Statistical Analysis

To verify that both the Cu and Zn extractions were due to the citric acid produced by *A. niger* and to the oxidant agent added ($Fe^{3+}$ or $Mn^{7+}$), chemical controls were carried out reproducing the bioleaching conditions. The results in Figure 7.4 confirmed the positive effect of $Fe^{3+}$ addition with final yields of 59.4 ± 0.9% and 24.6 ± 5.0% for Cu and Zn, respectively. The statistical analysis, carried out to compare the chemical results with the corresponding bioleaching ones, demonstrated that the results were not statistically different, with a P value higher than 0.05 for both the metal targets (Table 7.1). These results confirmed that the Cu and Zn leaching from PCBs were due to the concurrent effects of citric acid and oxidant agent and excluded the possible effect of glucose or other organic acids (such as oxalic or gluconic acid) produced by *A. niger*.

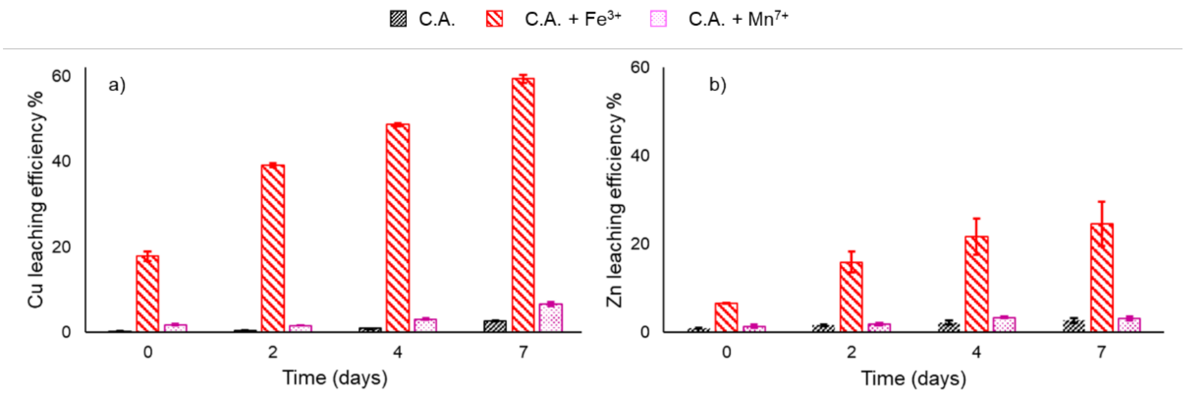


Figure 7.4. Cu (a) and Zn (b) leaching efficiency in the chemical control carried out with citric acid (C.A.) and oxidant agents ($Fe^{3+}$ or $Mn^{7+}$).

Table 7.1. The variance analysis comparing the chemical controls with the respective bioleaching experiments.

| Treatment | df | Statistical analysis (ANOVA) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | | | Zn | | |
| | | MS | F | P | MS | F | P |
| C.A. | 1 | 2.98 | 3.74 | 0.19 | 2.06 | 50.75 | 0.05 |
| C.A.+$Fe^{3+}$ | 1 | 5.74 | 0.46 | 0.57 | 140.6 | 2.98 | 0.23 |
| C.A.+$Mn^{7+}$ | 1 | 0.58 | 0.33 | 0.62 | 1.48 | 8.74 | 0.10 |

### 3.3. Citric Acid Production using Alternative Carbon Sources

When food wastes (olive wastewater or milk whey) were used for the fungal growth, only 0.13 ± 0.01 g/L of citric acid was produced (Figure 7.5). The main problems were the toxic effect of phenols on fungal metabolism in the case of olive wastewater (Andreozzi et al., 1998; Hamdi et al., 1991; Öngen et al., 2007) and the low availability of lactose as a carbon source for fungal metabolism in the milk whey experiment (Dhillon et al., 2011; Murad and Khalaf, 2003; Rivas et al., 2008; Santoro et al., 1999; Xu et al., 1989). The additional pretreatment by ozonation allowed reducing the concentration of phenols and decomposing lactose in a more available saccharide such as glucose, galactose, or fructose. After the pretreatment step, *A. niger* produced around 6.1 ± 0.1 and 13.7 ± 4.4 g/L of citric acid after 14 fermentation days with olive wastewater and milk whey, respectively. These quantities were enough to complete the leaching of the two metal targets. The decrease of the final COD concentration in both food wastes simplified the final sludge management.

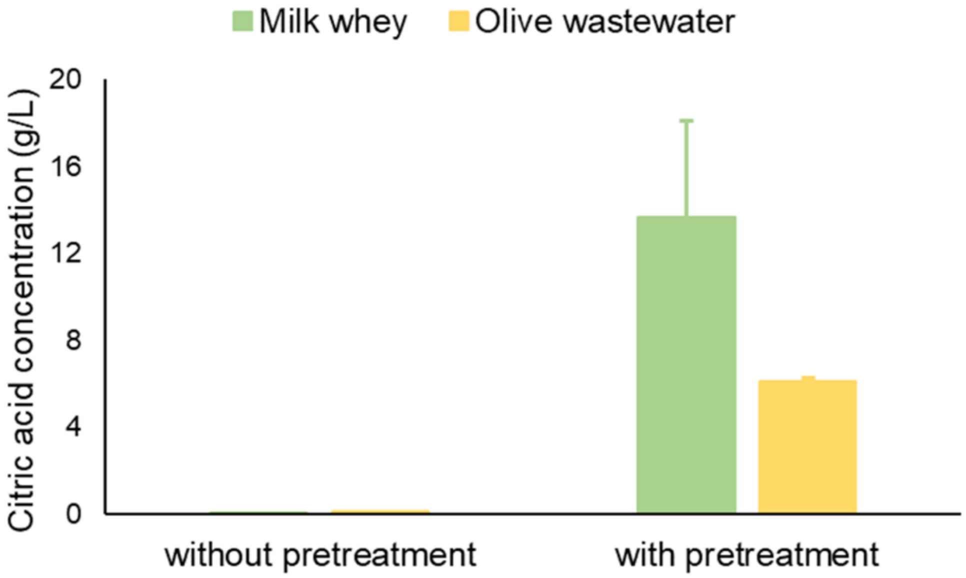


Figure 7.5. Citric acid produced by *A. niger* using agricultural and food wastes without and with pretreatment with ozonation after 14 days of fermentation.

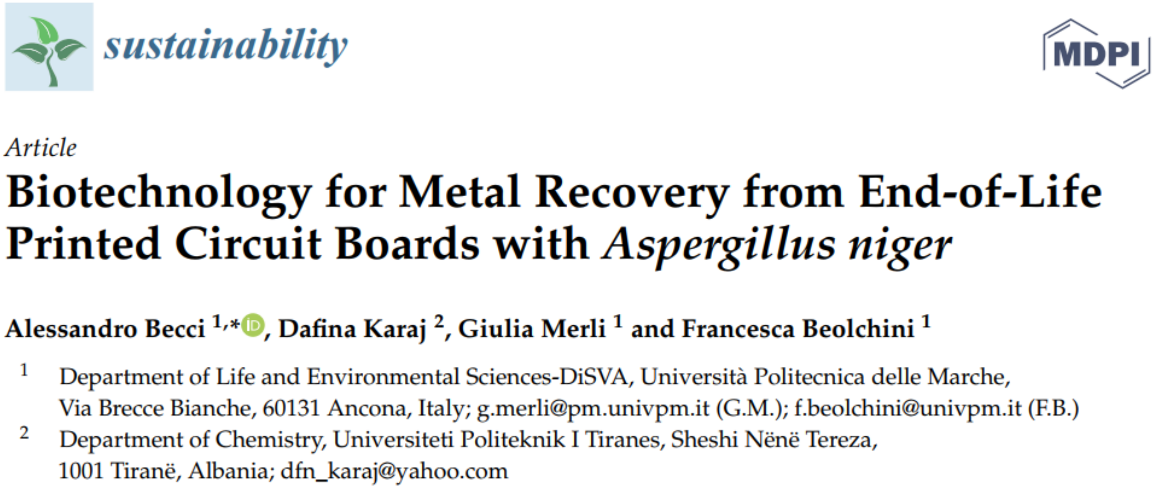
## 4. Discussion and Conclusions

This work proposed a bioleaching process for metal extraction from end-of-life PCBs with *A. niger*. The results prove the possibility to increase the treated PCB amount from 0.5% to 2.5% w/v without Cu efficiency decrease (Brandl et al., 2001; Faraji et al., 2018). The relevance of the proposed approach is confirmed by two main reasons: (i) Cu is the main metal of interest in PCBs, after precious metals (Au and Pd). The economic sustainability of its extraction from these scraps is also connected to its high concentration (Evangelopoulos et al., 2019; Kaya, 2019; Manjengwa, 2019). (ii) Cu is the main interferent in Au leaching; therefore, its previous extraction allows for a significant increase of both efficiency and purity in the Au recovery (Natarajan and Ting, 2015; Pradhan and Kumar, 2012; Tay et al., 2013). The present paper represents an example of success in the implementation of circular economy principles described by the New European Circular Economy Action Plan (European Commission, 2020). Indeed, PCBs are included within the list of key products in the documents (electronics and ICT). Furthermore, the biotechnological implementation allows the substitution of hazardous chemicals to protect citizens and the environment. The chance to give value to the waste (both PCBs and food waste for the citric acid production) pushes the market towards the creation of a secondary raw materials market, while avoiding export to non-European countries, in agreement with the modern circular policies. More comprehensively, the biotechnological approach using the *A. niger* strain allowed the exploitation of end-of-life PCBs at a low temperature, reducing the consumption of chemical agents. High efficiencies, around 60% and 40% for Cu and Zn, respectively, were achieved at the best selected conditions: addition of PCBs and $Fe^{3+}$ (oxidant agent) 14 days after the start of fermentation (when *A. niger* reached the exponential growth phase and produced the maximum amount of citric acid (around 15 g/L)), 30°C, 7 days leaching time, and 2.5%

(w/v) PCB concentration. A further recovery process allows for the new metal's placement on the market, while avoiding the depletion of raw materials. Moreover, the environmental sustainability of the treatment was enhanced by the use of food wastes (milk whey and olive wastewater) for *A. niger* metabolism and was able to replace the glucose from primary sources, solving the criticalities connected to their management.

## 5. Products

Results illustrated and discussed in this chapter were used to support a peer reviewed article published on Sustainability, MDPI (Figure 7.6).



sustainability

MDPI

*Article*

**Biotechnology for Metal Recovery from End-of-Life Printed Circuit Boards with *Aspergillus niger***

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Figure 7.6. Articles published on *Sustainability*.

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