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Innovative green technologies for the recovery of strategic metals from residues

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Content

Dissertation outline

1. Overview

Electronic waste (e-waste) is considering to be one of the fastest growing categories of waste of the last decade (Liu et al., 2016). In 2019 the global annual production of e-waste was 53.6 million metric tons (Mt) (Forti et al., 2020). Most of these waste come from Asia (25 Mt), followed by Americas (13.1 Mt) and Europe (12 Mt) (Figure 1) (Forti et al., 2020). China alone hold the highest average annual growth rate of e-waste generation, which in 2017 amounted to 5-10%, while in all the Europe was around 3-5% (Wu et al., 2017).

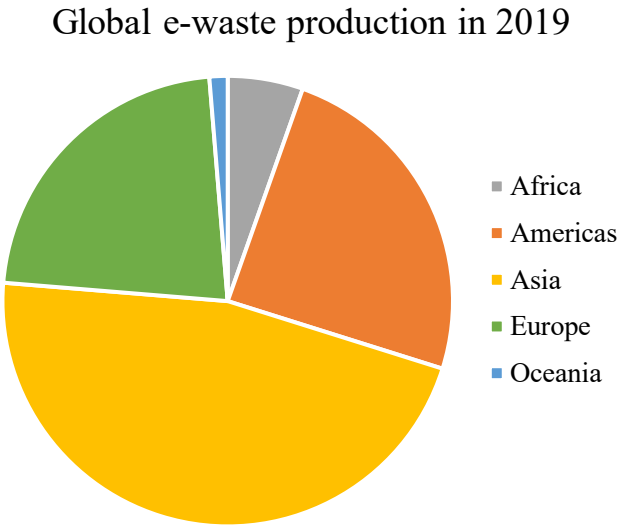

Figure 1. Global production of e-waste in 2019 calculated in million metric tons (Forti et al., 2020).

A higher increase than previously estimated is expected in the future as in the first 9 months of the 2020, due to COVID-19 pandemic, the consumption of game consoles, cell phones and laptop and electrical ovens increased in the high-income countries, adding 0.3 Mt to the consumption of electronic devices and hence, to the future e-waste generation (Baldé and Kuehr, 2021). On the other hand, the other products showed a decrease by 4.9 Mt, principally in the low- or middle-income countries. Despite this seems to be a positive data, as these regions have no or inadequate e-waste management infrastructures, this effect is supposed to be only temporary, and e-waste is still expected to grow in the coming years, up to 74 Mt by 2030 (Baldé and Kuehr, 2021).

The lifespan of electronic and electric equipment (WEEE) is shorter compared to the devices produced in the past (Wu et al., 2017), as technological innovation continually brings increasingly efficient and modern products to the market that make the electronic devices becoming redundant and obsolete rapidly (Liu et al., 2016).

The WEEE are made with valuable resources mixed with heavy metals and hazardous materials. Generally, metals in e-waste can be grouped into base metals (Cu, Al, Ni, Sn, Zn, and Fe), precious

metals (Au, Ag), platinum groups metals (Pd, Pt, Rh, Ir, and Ru), metals of concern/hazardous (Hg, Be, In, Pb, Cd, As, and Sb), and special metals/scarce elements (Te, Ga, Se, Ta, and Ge) (Kaya, 2019). The presence of heavy metals and hazardous materials makes them as potential environmental contaminants. However, the WEEE are considered as "urban mines" because they represent an important source of raw materials. The term urban mining describes the recovery of resources from waste material generated within urban environments and differs from the conventional mining first in the source of materials, for the recovery and for the appropriate mining process (Kaya, 2019).
The content of precious metals in the electronic devices average around 320 t of Au (11% of world Au production) and around 7500 t of Ag per year (Kaya, 2019). The recycling of e-waste, therefore, is becoming an increasingly important topic not only from the point of view of waste treatment, in order to make it more environmentally sustainable, but also for the recovery of precious materials (Cui and Zhang, 2008).
A huge criticality is the incorrect and unregulated disposal of the WEEE in some part of the word, especially in China, India and some areas of Africa, where primitive crude recycling methods such as dismantling without protection, open burning or dumping, are practised despite prohibited by domestic regulations (Kaya, 2019). The world attention is increasing towards an implementation of sustainable technologies for the WEEE recycling and for developing a proper metal and non-metals recovering technology suitable with environmental safety and human health protection.
One of the principal conventions related to the correct disposal of WEEE is the Basel Convention, entered in force in 1992. The key issues are the promotion of sustainable handling techniques at the place of disposal, to the disadvantage of the transboundary movements banned or strictly regulated, and the reduction of hazardous waste (Kaya, 2019). Between 2001 and 2016 the European countries, Japan and OECD countries adopted laws and directives related to the disposal of e-waste.

2. Waste printed circuit boards

Waste printed circuit boards (WPCBs) represent the most valuable part of the electronic industry and constitute the 5% of all the e-waste. WPCBs are particularly interesting from the point of view of the composition because they are made with a high metal content (around 40%, including precious metals, around 0.1%), mixed with plastics (30%) and ceramics (30%) (Cui and Zhang, 2008; Wu et al., 2017). Typically, PCBs are composed of 3 main layers, an upper and lower surface that are an assembly of woven fiberglass layers, solder joints, conductive tracks, adhered by epoxy resin and an internal substrate, composed by layers of woven fiberglass and Cu foils (Kaya, 2019).
Interestingly the purity and the abundance of metals are higher in the electronic residues compared to the natural ores, for example Au concentration in WPCBs is 70% times higher compared to the natural

minerals (Liu et al., 2016; Wu et al., 2017). WPCBs from mobile phone contains the highest content of precious metals, followed by WPCBs from personal computers, in 1 ton of laptops' WPCBs the precious metals content is around: 250 g Au, 1000 g Ag and 100 g Pd (Kaya, 2019). By contrast precious metals in the Earth crust are very rare, amount at around 0.004 ppm for Au, 0.07 ppm for Ag and 0.01 ppm for Pd (Kaya, 2019). Therefore, the recovery of metals from e-waste is fundamental and necessary for the conservation of resources since it delays the exhaustion of precious metals from mineral ores and also avoid the environmental problems of mining, especially as the demand for these metals continues to increase (Pham and Ting, 2009).

3. Precious metals recovery

The WEEE recycling process is classified in 4 principal steps:

- Collection and pre-sorting
- Pre-treatment (separation, dismantling, size reduction)
- Pre-processing (metal extraction)
- End processing (purification)

The collection takes place with collective municipal and commercial collection or by individual producer and retailers. The efficiency of the collection program depends on collection method and policy and social and societal factors (Kaya, 2019).

The pre-treatment step separate materials in different streams and remove hazardous components, in particular mechanical processes (shredding, grinding, gravity and magnetic separation) are utilized for the separation of the metallic components from WPCBs (Kaya, 2019).

The most common method for treating e-waste for metal recovery has always been pyrometallurgy, based on the utilization of high temperature above 2000°C, to control the chemical reactions and the smelting processes (Camelino et al., 2015; Cui and Zhang, 2008). To overcome the environmental problems caused by gas pollution, hydrometallurgical and bio-hydrometallurgical methods have been taken into consideration (Camelino et al., 2015). The last step, the purification and refining, is essential for selective recovery of metals (Kaya, 2019). Several methods for precious metals recovery such as activated carbon adsorption, cementation, solvent extraction, ion exchange resins and electrodeposition have been investigated and proposed (Cui and Zhang, 2008; Wu et al., 2017; Xu et al., 2017).

3.1. Hydrometallurgy

Hydrometallurgy is a technique in the field of extractive metallurgy that allows to obtain precious metals from minerals and from e-waste, performed by leaching the target metal using a variety of chemical reagents. Among them the most common solution for the leaching of precious metals is

cyanide, employed for over a century in the mining industry. Cyanide has long been used as the main leaching agent for its benefits, including the low labour cost and the consumption of a little dose of reagent due to the strong stability and selectivity of the cyanide-gold complex, which allows an excellent leaching even at low concentrations (Kulandaisamy et al., 2003; Zhang et al., 2012). The problem of cyanide is its toxicity. Further lixiviants and various substitutes have been proposed, such as thiourea, thiosulfate and halide (Breuer and Jeffrey, 2003; Petter et al., 2014). In particular, thiosulfate is considered a valid substitute of cyanide for the extraction of precious metals (Camelino et al., 2015; Jing-ying et al., 2012; Zhang et al., 2012). The dissolution of precious metals in the ammonia thiosulphate solution is an electrochemical reaction catalysed by the presence of cupric ions, which act as a catalyst, allowing the formation of a stable metal-thiosulfate complex (Cui and Zhang, 2008) The advantages of using thiosulfate rather than cyanide are high selectivity and the non-toxicity or corrosivity (Cui and Zhang, 2008; Zhang et al., 2012).

3.2. Biohydrometallurgy

Biohydrometallurgy is a process employing a natural ability of microorganisms to transform metals present in the ores from a solid form to a dissolved one (Willner and Fornalczyk, 2013). This process, also known as bioleaching or biomining, is an indirect process and involves the microbial production of metabolites that dissolve metals by the formation of soluble metallic complexes. Biohydrometallurgy is considered to be responsible of the 10-15% of the world's mined copper production and is widely used also for gold extraction, approximately 5% of Au is recovered through bioleaching (Kaksonen et al., 2018).

Precious metals bioleaching is carried out by cyanogenic bacteria, which produce cyanide by the oxidative decarboxylation of glycine using the HCN synthase enzyme, in a process known as biocyanidation (Brandl et al., 2008). The most employed bacteria for this process are *Chromobacterium violaceum*, *Bacillus megaterium* and different species of *Pseudomonas.* Cyanide generation is maximum when the microorganisms reach the logarithmic and early stationary phase of the growth cycle and decreases in the subsequent late stationary and decay phase (Habibi et al., 2020). It has been reported that some microorganisms can produce an enzyme, B-cyanoalanine synthase, which is responsible of cyanide degradation (Habibi et al., 2020; Işıldar et al., 2016).

The bioleaching process reduces the problems associated with the treatment of waste flows (rich in toxic cyanide) which characterize the most common chemical processes. Indeed, the non-reacted cyanide is used by bacteria metabolism avoiding its possible release in the environment.

3.3. Recovery

Cementation is a technique based on the precipitation of a metal by other more electropositive metals (Kaya, 2019). In particular for Au recovery, cementation employs pulverized materials, such as zinc,

to precipitate Au by a displacement reaction. The involved reactions, which occur at the surface of zinc, are a cathodic deposition of Au and an anodic corrosion of zinc (Cui & Zhang, 2008). The advantage of cementation process is that avoid contamination by other ions employing metals already present in the solution (Kaya, 2019). Solvent extraction is based on the utilization of an organic solvent to extract a substance from an immiscible phase and a number of extraction system, including organophosphorus derivatives, guanidine derivations amine oxides among the others, have been analyzed (Cui & Zhang, 2008; Grosse et al., 2003). The problem of these techniques is the low selectivity, as Cu ions in the solutions can also precipitate with these powders, competing with the recovery of the precious metals (Dong et al., 2017; Yu et al., 2018). Ion exchange is a reversible interchange of charged particles or ions with those of like charge. It is a very attractive technique, due to high adsorption and recovery rates, and desorption and regeneration can be conducted under ambient temperatures and pressures (Wu et al., 2017). Electrodeposition or electrowinning employs an electrode to generate a direct current in the solution containing precious metals, so that the precious metals can be reduced by the electrons from the current to metallic deposits on the cathode (Kaya, 2019). This method offers more purity and selectivity, but a set of unwanted secondary reactions can decrease the efficiency of the process (Wu et al., 2017; Xu et al., 2017). After all these processes the products are suitable for the market (Kaya, 2019).

4. Aim and objective of the PhD research activity

Given the global situation of e-waste production, the increase in electronic devices production and consumption, the risks, and the importance of a proper disposal of this kind of waste, the objective of this thesis was to explore, optimize and compare different processes for precious metals recovery from WPCBs.

The general aim was the development of high efficiency and low toxicity technology and biotechnology for precious metals recovery from end-of-life PCBs. Specific targets are:

- The identification of the best conditions for a complete Au, Ag and Pd extraction with thiosulfate and biogenic cyanide;
- Preliminary studies on precious metals recovery from thiosulfate solution;
- The environmental assessment of different strategies for WPCBs management.

The aim of this research is to find environmentally sustainable procedures and optimize processes to make them as suitable as possible for large-scale application. The objective is to balance low cost and toxicity with the highest process efficiency.

The following chapters are related to a single part of the work and can be read independently. For each chapter the specific scientific background and the related materials and methods are described.

Among the method to avoid cyanide and active carbons utilization for the recovery of precious metals in mining industry, the patent W02016/168930A1 claimed a mixture of acetic acid, hydrogen peroxide, sulfuric acid and salt for the extraction of Au and Pd (Foley et al., 2016). In this context, Chapter 1 evaluates the different techniques for acetic acid production, employing bacteria and alternative substrates, abundant and economic. In more details, it reviews the progress of the technological innovation of the last 30 years and evaluates the more environmentally friendly approaches, in view of a possible application of acetic acid as a biotechnology for Au recovery. Among the most evaluated biotechnologies for precious metals mobilization there are the cyanogenic bacteria and Chapter 2 studies the Ag bioleaching with *P. aeruginosa*. Cyanide production was optimized, and 90% of Ag was mobilized with the addition of 1 g/L of glycine. In addition, Au bioleaching was observed with an efficiency of 20% and so, in Chapter 3, Au bioleaching was evaluated in more details, together with Ag, with the small WPCBs size. Cyanide synthesis was improved and different strategies for precious metals recovery were performed.

Chapter 4 analyzes the utilization of chemical thiosulfate as a leaching agent for precious metals recovery to increase the selectivity of the process and reduce reagents consumption, with the aim of evaluating the possibility of producing it biologically. In particular, 3 different WPCBs sizes were tested. Chapter 5 focuses on preliminary studies for the last phase of the precious metals recycling process from a thiosulfate solution, in more details with the anion resins for metals absorption and concentration and the electrodeposition process for the final recovery of solid metals.

In Chapter 6 the carbon footprint of the bioleaching and the leaching with thiosulfate processes was evaluated with the life cycle assessment (LCA) tools. Furthermore, the combination of leaching processes in series (bio- and chemical leaching) was theoretically evaluated with the LCA analysis and the results were verified with experimental analysis.

5. Products

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

- Becci, A.; Karaj, D.; Merli, G.; Beolchini, F.; 2020. Biotechnology for metal recovery from end-of-life printed circuit boards with *Aspergillus niger*. *Sustainability* 12(16):6482. https://doi.org/10.3390/su12166482
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Chapter 1:
ACETIC ACID BIOPRODUCTION: THE TECHNOLOGICAL INNOVATION CHANGE

Abstract

Acetic acid is an organic acid of great importance globally and the demand of this product is currently increasing. The production of this acid has consequently aroused more and more interest over the years, especially for new and more sustainable processes. Feedstock for acetic acid synthesis are depleting, therefore the transition from chemical processes, based on the use of non-renewable sources, to biological processes is of fundamental importance. From a biological point of view, acetic acid can be produced by two different processes: acetogenesis using inorganic substrates like CO_2 or CO (with acetogenic bacteria) and aerobic fermentation (with acetic acid bacteria or fungi). In this review, several works of literature that consider the influence of operating conditions (T, pH, oxygenation) have been analysed for each process, with a particular focus on the use of innovative substrates such as synthesis gas or food waste. In addition, with the aim of investigating the progress of technological innovation in the last 30 years, the 1990-2020 international patents have also been considered, by using the Espacenet platform, that ensured a worldwide invention overview. These patents are mainly based on strategies to increase the concentration of acetic acid, which consider different methods of culture, innovative methodologies and, especially in recent years, the use of waste substrates or genetic engineering. These innovations will be significantly important for the environment by increasing the process sustainability and the annual yield of acetic acid and by lowering the production costs.

Content:

1. Introduction

Acetic acid is an important industrial feedstock, primarily produced from mineral oil or natural gas. Since oil, the most important acetic acid feedstock, is depleting, renewable resources should be taken into consideration to produce acetic acid (Li et al., 2015). Acetic acid is mainly produced by three chemical processes which employ mineral oil or natural gas: (a) *n*-butane oxidation, (b) methanol carbonylation and (c) acetaldehyde oxidation (Cheryan et al., 1997). The global goal is to develop an alternative way for acetic acid production: from non-renewable feedstock to renewable feedstock (biomass). Biological acetic acid production from bacteria has several advantages in terms of the lowest required costs and the highest specificity, furthermore bacteria possess the greatest resistance to poisoning and permit a minimum pollutants emission (Sim et al., 2007). In recent years, aerobic fermentation technology has also been studied to obtain acetic acid from the food waste treatment. The generation of high added value products from food waste will have decisive significance for the environment, not only in terms of reducing food waste, but it can also increase the annual yield of acetic acid, lowering the production cost (Li et al., 2015).

Although acetic acid is very important in the industrial sector, it is principally known for vinegar production. Today acetic acid is widely employed to produce vinyl acetate, which is then utilized for vinyl plastic, adhesives, textile finishes and latex paints, a market that is growing rapidly due to the demand for synthetic fibers. In 1979, calcium magnesium acetate (CMA) was identified as a non-corrosive alternative to chloride salts for defrosting the streets. Street salt consumption is around 10-12 million tons per year only in the United States and CMA in solid form, more sustainable, could be a viable alternative (Cheryan et al., 1997). Liquid potassium acetate is used as a deicer for airport runways and as a heat exchange fluid, partially replacing ethylene glycol. In addition, it has been reported that CMA or calcium acetate can be used as additives for coal-fired combustion units, e.g. for boilers. If these substitutions took place globally, the demand for acetic acid would enormously increase (Cheryan et al., 1997).

As an alternative to the chemical processes acetic acid can be produced by biological routes:

a) anaerobic process;
b) aerobic process.

In the literature there are several reviews concerning the acetic acid production that are based on specific aspects, such as the description of a process (Gullo et al., 2014; Ljungdahl, 1986; Muller, 2003; Ragsdale and Pierce, 2008), bacterial physiology and metabolism (Cheryan et al., 1997; Ljungdahl, 1986; Mamlouk and Gullo, 2013) or biotechnological application (Raspor and Goranovič, 2008). This paper has critically reviewed the three main acetic acid biological production processes, offering an overview of the most sustainable and effective strategies for the acetic acid production,

while simultaneously taken into consideration the registered patents, which represent the state of art of the technological innovation changes (Figure 1). The patents have been investigated by the free access Espacenet platform as the main information source with the keywords: “acetogenesis”, “acetic acid acetogenic bacteria”, “acetic acid syngas”, “acetic acid fermentation”, “acetic acid *Acetobacterium aceti*”, “acetic acid food waste”, “acetic acid yeast fermentation”, “acetic acid *Brettanomyces/Dekkera*”, “acetic acid apiculate yeast”. This information source, created by the European patent office, ensured a worldwide invention overview (Amato et al., 2020; Amato and Beolchini, 2018).

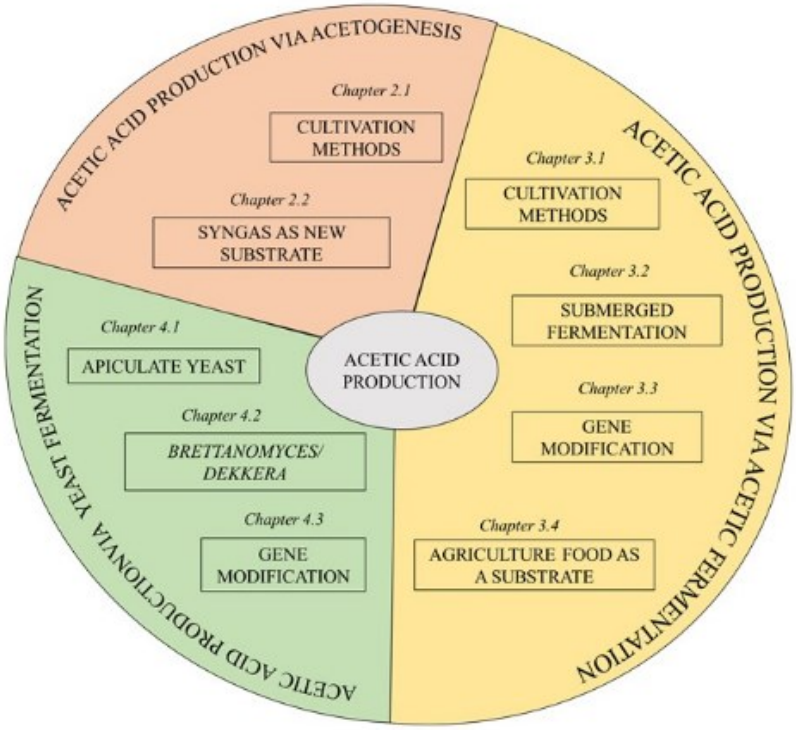

Figure 1. Roadmap of the present review about the technological innovation change of the acetic acid bioproduction.

2. Acetic acid production via acetogenesis

Organisms that are capable of reducing CO_2 to acetate through acetyl coenzyme A (acetyl-CoA) or the Wood-Ljungdahl (WL) pathway are called acetogens (Muller, 2003). Acetogens are strictly anaerobic bacteria that can grow by converting compounds with one atom of carbon, such as CO_2, CO or formate (HCO_2) to acetate. Furthermore, they can use a variety of substrates, including the biodegradation products of sugars, alcohols, aromatic compounds. (Ljungdahl, 1986). Globally, acetogens produce over 1013 kg of acetic acid annually, which reduces the total production of the world chemical industry (Ragsdale, 2008). Therefore, acetogens play an important role in the global carbon cycle. Another driving force to search for new energy compounds and technologies is the alarming effect of the increasing CO_2 concentration in the atmosphere, due to the burning of fossil

resources. One way of facing this problem is the biotechnological use of these bacteria that grow at the expense of CO_2 (Köpke et al., 2010). Phylogenetically, acetogens are quite diverse and, up to 2003, 19 different bacterial genera have been described (Muller, 2003). The microbial species most involved in acetic acid production, as reported in the literature, are *Clostridium aceticum, Acetobacterium woodii, C. thermoaceticum, Thermoanaerobacter kivui* and *A. wieringae*. The best conditions for the acetic acid production are a temperature of 30°C and a pH around the neutrality (6-8) (Braun and Gottschalk, 1981, 1982; Demler and Weuster-Botz, 2011; Morinaga and Kawada, 1990; Straub et al., 2014). It has been reported that pH control is necessary to achieve the highest product concentrations, around 44 g/L (Demler and Weuster-Botz, 2011). The thermophilic species *C. thermoaceticum* and *T. kivui*, on the other hand, require higher temperatures, between 55 and 66°C (Daniel et al., 1990; Hu et al., 2013; Sakai et al., 2005). Moreover, *C. thermoaceticum* shows the highest acetic acid production in the presence of $CO:CO_2$ (70:30) as substrate, with a production ranging between 20 and 30 g/L (Hu et al., 2013; Sakai et al., 2005). For the other analysed species, the acetic acid concentrations are significantly lower, with values around 1.2-9 g/L for *C. aceticum* (Sim et al., 2008, 2007), 0.9-1 g/L for *T. kivui* (Daniel et al., 1990) and 18 g/L for *A. wieringae* (Braun and Gottschalk, 1982). Alternatively, another method to increase the acetic acid production is the use of modified strains (Annie Modestra et al., 2020; Straub et al., 2014). The importance of acetogens is confirmed by the large number of patents related to the strategies for improving the acetic acid production (Figure 2).

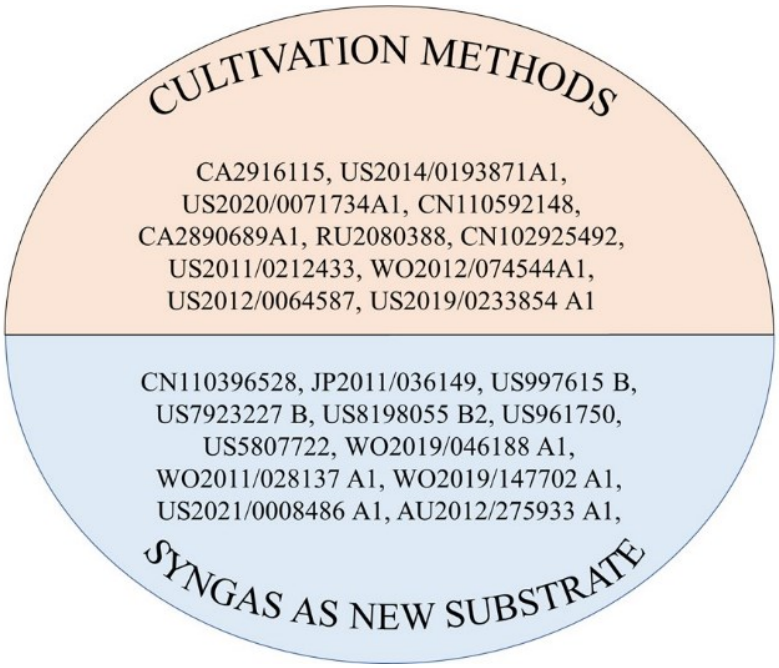

Figure 2. Patents related to the acetic acid production via acetogenesis.

2.1. Cultivation methods

The following inventions are related to methods for culturing acetogenic bacteria to improve the acetic acid production. Some patents reported the addition to the fermentation broth of particular compounds to improve bacteria metabolism, such as cysteine, as reported in invention CA2916115A1 (Haas et al., 2015). Moreover, lactic acid and salt, as claimed in invention US20140193871A1, led to the production of 9.15 g/L of acetic acid (Chen et al., 2014), and sodium ions, claimed in invention US20200071734A1, allowed to reach a final acid concentration of about 23 g/L (Senaratne et al., 2020). The acetic acid production can be promoted by the addition of auxiliary materials, such as mesoporous carbon nitride, to the medium and by controlling the reaction under light conditions, as reported in invention CN110592148 (Yinguang et al., 2019). Another method for cultivating acetogenic bacteria is proposed in patent CA2890689A1, based on bacteria germination and sporulation with a selected medium. This method includes a first step to adapt the bacterial strain to the selected substrate. After this phase, the formed spores are inoculated with a concentrated medium to carry out the acetic acid production (Senaratne, 2013). Differently, the invention RU2080388 is related to the production of acetate in the absence of bacterial growth. *T. kivui* cells are embedded in an immobilization medium composed of 8-12% polyvinyl alcohol (PVA) cryogel. The formation of granules occurs in a cryoimmobilization column filled with pentane (at -30°C), into which a mixture of polymer solution with cells is supplied. The acetic acid production increased two times in the system with immobilized cells due to a more complete consumption of the substrate thanks to the prolongation of the synthetically active phase of the cells (Detkova et al., 1997).

In order to find a cheaper and easier way to produce catabolites, several production methods were investigated. Invention CN102925492 provided a method for regulating the metabolic products by using cathodic polarization potential. The operations included the introduction of a mixed gas of CO_2 and H_2 and the setting of the cathode polarization potential to -850 mV ~ -1150 mV. When the cathode polarization potential is lower than -950 mV, acetic acid starts to accumulate, otherwise methane accumulates (Xiaohong et al., 2013). The invention US20110212433 A1 is related to a method for optimizing alcohols and acids production by controlling substrate supply. The concentration of acid in the bioreactor is determined at two different time points, if a change in acid levels occurs between the first and the second time points, an adjustment means can increase the substrate supplied to the bioreactor (Barker et al., 2011). Another method for continuous fermentation of gaseous substrate, with a constant nutrient medium supply, is reported in invention WO2012074544 A1. A concentration of 6.93 g/L of acetic acid has been obtained with this method (Senaratne et al., 2012).

Acetic acid production can be also improved with genetic engineering methods. In invention US20120064587, a culture method for a recombinant *Clostridium* expressing one or more

heterologous WL genes is developed. This recombinant *Clostridium* is derived from a recipient *Clostridium* that does not have a functional WL pathway. The reported method comprises culturing the recombinant *Clostridium* to produce the metabolite at an increased level compared with the recipient organism (Papoutsakis et al., 2012). Another method, claimed in invention US20190233854A1, consists in the creation of a recombinant microorganism, capable of proliferating using CO or CO_2 as a sole source, into which a foreign gene encoding for a target protein is introduced (Matsushima et al., 2019).

2.2. Syngas as new substrate

Acetogens can produce chemical and fuels using synthesis gas (syngas), which is a mixture of mostly H_2, CO and CO_2, as a carbon and energy source for fermentation (Daniell et al., 2012; Younesi et al., 2005). Syngas is a simple, abundant, and inexpensive substrate. It can be easily generated not only from natural gas and by gasification of coal and oil, but also from biomass, municipal waste, or by plastics (Younesi et al., 2005). In the past decade, an increasing interest in biological usage of gaseous substrates has developed in several bioprocesses for fuel synthesis. The products of syngas fermentation can also include acetic acid, ethanol, 2,3 butandiol, butyric acid and butanol (Bengelsdorf et al., 2013; Daniell et al., 2012). Acetogenic bacteria employing the WL pathway can be used as biocatalysts in syngas fermentation. A fermentation process using a biocatalyst has several advantages compared with a thermochemical route: low temperature, low pressure, the tolerance of a biocatalyst to several impurities in syngas and the ability to use flexible syngas compositions. However, syngas fermentation has also a major limitation, which is the lack of genome knowledge, that would allow the construction of tailor-made strains suitable for a whole variety of different production processes (Bengelsdorf et al., 2013).

Syngas can be produced by different sources and utilized in the same system to produce acetic acid. The invention CN110396528 provided a method to produce synthesis gas by gasification of biomass and the syngas thus produced is utilized to synthetise acetic acid. This method is based on gradual pH and substrate changing, so that methanogenesis is suppressed. At the end of the process only acetic acid will be obtained, in a concentration of 4.32 g/L (Yafeng and Jitao, 2019). An objective of the invention JP2011036149 is to create a more practical method for producing acetic acid using petroleum asphalt as raw material (Naoki et al., 2011). The asphalt was gasified and the obtained gases were cooled with water while the unreacted carbon cord was recovered and reused. CO and H_2 can be separated in the purification column and used as a substrate for *A. woodii* fermentation. The operation was carried out for 4 hours, producing 10.3 g/L of acetic acid (Naoki et al., 2011). Invention US20190203235 reported the conversion of a methane feedstock into a substrate comprising CO and

H_2, that is then fermented in a bioreactor by microbial cultures in order to produce acetic acid (Matsushima et al., 2019).

The major challenge in syngas fermentation is gas-liquid mass transfer because of the low solubility of CO and H_2 in water (Sun et al., 2019). The strategies for enhance gas-liquid mass transfer are based on the exploration of various and different reactor configurations, such as continuous stirred tank reactor (CSTR) (US9976158) (Bell and Ko, 2018), hollow fiber membrane reactor (HFMR) (US7923227B, US8198055B2) (Datta et al., 2012; Hickey et al., 2011), bubble column reactor (US961750)(Li et al., 2017), trickle bed reactor (TBR) (US5807722, WO2019046188A1) (Bennett and Orr, 2019; Gaddy, 1998).

More in details, in patent US9976158 syngas was introduced into a CSTR vessel through a gas sparger, located below the liquid level, at a flow rate effective for maintaining a pressure inside the reactor vessel of at least about 1 psig. The process was effective for providing a volumetric CO mass transfer coefficient of about 100 to 1500 per hour (Bell and Ko, 2018). In invention US8198055B2 a combination of microporous membrane layer and a non-porous membrane layer was used to transfer syngas into direct contact with microorganisms. The microporous membrane layer can serve as the support upon which the bacteria grow in a concentrated layer as a biofilm. The gas pressure on the gas phase side of the membrane must be kept above the pressure on the liquid phase side to enhance the direct contact. The reported result is a highly efficient and economical transfer of the syngas (Datta et al., 2012).

In patent US9617509 the bubble column reactor, a reactor that has a high gas-liquid mass transfer capability, was improved with the introduction of a secondary circulating loop in a forced-circulation loop vessel, greatly enhancing the gas-liquid mass transfer (Li et al., 2017). In invention US5807722A1 the conversion rate of waste gases introduced into a TBR, was maximized by the use of centrifuges, hollow fiber membranes or other means of ultrafiltration to recycle bacteria, insuring the highest possible cell concentration. In this patent, 20 g/L of acetic acid can be obtained when the waste gas is composed by 13% CO_2, 14% H_2, 5% CO and 68% N_2 (Gaddy, 1998). The invention WO2011028137A1 claimed a bioreactor configured to improve the fermentation efficiency on CO substrates. The gaseous substrate is added to a circulated liquid loop bioreactor including a gas/liquid contact module with multiple channels containing a culture of microorganisms and configured to produce acetic acid. The amount of substrate transferred into the broth is controlled to ensure high production rate and avoid inhibition. A concentration of 11.7 g/L of acetic acid can be obtained with a gas composition of 2% H_2, 30% N_2, 47% CO, 21% CO_2 (Trevethick et al., 2011).

Two-stage continuous fermentation or multi-stage fermentation performed in two different types of reactors, can improve ethanol and acetic acid yield compared to single stage fermentation (Gao et al.,

2021; Jensen et al., 2019; Sun et al., 2019). Two or more different types of reactors can be used together to reduce the amount of time needed to inoculate a main reactor, as reported in patent AU2012275933, which is based on propagating a culture of acetogenic bacteria through different reactors to adapt the microorganism to the used syngas. (Bell and Ko, 2013).

3. Acetic acid production via acetic fermentation

In aerobic fermentation, acetic acid is produced by two steps; the first step is the production of ethanol from a carbohydrate source, such as glucose, generally with the anaerobic yeast *Saccharomyces cerevisiae* (Raspor and Goranovič, 2008). Efficient ethanol production requires four components: fermentable carbohydrates, an efficient yeast strain, some nutrients and simple growing conditions (Saha and Banerjee, 2013). The second step is the oxidation of ethanol to acetic acid, catalyzed by acetic acid bacteria (AAB) (Raspor and Goranovič, 2008). This fermentation is not a complete oxidation because the reducing equivalents generated are transferred to oxygen and not to CO_2, according to the reaction (Eq. 1) (Cheryan et al., 1997):

$$2CH_3CH_2OH + O_2 \rightarrow 2CH_3COOH + 2H_2O \quad (1)$$

AAB have been unknowingly used for a long time before their role in acetic fermentation was discovered, because in addition to playing a positive role in the production of selected foods and drinks, they can also spoil them. At present they are represented by the following genera: *Acetobacter*, *Acidomonas*, *Ameyamaea*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter*, *Kozakia*, *Neoasaia*, *Neokomagataea*, *Saccharibacter*, *Swaminathania* and *Tanticharoenia* (Mamlouk and Gullo, 2013). Among the genera, the AAB recovered from vinegar fermentation are mainly distributed in the genera *Acetobacter* and *Gluconacetobacter* (Gullo et al., 2006; Mamlouk and Gullo, 2013; Raspor and Goranovič, 2008). *Acetobacter* species can use sugars via the hexose monophosphate pathway and also through the Embden-Meyerhof-Parnas and Entner-Doudoroff paths. On the other hand, *Gluconobacter* can obtain energy more efficiently through the metabolization of sugars by pentose phosphate pathway (Gullo et al., 2006). The oxidative conversion of ethanol-containing solutions by AAB leads to the production of vinegar (Ebner and Enenkel, 1978; Raspor and Goranovič, 2008). Vinegar is traditionally a product of the fermentation of natural alcoholic solutions, which contain 10% - 15% by volume of ethyl alcohol. The raw materials can be various: wine, cider and other liqueurs deriving from the alcoholic fermentation of cereals, fruit, sugary solutions, or even pure ethanol with the addition of nutrients. Industrial vinegar manufacturing processes fall into three main categories: slow or traditional processes (Orleans or French), quick processes (or German), and submerged processes (Raspor and Goranovič, 2008). The various acetification techniques differ in the way in which the three components (ethanol, bacteria, and

oxygen) interact and are combined with each other. The main AAB characteristics are the resistance to high acetic acid concentrations and low pH, even if the optimum pH for the bacteria growth is 5.5 to 6.3 (Raspor and Goranovič, 2008). The optimum growth temperature is in the range 25-30°C (Raspor and Goranovič, 2008). However, thermotolerant AAB, which are able to grow at 37- 40°C, have been isolated and this could represent a very important feature (Ohmori et al., 1980; Raspor and Goranovič, 2008). Complete aeration and rigorous control of the oxygen concentration during the bioprocess are fundamental in aerobic fermentation to maximize yields and keep bacteria viable (Cheryan et al., 1997). Nowadays AAB represent a widely used resource in the biotechnology field and a lot of efforts have been made to improve identification, knowledge of their physiology and strategies to increase the production of acetic acid (Figure 3) (Mamlouk and Gullo, 2013; Raspor and Goranovič, 2008).

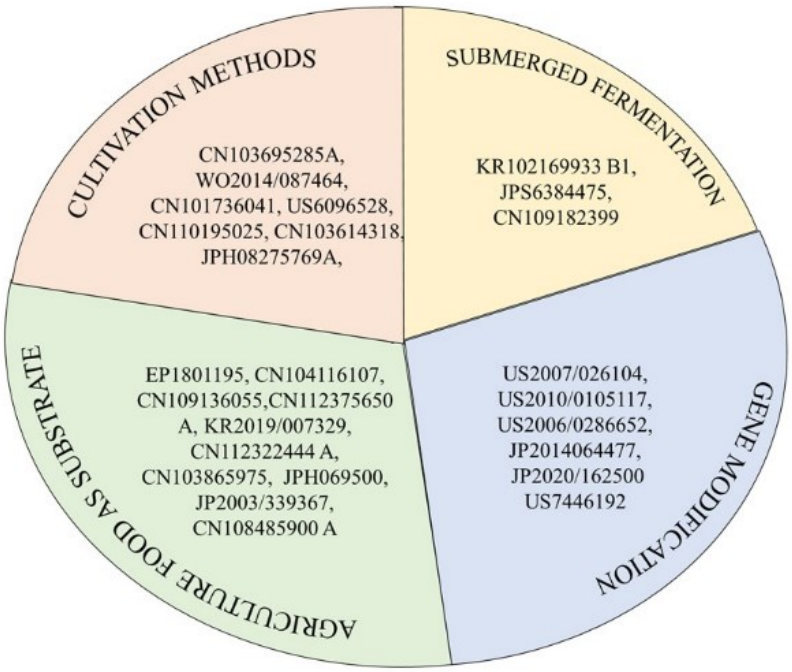

Figure 3. Patents related to the acetic acid production via acetic fermentation.

3.1. Cultivation methods

The AAB fermentation mainly uses the residual nutrient sources of wine fermentation supplemented with glucose, yeast extract, peptone. The Chinese patent 103695285 is directed to solving the problems of low efficiency and low performance of acetic acid fermentation medium. It provided a medium enriched with the nutrients required for the fermentation process, which can shorten the cycle, increase the production rate and reduce the production costs (Hong and Wang, 2014). In the production of high acidity vinegar, it is required to further improve the fermentation ability of AAB. As a result, invention WO2014087464 discovered that fermentation efficiency is remarkably

improved when at a culture solution is added a specific compound having a thiol group or a S-substituted derivative or a compound having a disulphide bond as an additive (Nakano and Ohno, 2014). A method to increase the concentration of one or more products is the use of mixed cultures. The invention CN101736041 disclosed a method for co-producing two beneficial products (acetic acid and bacterial cellulose) by mixed culture of AAB and *Acetobacter xylinum*. At the end of the fermentation, acetic acid and bacterial cellulose can be obtained with final concentrations higher than the ones obtained with single bacterial cultures, of 39.5 and 4.95 g/L respectively. Generally, in the process of acetic acid production with single AAB culture a peroxidation reaction occurs on the 4th day when acetic acid is used by bacteria to generate CO_2 and H_2O. The mixed culture solves this problem, in fact after 3 days of fermentation the output of acetic acid will not decrease but will continue to increase (Hongmei and Jinghong, 2010).

A limitation of the fermentation process is that it requires precise temperature control and sometimes a large-scale cooling system is necessary to maintain the optimum temperature, with a great cost. However, if fermentation can be conduct at a higher temperature, using thermophilic strains, the costs may be reduced (Ohmori et al., 1980). Patent US6096528 is related to a novel thermotolerant species of bacterium named *Acetobacter sp.* I14-2 useful for producing up to 43.6 g/L of acetic acid from an ethanol-containing substrate (Lu, S.F. et al., 2000). Other inventions disclose a heat-resistant *Acetobacter pasteurianus* (CN110195025A), which is able to produce fruit vinegar improving the efficiency of the fermentation tank without diluting fruit wine (Gao et al., 2019) and a high temperature resistant *Acetobacter sinensis* (CN103614318) which produce an acetic acid concentration of 60-180 g/L, with a promising alcohol conversion rate of over 85% (Han et al., 2015). However, the fermentation can be conducted also at low temperature. In invention JPH08275769 is provided a method to produce an ultra-high acidity vinegar with an acetic acid concentration of 215 g/L, characterized by the temperature control. The temperature is gradually lowered to 18°C to perform fed-batch culture, and then it is further gradually lowered to 15°C to perform acetic acid fermentation. In the present invention *Acetobacter polyoxogenes* is used, capable of carried out the acetic fermentation even at a low temperature (Higashide et al., 1995).

3.2. Submerged fermentation

Submerged fermentation was first applied in the 1950s leading to an increase in acetic acid production compared to surface fermentation (Hromatka and Ebner, 1959). Nevertheless, the process suffered from several disadvantages, such as long duration, low efficiency due to the involvement of unproductive stages and high costs of the raw materials, whereas the high purity of the end product was not always guaranteed. Currently the most used apparatus for speeding up the acetic acid synthesis rate is the Frings acetator, a semi-continuously operated tank, with a conversion efficiency

of ethanol into acetic acid of around 96-98%. However this process is still too slow, requiring about a week to produce the desired concentration (Hidalgo et al., 2010). Patent KR102169933B1 discovered the *Acetobacter pasteurianus BGK2018* strain, which exhibits an excellent acetic acid production ability even at a low ethanol content compared to the other strains, useful for shortening the time required for the production of vinegar (Jinman et al., 2020). One of the risks connected with submerged fermentation is that the bacteria or a substantial part thereof, are taken along with the final product, therefore it can be beneficial apply immobilisation techniques for bacterial strains, as reported in patents JPS6384475 and CN109182399. The former is related to AAB adhered and fixed on ceramics treated with a mineral acid or an organic acid. First the ceramic is treated with acid, then the medium for acetic acid fermentation is injected from the inlet and oxygen-enriched air is supplied. Thereafter, a seed culture solution of AAB is added and cultured and, after attachment, the fermentation starts (Kamiya et al., 1988). The other patent, invention CN109182399, adopted a reconstructed wall membrane complex (yeast cell wall, AAB cell membrane of fermentation end point) to cover corncob microparticles as a carrier, to improve bacteria ability to resist to acetic acid and significantly improve the final production. An acetic acid concentration of 35 g/L was reached at end of the process (Gong et al., 2019).

3.3. Gene modification

Genetic engineering can improve the acetic acid production, lowering the fermentation time and the required costs. In invention US20070026104 a novel alcohol dehydrogenase gene, encoding a protein capable of improving the growth-promoting function, has been modified and the new AAB strain showed a better growth-promoting function. In this way it was made possible to significantly shorten the growth period and improve the acetic acid fermentation rate, reaching an acid concentration from 105 to 173 g/L (Nakano, 2007). Invention US20100105117 is related to an AAB in which foam formation has been suppressed by reducing or deleting the function of the protein encoded by the gene involved in that process. With this modified AAB, an acetic acid concentration of 43.2 g/L can be obtained (Iida, 2010). Patents US2006286652, JP2014064477 and JP2020162500 developed methods to obtain AAB able to grow at high temperature, improving the fermentation efficiency and lowering the culturing costs. In the first one a gene involved in temperature tolerance was obtained by constructing a chromosomal DNA library and a non-thermotolerant AAB was transformed with this gene. The maximum concentration of acetic acid obtained with this mutagenic AAB is reported to be 172 g/L (Goto, 2006). The invention JP2014064477 reported a method for culturing a mutated AAB strain (*Acetobacter pasteurianus TI*), obtained by modifying the genes involved in acetic acid resistance and heat resistance from *Acetobacter pasteurianus* SKU1108 strain, so that the function of the proteins encoded is reduced or deleted (Gunjana et al., 2014). In invention JP2020162500 the

Acetobacter strain was modified by disrupting and suppressing the expression of one of the gene encoding for histidine kinase, response control factor kdpE or a LysR type transcriptional regulator (Ogino and Shirai, 2020). Another invention, US7446192, is related to novel genes participating in acetic acid tolerance. These genes were cloned from AAB belonging to the genus *Gluconoacetobacter* by genes from chromosomal DNA library that enable to grow on the medium at high acetic acid concentration. Further, in mutagenic strain, acetic acid tolerance is remarkably increased and when the transformants are subject to aeration the final acetic acid concentration can be improved, reaching 162 g/L (Goto and Nakano, 2008).

3.4. Agriculture food as substrate

Recently the research is focusing on alternative and cheaper carbon sources to reduce the cost of the raw materials. Several patents developed beverages which are rich in nutrition properties and with unique flavours, made by the acetic fermentation of fruits or juices extract from cereals and vegetables. The invention EP1801195 reported a fermented cloudberry vinegar with an acetic acid content in the range of 50-150 g/L. Cloudberries were mixed with aqueous alcohol and the resulting mash, with the addition of sugars, was fermented in the presence of *Acetobacter aceti* (Mueller and Mueller, 2007). Similar procedure has been applied for the fermentation of other fruits such as strawberries, in patent CN104116107 (Lin, 2014) and persimmons, in patents CN109136055 and CN112375650A (Liu, 2019; Ren et al., 2021). Vegetables can also be utilised as a substrate for vinegar production. Invention KR20190007329 referred to a vinegar production using only 100% onion as a raw material, without using any additives, reaching a final acetic acid concentration of 53 g/L (Joung, 2019). Patent CN112322444A aimed to produce a nutrient-rich sweet potato vinegar beverage which is suitable for long-term drinking, prepared by combining sweet potatoes, mainly used for feeding due to their poor taste and high staple food processing cost, with tea leaves (Huang et al., 2021). Invention CN103865975 proposed an efficient two-step fermentation method, with high-activity dry yeasts and AAB, to treat kitchen waste. The advantages are a correct disposal of waste and the reduction of the acetic acid production costs. With this method an acetic acid concentration of 25.6 g/L was reached, which is higher than that without adding high active dry yeast and AAB (Chai et al., 2016).

Invention JPH069500 related to a method for producing vinegar from molasses by simultaneously perform alcoholic fermentation and acetic fermentation. The fermentation temperature has been set to exactly 30°C and the use ratio of the yeast: vinegar has been fixed to 2-3: 1, to start the yeast fermentation at the beginning. If the sugar content of molasses is 15% or more, or the alcohol content is 8% or more, acetic acid fermentation is not performed, so it is necessary to keep the concentrations constant. Molasses can cause excessive oxidation because it is mainly composed of sugar, therefore,

to prevent the acid reduction the air restriction is performed twice in different degree and acetic acid content in the vinegar at the end of fermentation is 62.5-70 g/L (Hayano, 1994). In addition to molasses, invention JP2003339367 and CN108485900A focused on brown sugar, rich in minerals, as a substrate (Huang et al., 2018; Izeki et al., 2003).

4. Acetic acid production via yeast fermentation

The different yeast species represent a factor that can exert a marked effect on the production of secondary fermentation products. The ability of these strains to produce different quantities of higher alcohols and acetic acid could be used as a determining factor in the selection of yeasts for industrial purposes (Romano et al., 1992). The characteristics and composition of the base wine can interfere with the fermentation and therefore affect the quality of the vinegar. Even the type of yeast used for fermentation can influence acetic fermentation, in particular the growth of AAB and the analytical profile of the vinegar thus obtained (Ciani, 1998). The base wines obtained from the fermentation of apiculate yeast, like *Candida stellata* and *Kloeckera apiculata* offered interesting substrates. Apiculate yeast can also directly produce acetic acid, but the concentrations are lower than those obtained with *Acetobacter*: 0.1-0.2 g/L by *Hanseniaspora guilliermondii* and *H. uvarum,* 0.3 g/L by *K. apiculate* (Ciani and Picciotti, 1995; Romano et al., 1992). Also the genus *Brettanomyces/Dekkera* is able to produce acetic acid by direct transformation of glucose, depending on the culture conditions (Aguilar Uscanga et al., 2003). However, there are not many information on the use of these yeasts for the explicit purpose of producing acetic acid (Figure 4).

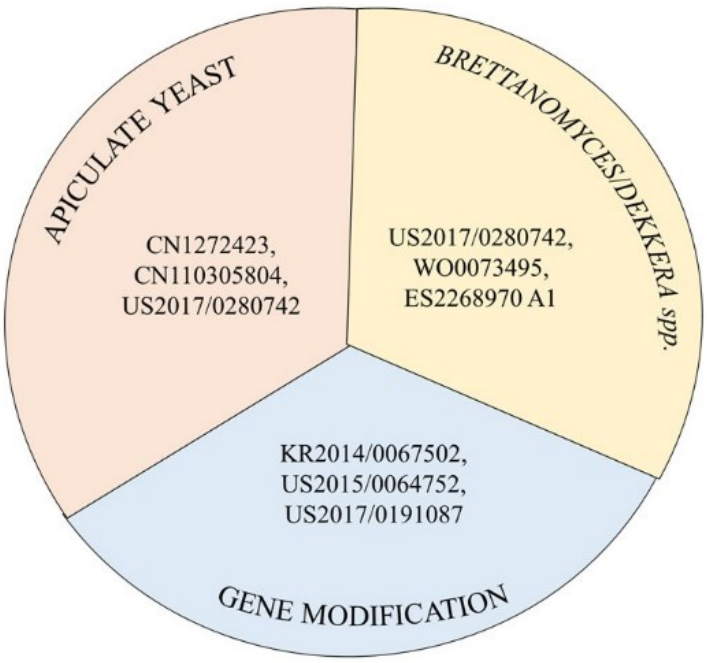

Figure 4. Patents related to the acetic acid production via yeast fermentation.

4.1 Apiculate yeast

Some yeasts, comprising *H. guillermondii.* as reported in invention US20170280742, are capable of fermenting cocoa beans producing both ethanol and acetic acid. These yeasts can be added as a single strain or as a mixture of yeasts. The fermentation process started with the addition of yeast to the mixture of beans and pulp and required from 2 to 7 days. The temperature of the process varied from 30°C to 50°C. With this process vinegar with a high acetic acid concentration (up to 2.5 g/L) can be produced (Fourcassie et al., 2017). The same process can be applied for citrus wine fermentation, as claimed in invention CN110305804, where *H. thailandica* is inoculated in a fermentation medium with 80 mg/L of honey. The final acetic acid concentration was 7.9 g/L. Nevertheless, compared with the dry Angel yeast (total acid of 8.5 g/L), *H. thailandica* showed a more significant acid reduction. This explain why at the present most of the yeasts in citrus fruit wine are extended wine yeast or dry wine yeast (Xiao et al., 2019). The mixed fermentation compared to single yeast culture is useful for the acetate production (Xu, 2006). The invention CN1272423C optimized the fermentation parameters, such as the inoculum amount, for the fermentation of a mixed culture of *H. valdensis* and *S. cerevisiae* CCTCC M201022. *H. valdensis* has been inoculated first, followed, after several days, by *S. cerevisiae*, and the fermentation has been conducted at 15°C. When the apparent sugar in the fermentation broth reached a constant value, the yeasts are removed by centrifugation. The total produced acetate was 1.07 g/L (Xu, 2006).

4.2 Brettanomyces/Dekkera spp.

Yeast belonging to the genera *Brettanomyces* and *Dekkera* are known for their ability to produce acetic acid (Freer, 2002). They are also associated with the deterioration of industrial alcoholic fermentation and their presence seems to inhibit the *Saccharomyces* growth (Phowchinda et al., 1995). The acetic acid is produced in these yeasts by the oxidation of acetaldehyde thanks to the action of aldehyde dehydrogenase and the subsequent activation to acetyl-CoA thanks to acetyl-CoA synthetase. The best conditions to increase the acetic acid production are the high glucose concentrations and the maintenance of high dissolved oxygen (Freer, 2002). The presence of oxygen in the medium is reported to be one of the most important parameter for improve acetic acid production (Aguilar Uscanga et al., 2003; Castro-Martinez et al., 2005; Ciani and Ferraro, 1997; Ciani and Maccarelli, 2003). Few studies have been conducted on the influence of temperature, despite being another important parameter, and it has been reported that in the range 15-32°C the acetic acid production reached the highest level, while over this range was strongly decreased (Brandam et al., 2008). The use of cheaper available carbohydrates as carbon sources, rather than glucose or ethanol, favours the possible acceptance of *B. bruxellensis* for industrial purposes (Aguilar Uscanga et al., 2007). This yeast grows well in complex culture medium (e.g. juices and molasses) (Aguilar Uscanga et al., 2007), but the highest acetic acid production (around 40 g/L) is reached when *B. bruxellensis*

used maltose and cellobiose as carbon source (Blomqvist et al., 2010; Blondin et al., 1982). As well as *H. guillermondii*, also *B. bruxellensis* can ferment cocoa beans in the same conditions, producing more than 12 g/L of acetic acid, as reported in patent US 20170280742 (Fourcassie et al., 2017). Overall, the efficiency of acetic acid production is reasonably low, and this is probably due in part to the inhibitory effects of acetic acid on yeasts (Freer, 2002).

A culture medium for the differential detection and enumeration of *Dekkera* and *Brettanomyces* genera is reported in invention WO0073495. This medium, containing ethanol and p-coumaric acid, allowed *Dekkera* and *Brettanomyces* yeasts to produce acetic acid in an exclusive form, compared to other yeasts (Loureiro et al., 2000). The trend of increasing acetic acid production in the presence of 20 mg/L of coumaric acid is reported also in the literature (Lucy Joseph et al., 2013). Other authors, with invention ES2268970A1, have discovered a culture medium that contributes to the isolation, detection and enumeration of strains of the species *B. bruxellensis,* composed by: 10-100 g/L of glucose, 20-30 g/L of calcium carbonate, 3-5 g/L of yeast extract, 50-60 g/L of ethanol, 0.5-0.6 g/L of chloramphenicol, 0.1-0.2 g/L of cycloheximide, 0.03-0.07 g/L of neutral red and 15-20 g/L of agar (Lopez Rodrigues Da Silva et al., 2007).

4.3 Gene modification

It has been reported that the presence of high concentration of acetic acid may inhibit the activity of yeasts (Freer, 2002). Therefore, some inventors have tried to find a way to improve acetic acid tolerance for yeast strain, principally for the purpose of bioethanol production and only few studies are related to the production of acetic acid. In the invention KR20140067502 an approach called 'global transcriptional machinery engineering' (gTME) is used. This method has been used for the first time to create strains with increased ethanol resistance by triggering mutations in the TATA-binding protein (TPP) encoded by the SPT15 gene, previously reported to be able to grow at lethal ethanol concentrations (Alper et al., 2006). Similarly, in this invention, two acetic acid-resistant strains have been obtained by screening a library that overexpresses the SPT15 mutant allele. As a result, the strains exposed to acetic acid showed a significant decrease in intracellular reactive oxygen species (ROS) levels (Choi and Kim, 2014). Invention US20150064752 focused on a genetically modified acid-tolerant yeast with an increased amount of polypeptide having an amino acid sequence with an identity of about 95% with respect to SEQ ID NO:1 and with an increased amount of beta-1,3-glucanosyltransferase, compared to a genetically unmodified yeast cell of the same species. The yeast cell may be a natural or a variant yeast cell capable of producing a product of interest, like acetic acid (Lee et al., 2015). Invention US20170191087 reported the use of a specific allele to confer acetic acid tolerance. The inventors reported that a specific allele of GLO1 is needed and sufficient to confer tolerance to relatively high acetic acid concentrations. Replacement of the allele results in an

improvement of the fermentation performance in presence of acetic acid. The use according to the invention is the overexpression of the protein, encoded by the specific allele (Thevelein et al., 2017).

5. Future and perspectives

This review analysed the technological innovation state of the art of the last 30 years regarding three different processes for the acetic acid production (acetogenesis, acetic fermentation and fungal fermentation), in order to verify which technologies can be used for the low-cost acetic acid production. Concerning the trend of technological innovation, an increase in the number of patents related to the acetic acid synthesis can be seen in all the three processes, especially in the last 10 years (Figure 5a). This observation confirms the growing interest in this topic and the need to find efficient and economically sustainable methods for the acetic acid production. The acetic fermentation is the most known process, practiced for a long time, as demonstrated by the presence of patents dating back to the last two decades (Figure 5a). Fermentation by yeasts, on the other hand, is a quite new process, in fact the number of patents is still limited (Figure 5a). As far as acetogenesis is concerned, the increasing number of patents from 2010 to the present day could be an indication of the greater attention paid to the problem of CO_2, as acetogenic bacteria are able to grow at the expense of this gas, confirming the importance of syngas as an alternative substrate (Figure 5a).

As regards acetogenesis, different strategies to increase the acetic acid production have been analysed. Some patents are based on culture methods or on the use of genetically modified strains which increase acetic acid production thanks to an overexpression of the enzymes involved in WL pathway. The enhancement of the gas-liquid mass transfer to improve the fermentation efficiency is one of the major focus of the research, based on the exploration of different and innovative reactor configurations. The development of these processes aimed at the direct conversion of CO_2 into organic compounds and could indicate a promising direction to utilize CO_2, whose rapid increase is considered to become a serious problem in the future. In this regard, the use of syngas as a carbon and energy source to produce commercial compounds could be very useful for reducing the carbon footprint and the energy used by some gasification industries.

Concerning acetic fermentation, the most used method is submerged fermentation. As a strategy for further increase the efficiency of the process, the immobilization method in which the AAB are adhered to different support materials, can be applied. These processes have been known and practised for several years, in fact some related patents date back to 80-90s (Figure 5b). A limit to the fermentation process is represented by the increase in the temperature. Since the last decade, to avoid this problem, the research has focused on new thermotolerant species, able to resist up to 40°C, also applying genetic engineering techniques (Figure 5b). Several new substrates have been analysed with

the aim of decreasing the cost of raw materials (Figure 5b). Among them, food wastes are of growing concern. Due to their high organic matter level their release can pollute water and air, improper disposal methods therefore affect human health and environmental safety (Li et al., 2015). The key of the successful food waste management is the development of appropriate ecological reprocessing technologies that can convert all the precious components into valuable products and reduce the quantity of waste destined to landfills (Roda et al., 2017).

The direct production of acetic acid via fungal fermentation has been little studied. Compared to the other processes, it leads to lower acetic acid production. The most important operational parameter seems to be the oxygenation, in fact the presence of the oxygen significantly increases acetic acid concentration. The use of alternative or waste substrates as carbon sources is promising, especially by using substrates rich in complex sugars, such as molasses or cellobiose. It has also been reported that high acetic acid concentrations can cause the inhibition of yeast fermentation, for this reason some recent patents have studied methods to increase resistance to acetic acid applying genetic engineering techniques.

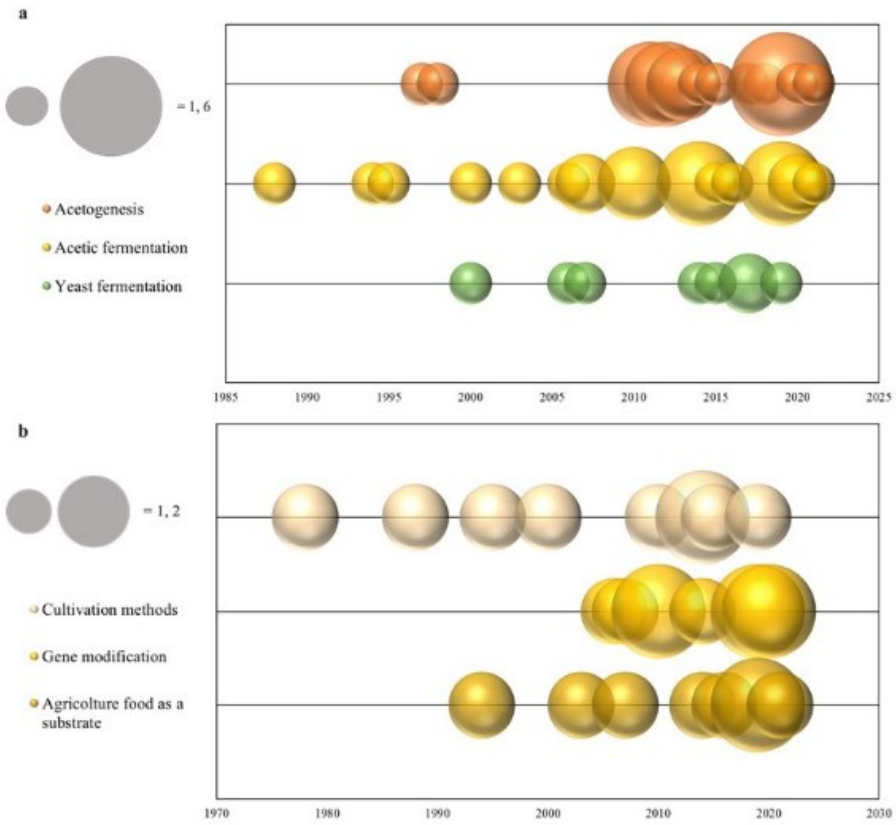

Figure 5. Technological evolution of the acetic acid production by the three different processes (a) and specific focus on acetic acid fermentation (b). The ball size is proportional to the number of the patents.

Over the last two decades, efforts have been made to study acetic acid synthesis by anaerobic and aerobic fermentative process, using cheap material and renewable carbon sources in an eco-friendly approach. Investigation for innovative methods and technologies is necessary as a strategy to increase the efficiency of the processes and the concentration and the purity of the final product. Given the prospect of a growing demand for acetic acid, its production via biological pathways could be more sustainable and convenient considering the cost/benefit ratio. In particular, the use of waste products as a substrate leads to a double benefit, on the one hand the sustainable disposal of food waste and on the other the increase in the annual yield of acetic acid with the consequent decrease in production costs.

6. Products


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Review

Acetic acid bioproduction: The technological innovation change

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Figure 6. Article published on Science of the Total Environment

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Chapter 2:
RECOVERY OF PRECIOUS METALS FROM PRINTED CIRCUIT BOARDS BY CYANOGENIC BACTERIA: OPTIMIZATION OF CYANIDE PRODUCTION BY STATISTICAL ANALYSIS

Abstract

The present work was focused on mobilizing Ag from the waste printed circuit boards (WPCBs) of discarded computers by bioleaching. In this context, bioleaching promoted by bacteria may be regarded as a potential alternative to conventional cyanidation and chemical leaching. The capacity of a cyanogenic bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*), for cyanide production and Ag mobilization from electronic waste was assessed. Also, in order to maximize cyanide production, the influence of the initial pH and glycine concentration was studied and optimized via central composite design of a response surface methodology (CCD-RSM). In the optimal conditions (pH 8 and 1 g/L of glycine) *P. aeruginosa* produced around 10 mg/L cyanide. Therefore, the bioleaching experiments were performed under the synergistic effect of glycine and cyanide through a two-step process. In the first step the bacteria were grown in the optimized conditions and in the second step, after the maximum cyanide production was reached, WPCBs were added to the growth medium and the pH was set to 9. The optimization resulted in 90% Ag mobilization. In addition, the Au bioleaching was investigated and observed with an efficiency around 20%. Considering the current availability of WPCBs on the market, the results are very promising for the development of recycling processes in the spirit of circular economy.

Content

1. Introduction

Electronic waste (e-waste) is currently considered to be the fastest growing category of waste of the last twenty years, due to the technological innovation which makes electronic devices becoming obsolete and redundant rapidly [1]. WPCBs represent an essential part of the electronic industry, constituting the 5% of all the e-waste [2]. According to the definition reported in the European Directive, e-waste can be defined as "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" [3]. Regarding the composition, the WPCBs consist of different metals, mixed with plastics and ceramics [2,4], but the purity and the amount of some metals are higher compared to the ores [2]. Indeed the amount of Ag in the natural mines is around 0.08% w/w, whilst in the WPCB could reach up to 0.12% w/w [5]. Also Au concentration is reported to be 50-100 times higher in WPCBs compared to the minerals [6]. Therefore, the recycling of WPCBs is becoming an increasingly important topic not only from the economic point of view of waste treatment, but also for the recovery of precious elements [4]. The main strategies for metal recovery from e-waste are based on pyrometallurgical treatments [4], that require temperatures between 300° and 900°C [7,8]. To overcome the environmental problems caused by the high temperatures and the resulting polluting gases, hydrometallurgical and bio-hydrometallurgical methods have been taken into consideration [4,9]. Cyanidation process is widely used to extract Au and Ag from both ores and e-waste, but due to its toxicity, thiosulfate and thiourea were investigated as alternative reagents [4,9]. The advantages of non-cyanide lixiviants are the non-toxicity and the high selectivity, whilst the main issue is the high consumption of reagents which makes these processes not economically favourable. One of the most promising innovations of the last decade for the recovery of precious metals is biohydrometallurgy [4]. This kind of treatment is an indirect process which involves the microbial production of metabolites that dissolve metals by the formation of soluble metallic complexes [10]. Precious metals bioleaching is carried out by cyanogenic bacteria, which produce cyanide [4,11].

More in detail, cyanide, in the form of hydrocyanic acid (HCN), is produced by cyanogenic bacteria as a secondary metabolite, by the oxidative decarboxylation of glycine with the HCN synthase enzyme [12,13], according to the following equation (Eq.1) [13]:

$$C_2H_5NO_2 + O_2 \rightarrow C_2H_3NO_3 + H_2O \rightarrow C_2HNO_2 + 2H_2O \rightarrow HCN + CO_2 + 2H_2O \quad (1)$$

The most employed microorganisms for this process are *Chromobacterium violaceum*, *Bacillus megaterium* and different species of *Pseudomonas (P. putida, P. aeruginosa, P. fluorescens, P. balearica).* The cyanide concentration varies with bacterial growth and incubation time: the cyanide production is reported to reach the maximum concentration between the late logarithmic and the early

stationary phase, and then it decreases in the subsequently phase [12,14]. The increase in the stationary phase length could allow a higher cyanide concentration in the nutrient solution. A chemically defined medium, with the carbon source, lipids, co-factors, metal ions, vitamins and amino acids, is fundamental for the maximum bacterial growth and for the cyanide overproduction [15].

The cyanide thus produced can act as a lixiviant for the mobilization of both Ag and Au from WPCBs [13]. Under alkaline conditions (pH 8–10), cyanide forms a water-soluble complexes with the precious metals present in WPCBs [16–18]. More in detail, cyanide produced by bacteria exists in equilibrium according to the following reaction (Eq. 2):

$$HCN \leftrightarrow H^+ + CN^- \quad (2)$$

At pH around 7-8 a quantity of cyanide may be lost in the form of HCN via volatilization; however, this range is considered the best to ensure both the bacterial growth and the cyanide production [19]. Since the pKa of HCN is 9.3, conducting the Au and Ag dissolution under alkaline condition increases the total cyanide ion available for metals bioleaching.

Although the application of bio-cyanidation to Au extraction from e-wastes was reported in some literature, little works were done on the Ag extraction from WPCBs by biosynthesized cyanide. Moreover, the ability of different strains of *Pseudomonas* to biosynthesize cyanide from glycine was exploited by Kumar [20,21] and Gorji et al [13], but this was never assessed for Ag mobilization by *P. aeruginosa*.

The present paper aims at the identification of the best operative conditions to optimize the cyanide production by *P. aeruginosa* and, under the best conditions, to assess Ag and Au bioleaching from WPCBs. Glycine was chosen as the main parameter to enhance cyanide synthesis, and its interaction with the pH was studied via CCD-RSM. The bioleaching was carried out in a two-step process. The bacteria were initially cultured in the medium without e-waste. The WPCBs were added to the bacterial culture in a second step, when the bacterial culture reached a significant cell abundance, and the cyanide production reached its peak.

2. Materials and methods

2.1 Preparation of waste printed circuit boards

The bioleaching tests were carried out on WPCBs, mainly from desktop computers from 90s, discarded by the Polytechnic University of Marche. The WPCBs were prepared as follow: the material was shredded using stainless steel blades and pliers after manually removing the main electronic components. Thereafter, the product was crushed to obtain dimensions lower than 2 mm (Figure 1). A consecutive washing with water saturated with NaCl (210 g/L), allowed the plastics

(such as polyethylene, polypropylene, polyesters, polycarbonates, phenol, formaldehyde, etc.) separation thanks to the difference in density. The presence of Cu in the WPCBs can interfere with the formation of complexes between cyanide and precious metals, binding the cyanide, with the consequent decrease of the leaching rate [11]. To solve this issue, a Cu oxidation by acidophilic bacteria or chemical agent, allows the preliminary removal of 90% of the element [7,11,22] with the resulting increase of the leaching efficiency of precious metals [11,19,23,24].

Therefore the WPCBs were pretreated by a bioleaching process using *Acidithiobacillus ferrooxidans* bacteria with 10 g/L Fe^{2+}, for the removal of Cu and Zn, as described by Becci et al. [7]. These metals were recovered with the following techniques: Fe precipitation by NaOH, Cu cementation by Zn powder and Zn precipitation by oxalic acid, as described by Amato et al. [25].

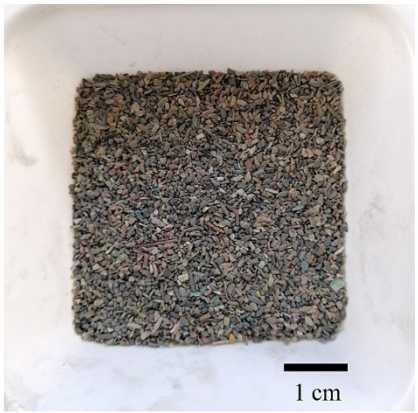

Figure 1. Crushed WPCBs, with dimension less than 2 mm, after the pre-treatment.

The results of metal content of WPCBs after the pre-treatment are presented in Table 1. The amount of Cu was in majority, but it was definitely lower than the concentrations reported before the pre-treatment and in literature characterizations (values around 20% w/w) [7,25]. The effectiveness of pre-treatment is proved by the precious metal concentration increase as a consequence of Cu removal.

Table 1. Average concentrations of WPCBs components before and after the pre-treatment.

Components		Concentration before physical pre-treatment	Concentration after bioleaching with *At. ferrooxidans*	Concentration after pre-treatment
Metals	Cu	~40%	24.7% w/w	5% w/w
	Au		100 ppm	200 ppm
	Ag		300 ppm	300 ppm
Plastics		~30% w/w	-	-
Ceramics		~30% w/w	-	-

2.1 Cyanide production

P. aeruginosa (PAO1-T) strains were provided by the microbiology laboratory of Polytechnic University of Marche. The bacterial colony was cultured in a 50 ml Nutrient broth (NB) medium containing peptone (5 g/L), meat extract (1 g/L), yeast extract (2 g/L) and sodium chloride (5 g/L), in a 100 mL Erlenmeyer flask and incubated at 30°C in a shaker incubator at 150 rpm.

A single-stage optimization process was followed to evaluate the effect of different glycine concentrations, selected as the main parameter to improve cyanide production by *P. aeruginosa*.

10 mL of the pre-grown *P. aeruginosa* were added in a 250 mL culture bottle containing 90 mL of NB medium. The initial pH, according to the literature, was adjusted to 8 by NaOH 1M [13] and glycine, in various concentration (0, 1, 2.5, 5 and 7.5 g/L), was added to the NB medium. The flasks were incubated in a shaker incubator at 30°C and 150 rpm for 48 hours and cyanide production was measured in the time span 18-34 hours every 2 hours.

2.2 Statistical analysis and design of experiments

The two-way analysis of variance (ANOVA) was chosen as statistical tool to calculate the individual interactions of all the parameters in experimental design (glycine concentration, time and their interaction). The fixed parameters were pH 8, temperature of 30°C and shaking speed of 150 rpm.

More in detail, statistical significance of the process parameters considered for the cyanide production was determined at a probability level of $p < 0.05$.

Additional post hoc tests were performed to explore differences between multiple-group means within the most significant conditions identified. The results of the ANOVA were helpful to implement the CCD-RSM to find the best operating conditions for the maximum cyanide production. The central points of the CCD-RSM (glycine concentration 2 g/L, and the pH value 8.5) were chosen based on the previous results. More in detail, the results of the ANOVA analysis highlighted a significant difference between 1 and 2.5 g/l of glycine, and so a midpoint was chosen. Furthermore, pH values higher than 7-8 (range of cyanide loss as HCN [19]) were studied to verify the possible increase of cyanide concentration. The CCD was used to fit a second-order model (Eq. 3)

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k-1} \sum_{j=1}^{k} b_{ij} X_i X_j + \sum_{i=1}^{k} b_{ii} X_i^2 + \varepsilon \quad (3)$$

where Y is the response variable predicted (cyanide production), X_i and X_j are the input variables (the evaluated factors, pH and glycine concentration, respectively), k is the number of input variables (factors, 2), b_0 is the offset term, b_i is the linear constant, b_{ii} is the second-order constant, b_{ij} is the constant related to the interaction between X_i and X_j and ε is random error. The number of the experiments required for the CCD analysis is calculated from the following equation (Eq. 4):

$$N = 2^k + 2k + cp \quad (4)$$

where cp is the central point number that determines the random error (0, 0), 2^k are the factorial points and are coded in ± 1 notation and 2k are the axial points. The axial points are located at (±α, 0) and (0, ±α), which indicate the distance between the axial and central points. The α is calculated by (Eq. 5):

$$\alpha = [2^k]^{\frac{1}{4}} \quad (5)$$

All variables were studied at five levels (-α, -1, 0, +1, +α). A total of 10 runs were selected with 4 full factorial tests (k =2), 4 axial points, 2 central points (cp =2) and α = 1.414 (Table 2).

Table 2. Factors and levels for the CCD-RSM.

Code	Factor	-α	-1	0	+1	+α
X_1	Glycine (g/L)	0.6	1.0	2.0	3.0	3.4
X_2	pH	7.8	8.0	8.5	9.0	9.2

The statistical analysis of the data was processed in Rstudio software (rsm).

2.3 Bioleaching experiments

In two-step bioleaching *P. aeruginosa* was initially grown in the absence of e-waste in a 1 L flask containing 450 mL of NB medium and 50 mL of bacterial culture stock. The growth conditions for maximum cyanide production were chosen considering the results of the CCD-RSM plan: 1 g/L of glycine was added to the growth medium and the pH for the bacterial growth was set at 8. After 20 hours of bacterial growth, time required for the maximum cyanide production, 1 g/L of WPCBs was added to the bacterial culture and the pH was adjusted to 9 by the addition of 2M NaOH. We used a low WPCBs quantity to be added into the medium because WPCBs contains toxic and harmful substances, which can affect the microbial growth and the ability to produce cyanide of *P. aeruginosa*. Moreover, increasing WPCBs content increase the amount of non-precious metals which can combine with cyanide, generating compounds much more stable than precious metals [26]. The flask was kept in a shaker incubator at 30°C and 150 rpm for 7 days after the addition of WPCBs. Samples were collected every 24 hours to analyse the concentration of Au and Ag. Cyanide concentration and pH were measured during the experiment. The microbial growth was analysed to verify the toxicity of WPCBs on bacterial metabolism.

2.4 Analytical methods

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

The microbial growth was analysed by a direct count on an epifluorescence microscope following the procedure described by Danovaro et al. [27]. A subsample (0.5 mL) was withdrawn periodically and was fixed in a 2% formalin solution. 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 using the following equation to determine the bacteria free in the leaching solution (Eq. 6):

$$Total\ prokaryote\ count/mL = (B.N.\cdot C.O.\cdot d)/mL \quad (6)$$

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

Free cyanide concentration was measured periodically by a spectrophotometer (Aqualytic AL450 Multidirect), with reagent test (Cyanid-11,-12,-13).

The quantification of metal content was performed by the Research Institute – Gruppo C.S.A. S.p.A. which used the method 3052 "microwave assisted acid digestion of siliceous and organically based matrices" and the method 6010D "inductively coupled plasma-optical emission spectrometry". The limit of quantification for Ag is 5 µg/L for the solid samples and 0.02 mg/Kg for the liquid samples and for Au is 10 µg/L and 10 mg/Kg for the solid and the liquid samples, respectively.

As concern the mobilization efficiencies, they were calculated following the equation (Eq. 7):

$$Efficiency\ (\%) = [(C_t)/(C_f+C_s/1000)] \times 100 \quad (7)$$

where C_t (mg/L) is the metal concentration in the leaching solution at t time, C_f (mg/L) is the final metal concentration in the leaching solution and C_s (mg/kg) is the metal concentration in the final solid, after the bioleaching process.

3. Results and discussion

3.1 Cyanide production

Figure 2 shows the effect of both glycine concentration and time on the cyanide production. Overall, the cyanide concentration showed a growing trend and a peak, followed by the cyanide decline with the time increase. This can be due to the degradation and utilization of cyanide by bacteria as carbon and nitrogen source during the late growth phase [23]. Cyanide can also be absorbed on the cell surface, react with metallic ions or exit as HCN [28]. On the experimental results basis, 1 g/L of glycine is the concentration that allowed to obtain the best cyanide concentration (around 10 mg/L) in the shortest time (20 hours), compared with the other concentrations. This condition ensured a bacterial survival rate around 1.24×10^9 bacteria/mL.

The decrease in pH in the first 6 hours could be attributed to the acidification that results from the conversion of glycine to carboxylic acids, such as oxamic acid. The increase in pH after this point was followed by the increase in cyanide synthesis due to the conversion of the intermediate products to cyanide (Eq. 1)[29].

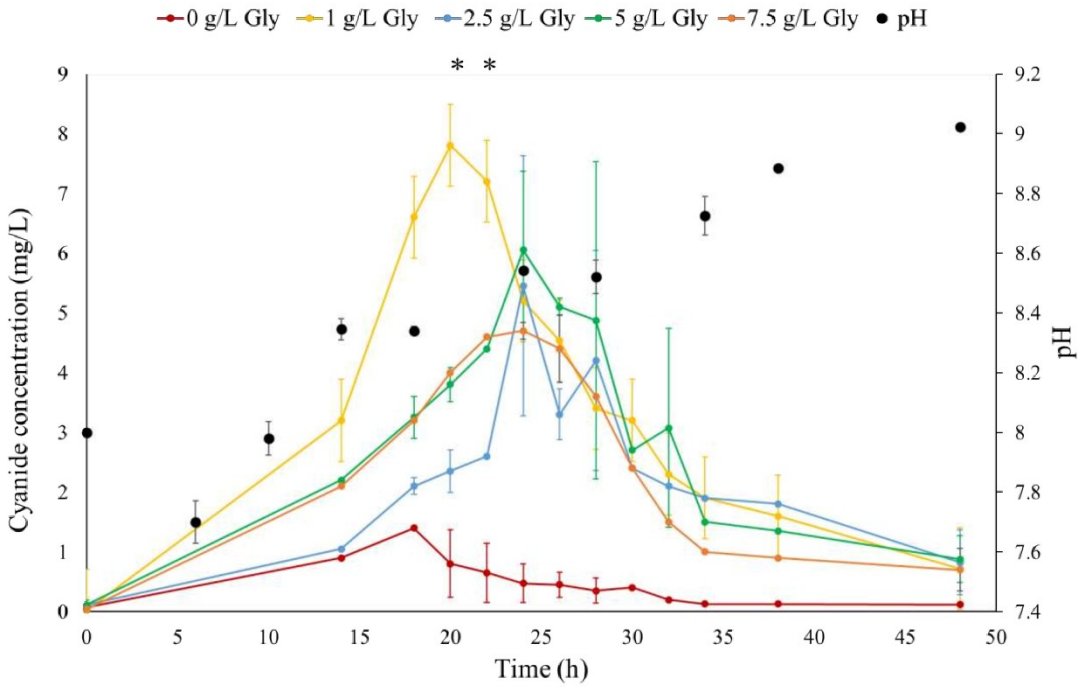

Figure 2. Trend of cyanide production and pH profile at different times and glycine concentration. The lines represent the average between the various real values (n=3 replicates for each sample). The black stars identify the time conditions affected by the glycine concentration where there is a difference statistically significant evaluated by the two-way ANOVA analysis

The effect of the considered factors (time, glycine concentration and their interaction) was supported by the statistical analysis which assessed p-values significantly below 0.05. The trend of cyanide production with 0 g/L glycine was included in the experimental plan since cyanide can be produced from alternative sources, like peptone, yeast or meat extract [13], as confirmed by the detected cyanide concentration around 1 mg/L in the absence of glycine. Anyway, the addition of glycine exerted a positive effect on cyanide production, mainly visible at 20 and 22 hours (as highlighted by the two-way ANOVA analysis which studied the time and glycine effect, resulting in a p-value below 0.05), corresponding to the late logarithmic and the early stationary bacterial growth phase [12,14]. An additional statistical analysis (post hoc tests) was focused on the results at 20 hours (selected as the best condition), to verify the differences between the single conditions of glycine concentration. As summarized in Table 3, the evaluation highlighted the positive effect of glycine addition, compared to its absence. Furthermore, the benefit of 1 g/L of glycine was confirmed, compared to 2.5, 5 and 7.5 g/L which led to a gradual decrease in cyanide concentration. In this regard, Faramarzi et al. (2004) reported that glycine concentrations greater than the optimum are toxic and they may lead to the inhibition of bacterial growth and metabolism [20,30]. This hypothesis is also supported by the not significant difference between results at 5 and 7.5 g/L of glycine (Table 3).

Table 3. Statistical significance resulting from post hoc test performed to compare couples of results at different glycine concentration at 20 hours. *** (statistically significant) NS (not significant)

Glycine conc. (g/L)	Glycine conc. (g/L)			
	1	2.5	5	7.5
0	***	***	***	***
1		***	***	***
2.5			***	***
5				NS

3.2 RSM-based optimization

The previous results showed as the glycine had a significant effect on the cyanide production by *P. aeruginosa*. The next tests were carried out to find the best conditions for the cyanide production evaluating the glycine concentration (between 0.6 and 3.4 g/L) and pH (between 7.8 and 9.2). The results of the 10 runs (Table 4) were statistically analysed to evaluate the effects of the two considered factors.

Table 4. CCD-RSM matrix for the two tested variables.

Experiment	Experimental design	X_1	X_2	Cyanide production (mg/L)
1	Factorial point	-1	-1	9.2
2	Factorial point	1	-1	6.4
3	Factorial point	-1	1	7.2
4	Factorial point	1	1	9.6
5	Central point	0	0	11.6
6	Central point	0	0	12.4
7	Axial point	-α	0	11.6
8	Axial point	+α	0	6.6
9	Axial point	0	-α	12.4
10	Axial point	0	+α	10.0

The cyanide production was modelled as a function of the initial glycine concentration and the starting pH of the medium. The cyanide produced by bacteria metabolism can be expressed by the following equation (Eq.8):

$$CN = 12.00 - 1.74516\,(glycine) - 1.09207\,(pH) + 2.92229\,(glycine)\,x\,(pH) - 1.55633\,(glycine)^2 - 0.513831\,(pH)^2 \quad (8)$$

This quadratic model fits very well with the experimental points with a R^2 of 0.98 (Figure 3).

Table 5 shows the data of the ANOVA analysis. The results showed as the glycine and pH exerted a significant effect on cyanide production. Eq. 8 shows as the glycine concentration and pH have an important negative effect on cyanide production. On the other hand, the interaction between glycine

concentration and pH had a positive effect on bacteria metabolism. Moreover, increasing the pH and glycine concentration the cyanide production decreased (Figure 4).

Table 5. Summary of ANOVA results for RSM.

Sources	Sum of squares	df	Mean square	F-value	p-value
Linear (X_1, X_2)	11.78	2	5.89	16.06	*
Interaction (X_1, X_2)	24.94	1	24.94	68.01	**
Quadratic (X_1, X_2)	10.80	2	5.40	14.73	*
Residual	1.10	3	0.37		
Lack of fit	0.78	2	0.39	1.22	0.54
Pure error	0.32	1	0.32		

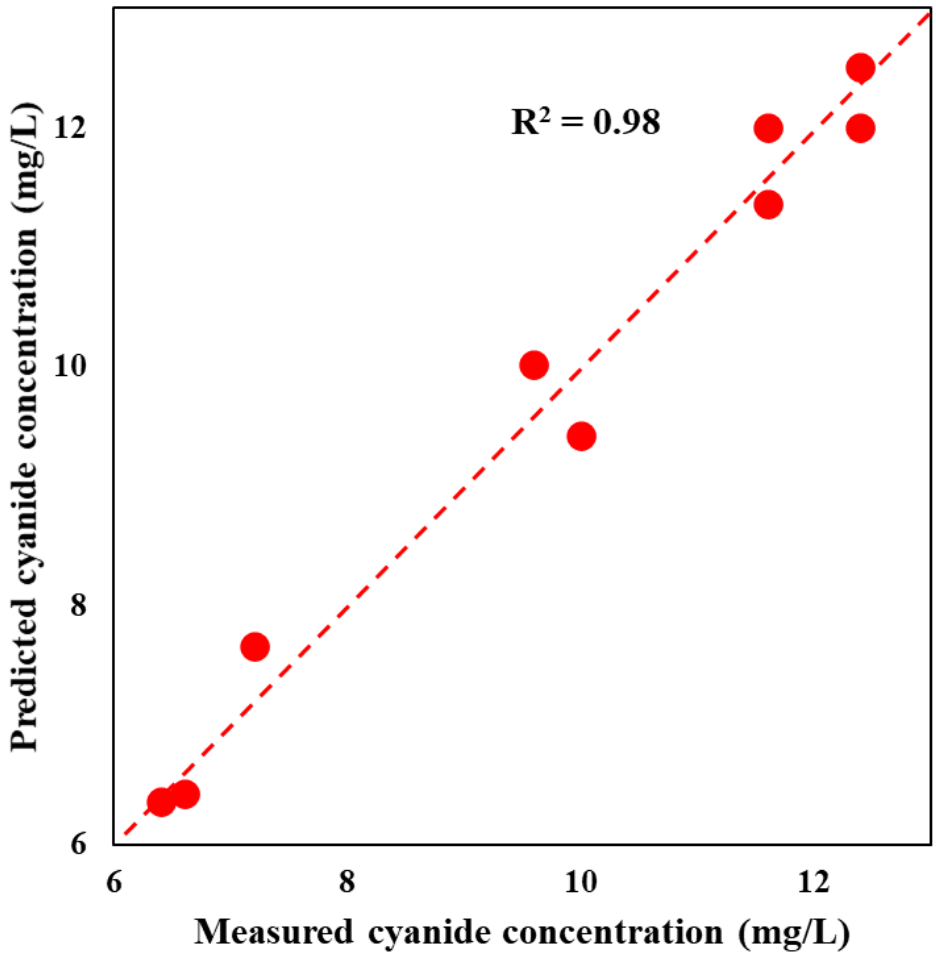

Figure 3. Predicted vs measured plots for cyanide production.

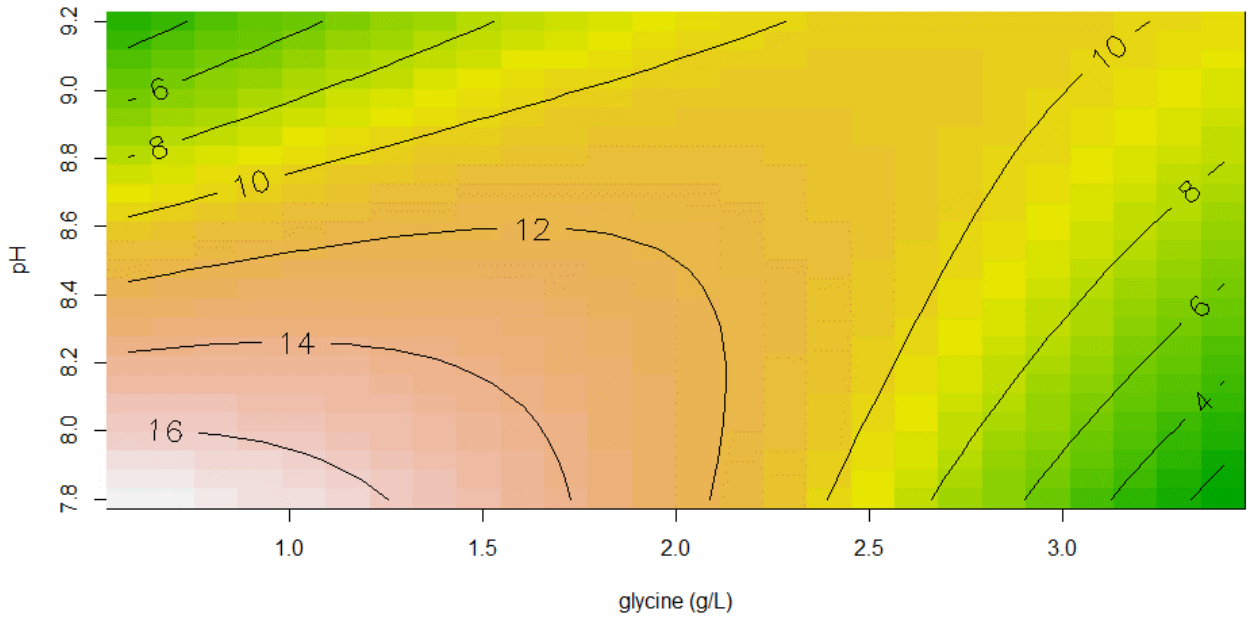

Figure 4. 2D contour plots for cyanide production (mg/L).

3.3 Bioleaching

Following the previous statistical results, the bioleaching experiment was carried out under these conditions: glycine concentration of 1 g/L, pH of 8, and addition of the WPCBs powder (1 g/L) after 20 h when the maximum cyanide concentration was reached.

Figure 5 reports the bacterial growth curve during bioleaching, in order to assess the possible toxicity due to the presence of heavy metals and other toxic pollutants, such as polybrominated diphenyl ethers, in WPCBs [20,26]. After 20 hours of growth in the absence of e-waste, the WPCBs were added to the culture (red arrow, Figure 5). Figure 5 highlights the immediate decrease in bacterial abundance after the addition of WPCBs, confirming the toxic effect of e-waste. Nevertheless, after around 8 hours the bacteria were able to recover and adapt to the new environment and they started growing again, entering in the stationary phase after around 24 hours from the addition of WPCBs. The comparison with the microbial growth of the control samples (without the addition of WPCBs) showed that in the presence of WPCBs the microbial abundance was lower. In the first 20 hours of growth the two curves were comparable, but the addition of WPCBs blocked the microbial growth and, despite the ability of bacteria to adapt to the new environment, the total number of bacteria was lower, 1.94×10^9 instead of 2.37×10^9 bacteria/mL. However, the stationary phase lasted longer, around 4 days, whilst in the control after 40 hours the death phase started. The difference between the death phase in the control and in the experimental could be due to a change in bacteria metabolism. In the

new environment *P. aeruginosa*, after a period of adaptation, has grown slower compared to the control with a less quick nutrient consumption rate.

In the control a sharp decrease in the microbial abundance was observed as the medium conditions become less favourable for the growth, the cells become less metabolically active and the number of dying cells continues to rise [29].

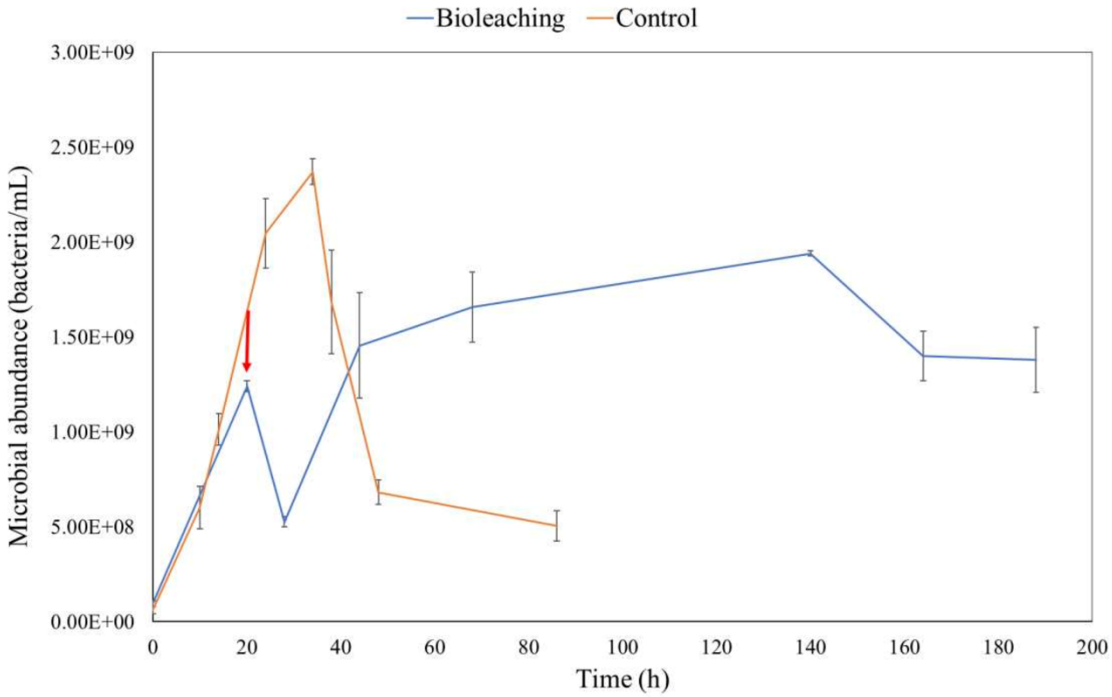

Figure 5. Variation in microbial growth (bacteria/mL) of the control and the bioleaching sample. The red arrow indicates the addition of WPCBs at the culture medium.

The cyanide concentration profile of the culture showed a peak of cyanide production 20 hours later the addition of WPCBs (Figure 6), corresponding to the onset of the logarithmic phase of bacterial growth. After that, there was a decline in cyanide concentration which could be attributed to the utilization of cyanide by bacteria or to the volatility of HCN.

The change in pH was observed during bioleaching of metals (Figure 6). The pH was set to 9 before the addition of WPCBs in order to favour the availability of cyanide ion for metals complexation. Indeed, cyanide concentration in the solution is highly dependent on pH [19]. The equilibrium between cyanide and gaseous HCN, showed in Eq. (2), indicates that cyanide is favoured at high pH, but bacterial growth is better at physiological pH, therefore the pH was risen only after the bacteria entered the logarithmic phase.

Considering the trend of pH, it showed a gradual decrease in the first day after the addition of WPCBs, and this could be due to the toxic and inhibitory effect on bacterial growth. Thereafter, an increase from 8.3 to 9.3 was observed over the course of the other 6 days (Figure 6), and this represents the

reaction of HCN with metals present in e-waste [20]. Hence, two-step bioleaching takes place when the availability of cyanide ion is favourable.

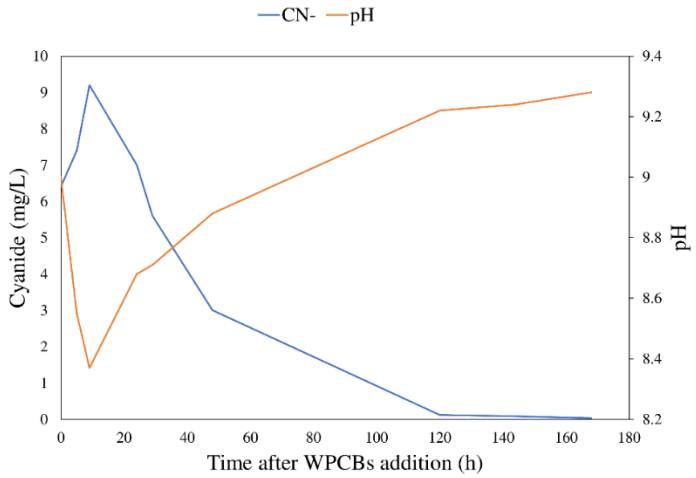

Figure 6. Changes in cyanide production and pH profile during bioleaching after WPCBs addition.

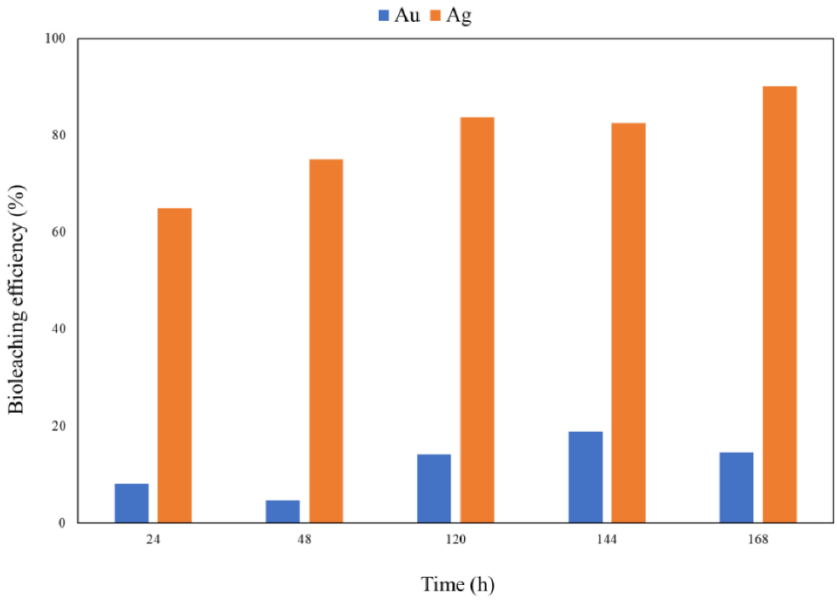

Figure 7. Ag and Au bioleaching efficiencies (%) during time.

Figure 7 showed the leaching profile of Ag and Au at different times (Figure 7). *P. aeruginosa* turned out to be a suitable microorganism for Ag bioleaching. Ag cyanidation follows a reaction similar to Au mobilization, with the formation of the dicyanoargentate during the bioleaching process (Eq. 9) [17,20,31].

$$4Ag + 8CN^- + O_2 + 2H_2O \rightarrow 4Ag(CN)^-_2 + 4OH^- \quad (9)$$

To assess a possible increase in metal mobilization with time the bioleaching test was performed for 7 days. The highest Ag mobilization was obtained after 7 days with an efficiency of 90%. An increasing trend in Ag mobilization was observed from 24 to 168 hours.

These results are very promising compared to the literature. Indeed, Ag bio-cyanidation has been little studied to date and in the previous works were reported extremely low efficiencies. The present study observed higher Ag mobilization (90%) as compared the previous studies (Table 6). Pradhan et al. (2012) described the abilities of different bacteria to mobilize metals and reported an Ag dissolution between 3.5 and 7% with *P. aeruginosa* and *C. violaceum*, respectively. Other *Pseudomonas* strains were tested for Ag bioleaching. *P. plecoglossicida* was reported to mobilized 5% Ag as dicyanoargentate from jewellery waste [17], a new strain, named *P. chlororaphis,* allowed to mobilize 12% of Ag in 72 hours [32], whilst *P. balearica* was reported to dissolve 33.8% of Ag [20]. A method using a CN^- producer *Pseudomonas* biofilm to leach Ag was also proposed, with a leaching efficiency of 14.7% after 7 days [31].

The highest Ag mobilization may be due to the presence of less amount of Cu in our e-waste, due to the first bioleaching, making the cyanide available for Ag bioleaching. In this work we find out that even with a lower glycine concentration (1 g/L) compared with previous studies we have been able to obtain a high Ag biocyanidation by *P. aeruginosa*.

Table 6. Comparison of maximum Ag bioleaching by different bacterial culture reported in the literature.

Bacterial culture	Glycine concentration	Maximum cyanide production	Maximum Ag bioleaching	Reference
P. aeruginosa	5 g/L	n.a.	3.5%	[33]
C. violaceum	5 g/L	n.a.	7%	[33]
P. plecoglossicida	5 g/L	~3 mg/L	5%	[17]
P. chlororaphis	4.4 g/L	~15 mg/L	12%	[32]
P. balearica	5 g/L	n.a	33.8%	[20]
P. balearica	6.8 g/L	n.a.	41.6%	[21]
Pseudomonas strains	4.4 g/L	~5 mg/L	14.7%	[31]

Since in the literature *P. aeruginosa* was successfully employed for Au bioleaching, the effect of bio-cyanidation on Au mobilization was analyzed. Au bioleaching showed a lower efficiency rate compared to Ag, around 20% after 144-168 hours. Even in this case a little effect of the time can be observed, with an increase in Au mobilization from 24 to 144 hours (Figure 7). The lower Au mobilization compared to Ag can be due to the pH used for the bioleaching test. A pH between 9 and 10 favors Ag bioleaching whilst Au mobilization required higher pH, between 10 and 11 [34], but the

possibility to work at a lower pH allowed to obtain pure Ag. Also, the limited amount of cyanide produced by *P. aeruginosa* could be a reason of the low Au bioleaching efficiency. The non-precious metals or the residual copper present in WPCBs powder had higher metal activity than Au and can easier bind cyanide to form more stable complexes with cyanide and interfere with Au cyanidation [14,26]. Considering the high Ag mobilization efficiencies, Au bioleaching could be improved by conducting a first bioleaching process to remove Ag, followed by Au bio-cyanidation. The use of *P. aeruginosa* for Au bioleaching in other studies showed variable efficiencies, comparable to the one we obtained. Pradhan et al. [33] reported Au dissolution around 50%, with *P. aeruginosa* at 1% (w/v) solid concentration. Au efficiency increased to around 80% when *P. aeruginosa* was mixed and cultured with *C. violaceum*. Natarajan and Ting (2015) described the two-step bioleaching of 0.5% WPCBs with *P. aeruginosa*. They obtained a maximum Au dissolution of 5.8% and a maximum cyanide production of 10 mg/L, which both increased to 10.2% and 15 mg/L respectively, when *P. aeruginosa* was mixed with *C. violaceum* [19]. Highest Au mobilization efficiencies were reported by Gorji et al. (2020) from pure Au powder and Au ore with *P. aeruginosa*. Performing the two-step bioleaching method Au mobilization efficiency was 31% which increased to 69% when the pH was raised from 8 to 10 [13].

The different mobilization of metals may be attributed to the heterogeneous nature of e-waste. Comparing metal recoveries directly from bioleaching studies is not easy since the metal composition of electronic residues is not always the same and varies with age, origin and producer and the acid digestion protocols used by the different researchers. Other factors such as the growth medium, the bioleaching time and the composition of toxic metals/non-metallic elements in the e-waste also influence cyanide production and consequently the recovery of metals [1].

4. Conclusions

The present study developed a high performance innovative biotechnological process aimed at filling the gap of scientific literature regarding the Ag recovery from WPCBs. Overall, the process includes the following phases:

- The cyanide production by *P. aeruginosa* (initial pH 8 and glycine concentration 1 g/L)
- The mechanical pre-treatment for crushing and plastic removal
- The bioleaching by *At. ferrooxidans* bacteria for the preliminary removal of Cu and Zn
- The two-step bioleaching of Ag from WPCBs by *P. Aeruginosa* (1g/L WPCBs powder added after 20 hours, pH increased to 9 during the second step)

The developed conditions allowed an Ag leaching efficiency of 90%. In addition to Ag bioleaching, P. *aeruginosa* was able to mobilize 20% of Au, confirming to be a suitable organism for precious

metal bioleaching. The Au recovery is not very satisfactory but may be improved by the further optimization of the cyanidation process or by separating the bioleaching process for the two metals, to avoid the interference of Ag with Au cyanidation.

The process does not present any potential environmental risks but represent an interesting alternative to solve the environmental sustainability issues of the most common chemical leaching.

Although the treated WPCBs concentration in bioleaching is lower than in chemical approach, this innovative treatment reduces the problems associated with the treatment of waste flows (rich in toxic cyanide) which characterize the most common chemical processes. Indeed, the non-reacted cyanide is used by bacteria metabolism avoiding its possible release in the environment. These results are promising to develop high-efficiency Ag bio-cyanidation as an industrial application of bioleaching in alkaline conditions, with a view to a transition to a circular economy.

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5. Products

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Recovery of precious metals from printed circuit boards by cyanogenic bacteria: Optimization of cyanide production by statistical analysis

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Figure 8. Article published on Journal of Environmental Chemical Engineering

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Chapter 3:
EXTRACTION OF PRECIOUS METALS FROM SMALL SIZE PRINTED CIRCUIT BOARDS BY CYANOGENIC BACTERIA

Abstract

The present work aimed to verify in more details the extraction of precious metals (Au and Ag) with the small wPCBs size (<0.25 mm). Firstly, the cyanide production was optimized by testing different concentration of the components of the nutrient broth and adding salts ($MgSO_4$ and Na_2HPO_4). The results suggested that the broth composed of 5 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract and 5 g/L NaCl with the addition of glycine and small amount of $MgSO_4$ enhanced cyanide synthesis. Four different techniques for the bioleaching were performed: the batch fermentation, the spent medium, the fed-batch and the two-steps wPCBs addition. The results showed different cyanide synthesis efficiencies, with 20 mg/L of cyanide produced during the batch fermentation and around 3 mg/L produced during the spent medium fermentation. These results were not reflected in the bioleaching efficiencies, as the same extraction efficiencies were observed for all the processes, with 90% Ag extraction and 20% Au extraction. These results highlighted the higher affinity of cyanide for Ag compared to Au, as Ag was extracted with high efficiencies even in the presence of low cyanide concentrations. A possible strategy for increase the extraction of Au can be the previous removal of Cu, that is one of the most interfering metals, or of Ag, thus the synthetized cyanide can complex more selectively with Au.

Content:

1. Introduction

Electronic waste (e-waste) production is continuously increasing globally. In 2019 the global annual production of e-waste was reported to reach around 53.6 million metric tons (Mt) (Forti et al., 2020; Li et al., 2020). The environmental issues related to e-waste are of great concern, as the electronic devices are made with valuable resources mixed with heavy metals and hazardous materials (Kaya, 2019; Li et al., 2020). The presence of heavy metals and hazardous materials makes these waste as potential environmental contaminants. However, for the content of valuable resources, e-waste is considered as an urban mine, as it represents an important source of raw materials (Kaya, 2019; Kumar et al., 2018; Li et al., 2020). The waste printed circuit boards (wPCBs) represent one of the most relevant fraction of e-waste due to their composition, made by valuable metals content and hazardous substances (Becci et al., 2020a; Cui and Zhang, 2008; Ghosh et al., 2015).

The current technologies for precious metals recovery from e-waste include pyrometallurgy, hydrometallurgy and biohydrometallurgy (Becci et al., 2020a; Li et al., 2020). Pyrometallurgy is based on the use of incineration or smelting technologies and requires high temperatures, resulting in high energy consumption and pollution to the environment (Becci et al., 2020a; Li et al., 2020; Rocchetti et al., 2013). The hydrometallurgical technologies use chemical leaching agents for metals dissolution, such as cyanide, with consequent environmental pollution and contaminated wastewater (Becci et al., 2020b; Behnamfard et al., 2013; Merli et al., 2022), or thiosulfate and thiourea, which are more sustainable than cyanide leaching but require a great amount of reagents (Becci et al., 2020b; Merli et al., 2022).

Bio-hydrometallurgical technologies are based on the exploitation of metabolites produced by microorganisms to dissolve metals (Willner and Fornalczyk, 2013). Currently, bio-hydrometallurgical strategies have gained increasing interest in the field of metal extraction form e-waste, due to the lower costs and to a greater environmental sustainability compared to the chemical leaching (Becci et al., 2020a; Cui and Zhang, 2008; Pham and Ting, 2009). In more details, precious metals bioleaching is performed by microorganisms capable of producing cyanide (Chi et al., 2011; Kaya, 2019; Merli et al., 2022). The employment of bacteria, with respect to chemical cyanide, has the advantage of avoiding cyanide loss in the environment, as the cyanogenic bacteria are also cyano-lytic and capable of cyanide degradation (Merli et al., 2022; Yew et al., 2020).

The production of cyanide depends on the medium in which these bacteria are grown; peptone and yeast extract are the main source of nutrition for *Chromobacterium violaceum,* essential for the growth (Brandl et al., 1999; Chi et al., 2011; Pham and Ting, 2009; Tran et al., 2011), while *Bacillus megaterium* and *Pseudomonas aeruginosa* also require the addition of meat extract and NaCl (Gorji et al., 2020; Merli et al., 2022; Zhou et al., 2020). Glycine, being cyanide precursor, is fundamental

for cyanide synthesis. The cyanide production can be increased by adding nutrient salts, such as $FeSO_4$ and $MgSO_4$, which enhance the catalysis of cyanide-generating enzyme with the addition of sodium hydrogen phosphate (Na_2HPO_4) to avoid the free cyanide ions consumption (Gorji et al., 2020; Li et al., 2015; Tran et al., 2011; Zhou et al., 2020).

In this work cyanide synthesis was optimized by changing the ratio of the medium components and by testing the influence of $MgSO_4$ salt addition, to find the best conditions for maximum cyanide synthesis. Furthermore, 4 different approaches were examined for Ag and Au bioleaching by *P. aeruginosa*: two-step batch fermentation, spent medium, fed-batch and two-step wPCBs addition.

The specific objectives of this work are: (i) to examine cyanide production with the addition of different salts and to select the conditions which produce the highest cyanide concentration for bioleaching studies; (ii) to compare four different methods for Au and Ag bioleaching: batch fermentation, fed-batch fermentation, two-step wPCBs addition and spent medium leaching.

2. Materials and methods

2.1 Preparation of waste printed circuit boards

The wPCBs for the leaching tests were prepared as reported by Becci et al. (2020). The procedure comprises a mechanical treatment (shredding and grinding) and a density-based separation with NaCl for plastics removal. The Cu can bind cyanide and interfere with the bioleaching process, so the wPCBs were treated with 50 g/L Fe^{3+} for Cu leaching (Becci et al., 2020a, 2019). After the pre-treatment the wPCBs were crushed another time to obtain different dimensions and passed throughout a sieve (Ø=0.25 mm) to select the finer fraction (0.1<Ø<0.25 mm).

2.2 Cyanide optimization

The bacterial strain employed for the bioleaching test was *P. aeruginosa* (PAO1-T), provided by the microbiology laboratory of Polytechnic University of Marche. The culture medium (nutrient broth, NB) was composed by peptone (5 g/L), meat extract (1 g/L), yeast extract (2 g/L) and NaCl (5 g/L). The bacterial colony were incubated in a 100 mL Erlenmeyer flask and kept at 30°C and 150 rpm in a shaker incubator.

Firstly, it has been analysed the effect of some of the nutrient medium components, varying their quantities, to study the influence of different ratio on bacterial metabolism and cyanide production. The cyanide optimization experiments were conducted following a full factorial plan to investigate the effects of peptone concentration (1, 10 g/L), NaCl (0, 10 g/L), glycine (1, 5 g/L) and $MgSO_4$ (0.25-0.5 g/L) and the central points (peptone and NaCl 5 g/L, glycine 2.5 g/L and $MgSO_4$ 0.25 g/L).

10 mL of the pre-grown *P. aeruginosa* were added in a 250 mL Erlenmeyer flask containing 90 mL of the different medium.

After that, to better understand the effect of $MgSO_4$ on cyanide production, its influence was analysed in more detail, alone and in combination with Na_2HPO_4 (1:10 ratio). Na_2HPO_4 can be required to make complex with the Fe and Mg ions and prevent them from precipitation or complexation with cyanide (Gorji et al., 2020). Therefore, in the present study, both $MgSO_4$ and Na_2HPO_4 were applied, and the influences of these salts on the cyanide concentration was investigated at the best pH and glycine concentration derived from the previous optimization tests.

These experiments allowed to find out the best conditions for cyanide production to conduct the further bioleaching process.

2.3 Bioleaching process

For the bioleaching process we compared four different methods.

1) **Batch fermentation**. In the two-step batch fermentation, the microorganisms are grown in the absence of e-waste to reach the maximum cell density and cyanide production, and, after that, different concentrations of e-waste are added for metal mobilization (Gorji et al., 2020; Li et al., 2015; Merli et al., 2022; Natarajan and Ting, 2015, 2014; Pradhan and Kumar, 2012; Zhou et al., 2020). In this experiment *P. aeruginosa* was grown in the NB medium enriched with 2.5 g/L of glycine and 0.25 g/L $MgSO_4$ at pH 8 (the best identified condition in the previous experiments). After 26 hours, the pH was raised to 9 by the addition of 2 M NaOH and the wPCBs (1 g/L) were added to the bacterial culture.
2) **Spent medium**. In the spent medium method the bacteria are removed from the culture medium after reaching maximum cell density and maximum cyanide production, and only the bacterial cell-free metabolites are used for the leaching process (Liu et al., 2016; Natarajan and Ting, 2015). This technique was made by growing the bacteria in the same condition of the batch fermentation for 26 hours, then the bacteria were removed from the medium by centrifugation. The wPCBs (1 g/L) were added to the spent medium and the pH was adjusted to 9.
3) **Fed-batch**. In fed-batch fermentation, fresh medium was added to the culture, after the maximum cell density and cyanide production were reached. The advantage of feeding during cultivation is that it allows to overall achieve higher cell densities and prolongs product synthesis when the desired product is positively correlated with microbial growth (Shin et al., 2013). The process started as a batch fermentation which consumes substrates and nutrients. After 26 hours of bacterial growth the wPCBs were added (1 g/L), and the process of feeding

with the fed solution (identical to the initial solution, but with a pH risen to 9) was started and proceed for 50 hours, at around 5 mL/h speed.

4) **Two steps PCBs addition**. The addition of wPCBs at two different time of the process has never been tested. After the first addition of wPCBs the number of bacteria decrease and the cyanide reaches its maximum peak (Merli et al., 2022), therefore the addition of more wPCBs at this point could benefit from the maximum cyanide yield. First the bacteria were grown in the same condition as the batch fermentation for 26 hours. After that, the pH was risen to 9 and half of the wPCBs was added to the culture (0.5 g/L). After other 8 hours, the remaining part of PCBs (0.5 g/L) was added to the medium.

All the bioleaching tests were conducted in 1L flask with a volume of 500 mL (450 mL medium and 50 mL withdrawn from the bacterial stock), except for the fed-batch in which the initial volume was 250 mL (225 mL medium and 25 mL withdrawn from the bacterial stock) and the final volume of around 500 mL was reached after 50 hours from the start of the feeding process. A total volume of 1 g/L of wPCBs was added in all the experiments with size $0.1<Ø<0.25$ mm The bioleaching analysis were conducted for 7 days after the addition of wPCBs. Samples were withdrawn for metals analysis every 24 hours. Cyanide concentration was analysed every 4 hours in the first two days and then every 24 hours.

3. Results

3.1 Cyanide optimization

The optimization of cyanide synthesis was studied by changing the ratio of the medium components and by testing the influence of $MgSO_4$ and Na_2HPO_4 addition.

In a previous study it was reported as the modification of the culture medium could enhance cyanide production by cyanogenic bacteria, compared to the commercial culture medium (Wang et al., 2021). In this study, the best cyanide production was obtained with the central points, which represent the composition of the commercial culture medium (peptone 5 g/L, meat extract 1 g/L, yeast extract 2 g/L and NaCl 5 g/L, indicating that was the best medium for bacterial growth and metabolism, enriched with 2.5 g/L of glycine and 0.25 g/L of $MgSO_4$ (Table 2).

Table 2. Cyanide production testing different ratio of NB medium components

Peptone	NaCl	Glycine	$MgSO_4$	Maximum cyanide production (mg/L)
1	0	1	0	4.2
10	0	1	0	6
1	10	1	0	1.4
10	10	1	0	2.3
1	0	5	0	4.8
10	0	5	0	3

1	10	5	0	1.2
10	10	5	0	1.1
1	0	1	0.5	2.5
10	0	1	0.5	3.4
1	10	1	0.5	2.2
1	0	5	0.5	5.2
10	10	1	0.5	1.9
10	0	5	0.5	3
1	10	5	0.5	0.9
10	10	5	0.5	1.2
5	5	2.5	0.25	7
5	5	2.5	0.25	3.6
5	5	2.5	0.25	7.4
5	5	2.5	0.25	6.4

Cyanide synthesis can be enhanced by adding low amount of metal ions to the culture medium, as this was reported to enhance the enzymatic process (Li et al., 2015; Tran et al., 2011; Wang et al., 2021). To verify this hypothesis the influence of $MgSO_4$ was studied in more details alone with glycine and in combination with Na_2HPO_4. In previous works it was reported that the addition of $MgSO_4$ and $FeSO_4$ to the medium was found to be equally effective for cyanide generation and Au bioleaching by *C. violaceum* (Li et al., 2015; Tran et al., 2011) and *P. aeruginosa* (Gorji et al., 2020). On the other hand, the presence of Na_2HPO_4 and $Pb(NO_3)_2$ further enhanced cyanide generation and favoured copper bio-dissolution, but it was not effective for Au leaching (Tran et al., 2011). Our results showed that the addition of $MgSO_4$ significantly increased cyanide production only in combination with 2.5 g/L of glycine, whilst in the other tested conditions cyanide synthesis was lower compared to the cultures with 1 g/L glycine alone. In contrast with what reported by Tran, in our work the addition of Na_2HPO_4 did not enhance the cyanide production but on the contrary decreased it (Figure 1).

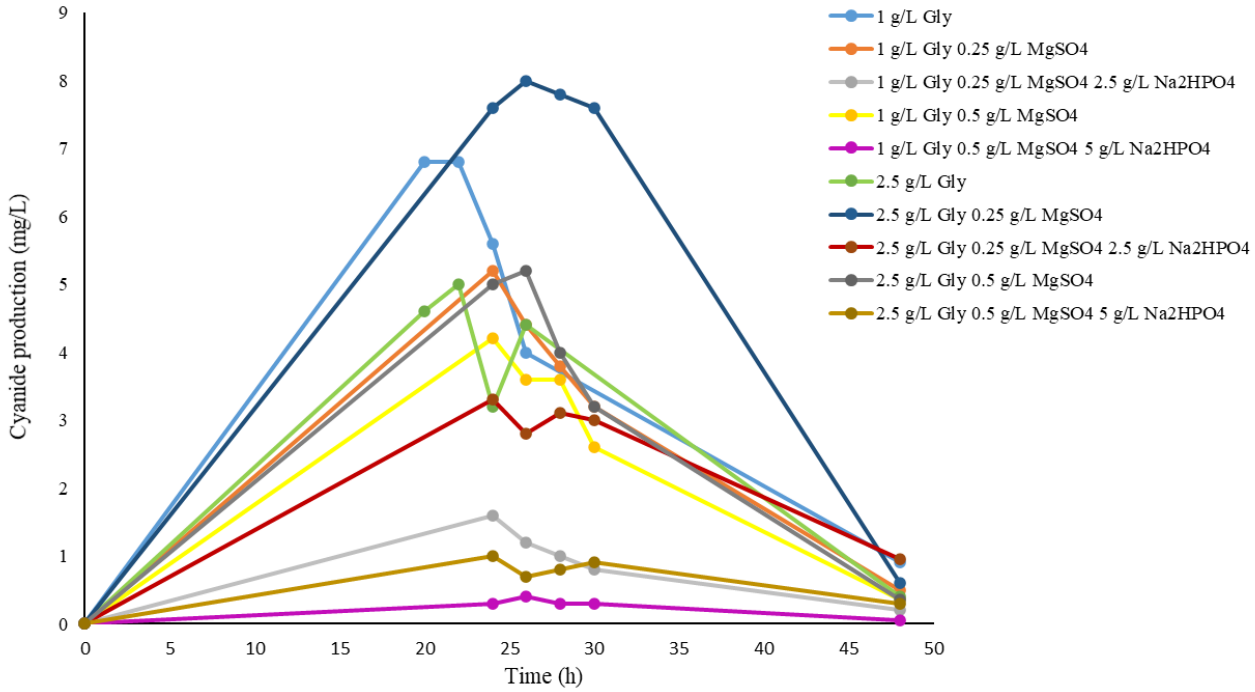

Figure 1. Cyanide production optimization in the presence of $MgSO_4$ and Na_2HPO_4

3.2 Bioleaching

The conditions for the bioleaching test (2.5 g/L glycine and 0.25 g/L $MgSO_4$) were selected from the previous experiments, and 4 different methods (batch fermentation, spent medium, two-steps wPCBs addiction, fed batch) were tested.

Furthermore, for these bioleaching processes a small size wPCBs was employed. Au extraction was reported to be limited by the particle size dimension, which does not allow a good mixing of the material (Birloaga et al., 2013). The small size should offer a large metal exposure area, promoting the contact between the wPCBs and the leaching agent (Tripathi et al., 2012; Birloaga et al., 2013). Cyanide production was analysed during the bioleaching processes (Figure 2).

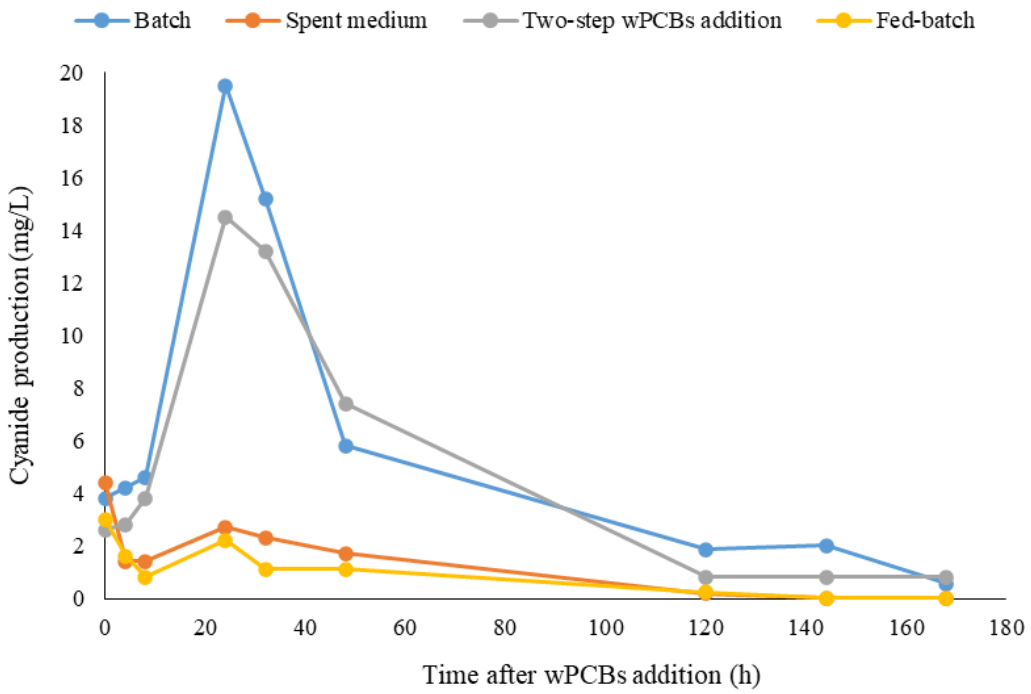

Figure 2. Cyanide production during the bioleaching process

The maximum cyanide concentration was obtained 24 hours after the addition of wPCBs. The highest cyanide synthesis was observed in the batch fermentation and in the two-step wPCBs addition, around 20 and 14 mg/L, respectively. In the other processes cyanide concentration decreased after the addition of wPCBs, in conjunction with the removal of bacteria and the start of the feeding process (time 0, Figure 2). In the spent medium process the removal of bacteria prevented the further increase in cyanide production, as it was produced by bacteria. On the other hand, a decrease in cyanide concentration was observed during the process confirming that the cyanide was employed for the chemical reaction for precious metals leaching. During the fed-batch fermentation the constant addition of fresh medium did not allow the bacteria to increase the cyanide production neither in the short nor in the long term. Production of cyanide by *P. aeruginosa* with fed-batch process for a bioleaching purpose has never been investigated. Anyway, fed-batch method was widely investigated for rhamnolipid production and compared to batch method (Avili et al., 2012). It was reported that the exclusion of a nutrient from the culture medium and the gradual introduction during the fermentation stimulated rhamnolipid production. In the case of cyanide synthesis the responsible amino acid is glycine, but it was reported that not all the glycine was consumed during the bioleaching process, making it a fundamental but non-limiting nutrient for the process (Gorji et al., 2020). Furthermore, we observed in a previous work that a high glycine concentration can be toxic for bacteria and results in a lower cyanide production compared to the lowest glycine concentration (Merli et al., 2022). Therefore, for the fed-batch fermentation during the fermentation was supplied the complete medium, which assess the best growing and cyanide synthesis conditions, and not only

one nutrient (glycine). The failure in cyanide increase may be because the bacteria are subjected continuously to a new culture condition and are not stimulated to produce cyanide, or it could be lost by volatilization.

However, the results of Au and Ag bioleaching showed no significant differences between the extraction efficiencies of the various processes (Figure 3). In more details, Ag extraction showed an increasing trend in the first 2 days, reaching 80-100% extraction efficiency in all the experiments, while Au extraction was the same in all the tested methods, with efficiency around 20% (Figure 3).

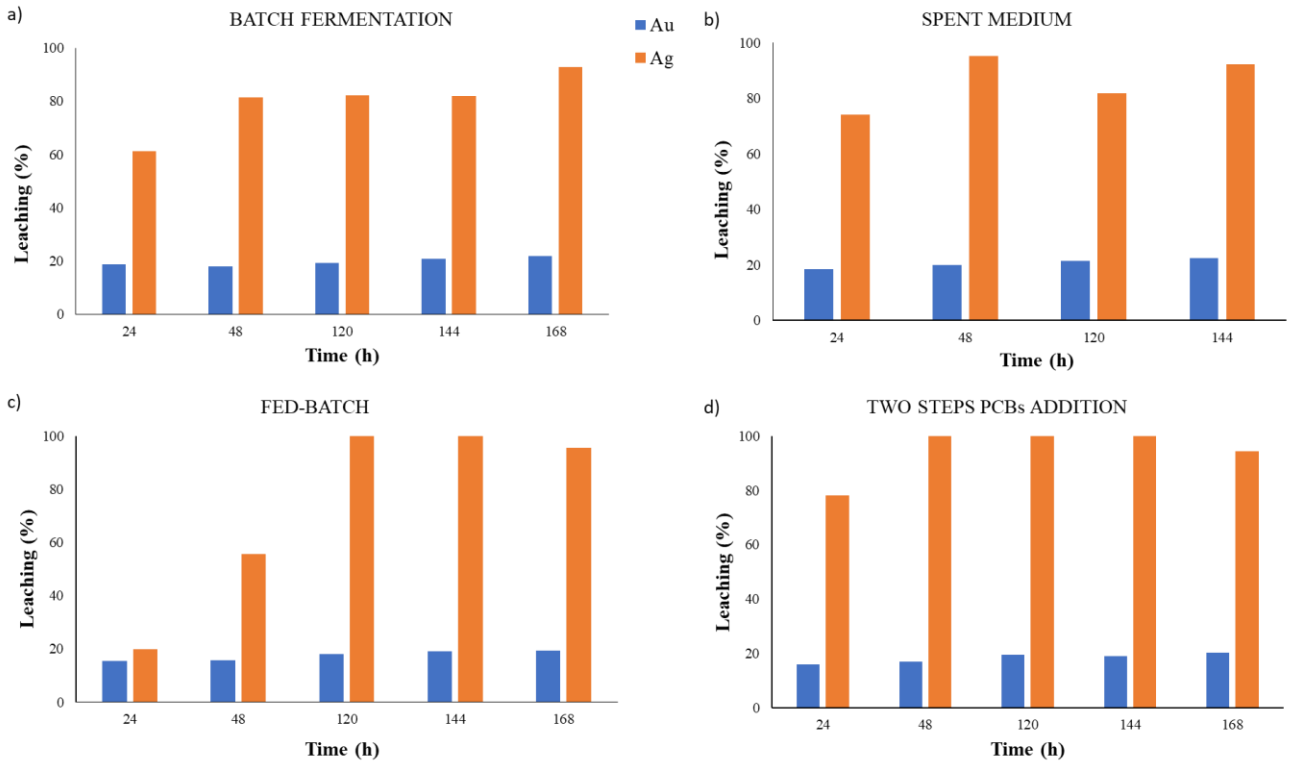

Figure 3. Metals extraction efficiencies with different bioleaching methods

The differences in cyanide concentration did not reflect the bioleaching efficiencies, since no differences were seen between the batch process (Figure 3a), in which 20 mg/L of cyanide were synthetized by bacteria, and fed-batch fermentation (Figure 3c) or spent medium (Figure 3b) processes, in which the concentration of cyanide after 24 hours was around 2 mg/L. A hypothesis to this results could be that the affinity of cyanide for Ag was greater than Au, and the higher concentrations of metal in the initial solid have allowed an almost total extraction of Ag even in the presence of low concentrations of cyanide. Concerning Au, an explanation could be that the extra cyanide bonded other metals, wearing away, in fact the selectivity of cyanide for Au is smaller rather than Cu and the presence of Cu in the solution caused a decrease in Au recovery. Furthermore, cyanide can make complexes with Fe, Zn, Ni and Ag faster than Au, making Au recovery more challenging (Arshadi and Mousavi, 2015).

These results support the observation reported in our previous work on the bioleaching with the big wPCBs size (>0.5 mm) (Merli et al., 2022). The resulted extraction efficiencies of precious metals were comparable, suggesting that there is no correlation between the size of the wPCBs and the extraction of the metals with cyanide.

4. Conclusions

The present work aimed to verify the extraction of precious metals in the presence of a small wPCBs size. The cyanide production was studied varying the nutrient broth composition and adding salts. The results suggested that the broth composed of 5 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract and 5 g/L NaCl with the addition of glycine was the best combination to enhance cyanide synthesis and that this increase in production was further exacerbated by the addition of a small amount of $MgSO_4$. The comparison of the four different methods for the bioleaching resulted in the same precious metals extraction efficiencies for all the processes, even if cyanide production was remarkably higher for the batch fermentation process. Considering these results, the batch fermentation should be preferred because it guarantees good extraction efficiencies (in particular for Ag) with less efforts and energy demand and reagents consumption. Anyway, these results confirmed the good affinity of cyanide for Ag and the high extraction efficiencies, irrespective for the fermentation conditions. Concerning Au, a possible strategy to increase the recovery can be the addition of an extra pre-treatment for the total removal of Cu, that is one of the most interfering metals. Furthermore, another possibility to be verified is bioleaching processes in series. A first bacterial leaching, which extract Ag with high efficiency, can be followed by another bioleaching process, thus the cyanide produced in the second process can focus more selectively on Au extraction.

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Chapter 4:
NON-TOXIC, HIGH SELECTIVITY PROCESS FOR THE EXTRACTION OF PRECIOUS METALS FROM WASTE PRINTED CIRCUIT BOARDS

Abstract

The present work focused on the extraction of gold (Au), silver (Ag) and palladium (Pd) from electronic waste using a solution of ammonium thiosulfate. Thiosulfate has been used as a valid alternative to cyanide for the extraction of precious metals, due to its non-toxicity and high selectivity. The interactions between sodium thiosulfate, total ammonia/ammonium, precious metal concentrations and the waste printed circuit boards (WPCBs) particle sizes were studied by the response surface methodology (RSM) and the principal component analysis (PCA), to maximize precious metal mobilization. Au extraction reached high efficiency with a granulometry less than 0.25 mm, but the consumption of reagents was high. On the other hand, Ag extraction did not depend either on thiosulfate/ammonia concentration or the granulometry of WPCBs and it showed efficiency of 90% also with the biggest particle size (0.50<Ø<1.00 mm). Pd extraction, similarly to Au, showed the best efficiency with the smallest and the medium WPCBs size, but required less reagents compared to Au. The results showed that precious metals leaching is a complex process (mainly for Au, which requires more severe conditions for the achievement of high extraction efficiencies) correlated with reagent concentrations, precious metal concentrations and WPCBs particle sizes. These results have a great potentiality, suggesting the possibility of a more selective recovery of precious metals based on the different granulometry of the WPCBs. Furthermore, the high extraction efficiencies obtained for all the metals are promising in the perspective of large-scale applications.

Content:

1. Introduction

A relevant fraction of the electronic industry waste is constituted by the waste printed circuit boards (WPCBs), which represent a 5% of all the electronic waste (e-waste) and contain substantial amounts of strategic metals. These metals often exceed the concentration in natural minerals (Liu et al., 2016; Wu et al., 2017). This is the case of Au, which shows concentrations in WPCBs up to 70%, greater than that in the ores (Liu et al., 2016; Wu et al., 2017). Therefore, the recovery of strategic metals from e-waste can be promising for the conservation of resources since it delays the exhaustion of metals from ores and also avoid the environmental, economic and social problems of mining (Pham and Ting, 2009). Furthermore, the implementation of techniques to recover metals from this kind of waste is considered crucial for a more effective waste management (Cui and Zhang, 2008; Ha et al., 2014). Traditional methods for e-waste management, such as landfilling, incineration and the exportation to underdeveloped countries (where safety standards are not complied with), can cause relevant pollution with harmful effects for atmosphere due to the gases resulting from thermal treatments, and for soil and water due to the possible dissolution of heavy metals (Akcil et al., 2015; Islam et al., 2020). The proper management of e-waste can overcome these problems and preserve the health of people and environment (Islam et al., 2020) with a possible reduction of air and water pollution up to 80% (Kaya, 2019).

Hydrometallurgy is an approach used in the field of extractive metallurgy to obtain precious metals from ores and from e-waste, performed by leaching the target metal using a variety of chemical reagents. Cyanide is one of the most common solutions for the leaching of precious metals, used for over a century in the mining industry for its advantages, like high efficiency and low consumption rate (Komnitsas and Pooley, 1991; Kulandaisamy et al., 2003; Zhang et al., 2012). However, the main problem of cyanide use is its toxicity. Therefore, the research has focused on the identification of alternative lixiviants, such as thiourea, thiosulfate and halide. Among these, thiosulfate is very attractive for its high selectivity for precious metals and its properties of non-toxicity or corrosivity. Thiosulfate has been used as a substitute to cyanide for the extraction of Au from ores (Cui and Zhang, 2008) and from e-waste (Senanayake, 2004; Ha et al., 2010; Jing-ying et al., 2012; Zhang et al., 2012; Petter et al., 2014; Camelino et al., 2015; Zhang and Senanayake, 2016). The dissolution of Au in the ammonia thiosulfate solution is an electrochemical reaction catalysed by the presence of cupric ions, which act as a catalyst (Cui and Zhang, 2008). The leaching mechanism of thiosulfate in the presence of cupric ions is described by the following reactions (Eq. 1):

$$Au + 5S_2O_3^{2-} + Cu(NH_3)_4^{2+} \rightarrow Au(S_2O_3)_2^{3-} + 4NH_3 + Cu(S_2O_3)_3^{5-} \quad \text{(Eq. 1)}$$

The copper-ammonia complexes acquire the electrons on the cathodic portion of Au surface and then they are reduced. At the same time, ammonia or thiosulfate ions react with the Au ions on the anodic

surface, forming the complexes $Au(NH_3)_4^{2-}$ or $Au(S_2O_3)_2^{3-}$. The dominant reaction depends on the concentrations of the species in solution (Aylmore and Muir, 2001; Cui and Zhang, 2008) but the formation of the more stable $Au(S_2O_3)_2^{3-}$ complex is favoured (Zhang et al., 2012). The Au-thiosulfate complex is quite stable once formed, but the presence of oxygen and the maintenance of the alkalinity conditions are necessary to avoid the decomposition of the thiosulfate and to guarantee the stability of the copper-ammonia complex (Cui and Zhang, 2008). Furthermore, in alkaline environment the tetrathionate, which is easily formed by the oxidation of thiosulphate, can be converted back into thiosulfate (Zhang et al., 2012).

The leaching mechanism for Ag by ammonia thiosulfate solution with cupric ions is similar to that of Au (Oh et al., 2003) (Eq. 2).

$$Ag + Cu(NH_3)_4^{2+} + 4S_2O_3^{2-} \rightarrow Ag(S_2O_3)_2^{3-} + 4NH_3 + Cu(S_2O_3)_2^{3-} \quad \text{(Eq. 2)}$$

Although its importance, Pd has received less attention compared to the other precious metals. It was demonstrated that Pd can form high stability complexes with thiosulfate ions (PdS_2O_3) and these complexes can be transferred to a dissolved state, as well as Au and Ag (Tyutyunnik et al., 2016).

The main problem due to the use of thiosulfate is the high consumption of reagents during metal mobilization which makes this process not economically favourable and not environmental-friendly (Cui and Zhang, 2008; Zhang et al., 2012).

In this study the ammonium thiosulfate mobilization efficiencies of Au, Ag and Pd from WPCBs of end-of-life computers were evaluated under different conditions. The influence of various parameters like sodium thiosulfate concentration, total ammonia/ammonium concentration, granulometry of WPCBs and their interaction with the leaching time were studied and optimized by the response surface methodology (RSM). The optimization by RSM helped to understand the interaction among the parameters at various levels. Furthermore, this work compared the effect of three different WPCBs particle sizes on metals mobilization by thiosulfate to verify the real effect of this parameter. Specifically, the objectives of this study were:

1) The study of the influence and the interaction of sodium thiosulfate concentration, total ammonia/ammonium concentration and leaching time on Au, Ag and Pd mobilization;
2) The analysis of the effect of different WPCB particle sizes;
3) The optimization of the leaching conditions for Au, Ag and Pd with the RSM.

2. Materials and methods

2.1 Printed circuit boards from computers

The WPCBs for the leaching tests were prepared as reported by Becci et al. (2020). More in detail, the WPCBs were shredded, crushed, and washed with water saturated with NaCl for plastics removal. The plastic can be re-used for plastic bottle production or converted into energy through incineration, pyrolysis and gasification (Kaya, 2019; Islam et al., 2020). To avoid the interference between Cu and thiosulfate, the WPCBs were pre-treated by a chemical leaching with 50 g/L Fe^{3+} (Becci et al., 2019, 2020). After the pre-treatment, Cu content was decreased from 25% w/w to 5% w/w and precious metals concentrations increased. The recovered Cu is suitable to be employed as a raw material for the current metal market (Kaya, 2019; Becci et al., 2020). Then the WPCBs were crushed another time to obtain different dimensions: 0.1<Ø<0.25 mm, 0.25<Ø<0.50 mm and 0.50<Ø<1 mm (Figure 1).

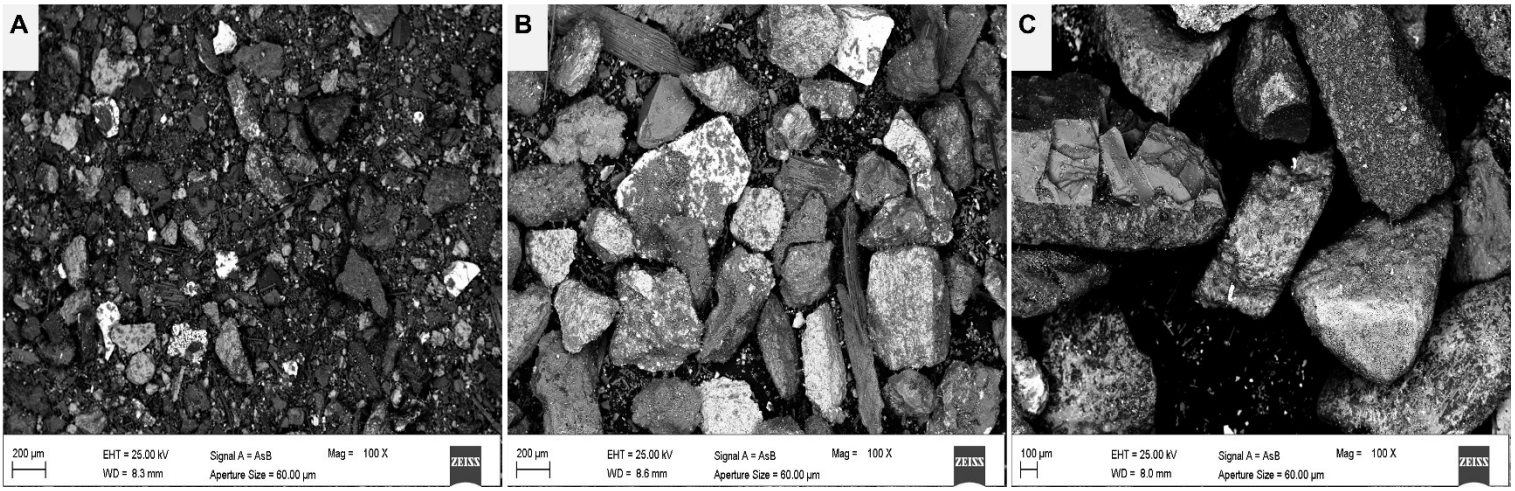

Figure 1. WPCBs with different granulometries: lower than 0.10<Ø<0.25 mm (A), 0.25<Ø<0.50 mm (B) and 0.50<Ø<1.00 mm (C) tested by SEM analysis.

2.2 Leaching test

Thiosulfate leaching experiments were conducted following a full factorial plan to investigate the effects of sodium thiosulfate concentration (0.1, 1 M), total ammonia/ammonium concentration (0.2, 1 M) and WPCBs particle size (0.1<Ø<0.25 mm; 0.50<Ø<1 mm) on the Au, Ag and Pd mobilization from crushed WPCBs. The reagents used were sodium thiosulfate ($Na_2S_2O_3$ anhydrous, 98%), ammonium hydroxide (NH_4OH, 30%), ammonium sulfate ($(NH_4)_2SO_4$, 99%) and copper sulfate ($CuSO_4 \cdot 5\ H_2O$) obtained from Carlo Erba Srl, Italy. Furthermore, the central points of the factorial plan were analysed (thiosulfate 0.55 M, ammonia/ammonium 0.6 M and WPCBs particle size 0.25<Ø<0.50 mm). $CuSO_4$ concentration was kept fixed at 10 mM. All the leaching experiments were

carried out by mixing 10 g/L of WPCBs and 0.5 L of ammonium thiosulfate solution. The pH was kept between 10 and 10.5 throughout the experiment by adding NaOH 10 M. The experiments were performed at an agitation speed of 120 rpm at 25 °C for 24 hours. The samples for chemical analysis were withdrawn every 4, 8, 16 and 24 hours to investigate the effects of the time.

2.3 Analytical determination and statistical analysis

Leaching solution samples (5 mL) and final solid samples (residues after the leaching experiments) were analysed to determine the metal concentrations by atomic absorption spectrophotometer (AAS). During all the leaching process the pH was analysed by means of a pH metro inoLab Multi 720 (WTW). The obtained results were optimized by central composite design (CCD) to assess the optimal experimental plan. The metals dissolution, represented by the concentration of the metals in the leach liquor (Y, µg/L), was taken as the response variables, whilst the initial ammonia/ammonium concentration (X_1, M), the initial thiosulfate concentration (X_2, M), and the granulometry of WPCBs (X_3, mm) were considered as the independent variables (factors). The influence of the leaching time (X_4, hours) was also considered as a factor. Table 1 reports their levels (Table 1). The required experimental runs were 10 ($2^k + 2k + n_0$), where k is the number of the factorial points (=2) and n_0 the central points (=2). The CCD model allowed to obtain experimental results that can be expressed by the Equation 3 (Eq. 3):

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k-1} \sum_{j=1}^{k} b_{ij} X_i X_j + \sum_{i=1}^{k} b_{ii} X_j^2 + \varepsilon \quad \text{(Eq. 3)}$$

where b_0 is the constant coefficient, b_i, b_{ii}, and b_{ij} are the linear, second order, and interaction constants and ε is random error.

Table 1. Factors and levels for the CCD-RSM.

Code	Factors	-1	0	+1
X_1	[Ammonia/ammonium] (M)	0.2	0.6	1
X_2	[Thiosulfate] (M)	0.1	0.55	1
X_3	Granulometry (mm)	0.1 < Ø < 0.25	0.25 < Ø < 0.50	0.50< Ø <1.00

Principal Component Analysis (PCA) was done using the rsm package in RStudio software. PCA was performed to understand in detail as the molar ratio between the ammonia or thiosulfate, the precious metals concentrations and the percentage of Au, Ag and Pd extraction (dependent variables) were correlated with the granulometry of the WPCB particle sizes (independent variable).

3. Results and discussion

3.1 Au leaching

The results in Figure 2 show the Au mobilization profiles in the liquor at different time with three WPCBs particle sizes (small: 0.10<Ø<0.25 mm, medium: 0.25<Ø<0.50 mm and big: 0.50<Ø<1 mm) and different concentrations of thiosulfate and ammonia/ammonium. The highest Au mobilization (2 mg/L) was achieved after 16 h with the smallest WPCBs particle size and 0.2 M ammonia/ammonium and 0.1 M thiosulfate concentrations or 1 M ammonia/ammonium and 1 M thiosulfate concentrations (Figure 2). Au dissolution was very low, less than 0.5 mg/L, using the medium and the biggest WPCBs particle size, at all the tested conditions. Au extraction was limited by the increase in particle size dimension, which does not allowed a good mixing of the material, decreasing the contact between the precious metal and the leaching reagent (Birloaga et al., 2013). It is evident that reducing the size of WPCBs exerted a significant effect on the metal dissolution. The smallest particles size ensured the contact between the WPCBs and the thiosulfate, offering a large metal exposure area (Tripathi et al., 2012; Birloaga et al., 2013). The best operating conditions were obtained using the molar ratio 1:1 between thiosulfate and ammonia/ammonium concentration. The increase in ammonia/ammonium concentration could improve Cu dissolution and the formation of a high concentration of copper-ammonia complexes which catalyse Au dissolution (Eq. 1). The enhanced dissolution of Cu could also increase the dissolution of Au-bearing Cu particles, which is the most common form of Au in the WPCBs, augmenting Au recovery (Jeon et al., 2020).

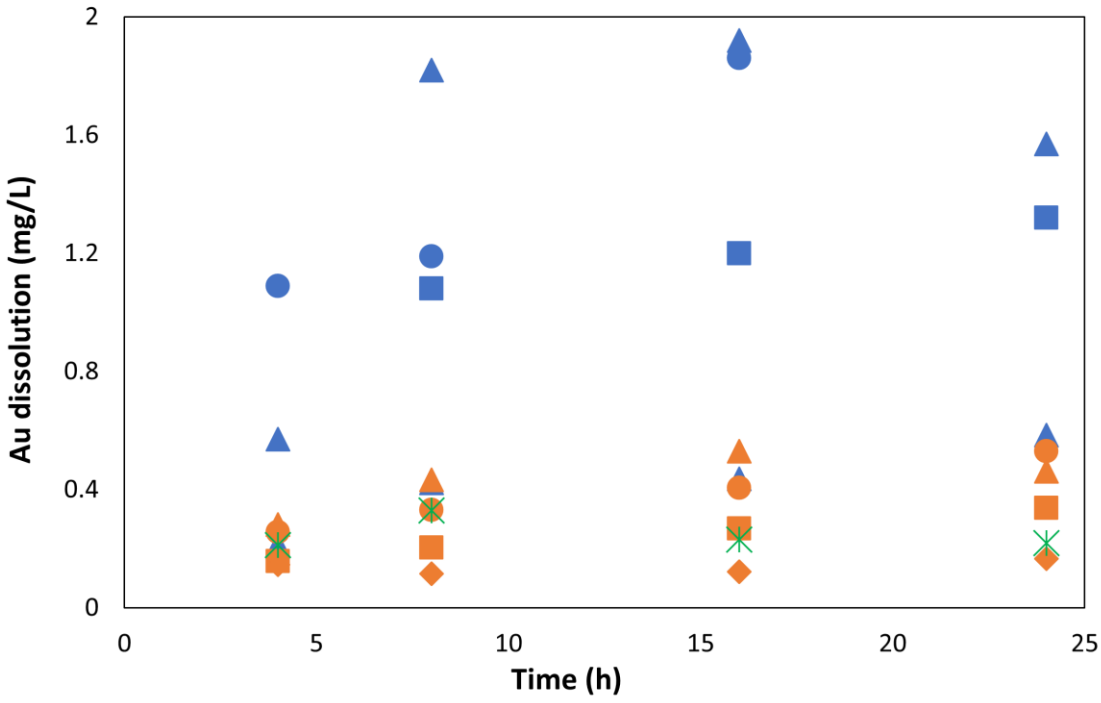

Figure 2. Au dissolution in the final liquor with small size WPCBs (0.10<Ø<0.25 mm) ● 0.2 M NH_3 0.1 M thiosulfate ■1 M NH_3 0.1 M thiosulfate ♦ 0.2 M NH_3 1 M thiosulfate ▲ 1 M NH_3 1 M thiosulfate, big size WPCBs (0.50<Ø<1.00 mm) ● 0.2 M NH_3 0.1 M thiosulfate ■1 M NH_3 0.1 M thiosulfate ♦ 0.2 M NH_3 1 M thiosulfate ▲ 1 M NH_3 1 M thiosulfate and medium size (0.25<Ø<0.50 mm) ⁎ 0.6 M ammonia/ammonium 0.55 M thiosulfate.

The results were analysed with CCD in order to optimize the considered factors. The dissolved Au concentration in the liquor was modelled as a function of initial ammonia/ammonium concentration, thiosulfate concentration, WPCBs particle size and leaching time, and can be expressed by the following equation (Eq. 4):

$$Au = 353.40 + 79.16X_1 - 57.66X_2 - 360.89X_3 + 98.69X_4 + 247.15X_1X_2 - 47.91X_1X_3 + 25.00X_1X_4 + 47.40X_2X_3 - 18.46X_2X_4 - 48.81X_3X_4 + 422.34X_3^2 - 34.75X_4^2 \quad \text{(Eq. 4)}$$

Figure 3 shows the normal probabilities plot for the performed analysis of variances, indicating the normal distribution of residuals and the validity of analysis (R^2 of 0.92) (Figure 3).

The ANOVA analysis highlighted as all the factors and their interactions affected Au mobilization (Table 2). In more details, a negative effect was exerted by thiosulfate concentration and granulometry whilst ammonia/ammonium concentration showed a positive effect (Eq. 5).

The statistical analysis confirmed that the best operative condition for Au chemical leaching was 1:1 molar ratio between thiosulfate and ammonia/ammonium concentration (Figure 4a). The increase of the granulometry reduced the Au dissolution in the liquor (Figure 4b).

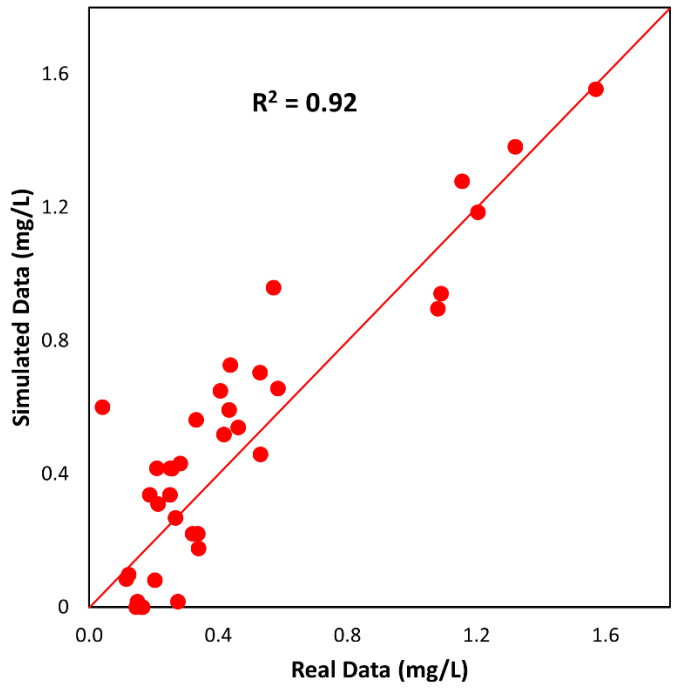

Figure 3. Simulated data vs real data for Au mobilization.

Table 2. Summary of ANOVA results for RSM for Au mobilization.

Sources	Sum of squares	df	Mean square	F-value	p-value
Linear (X_1, X_2, X_3, X_4)	4746626	4	1186656	15.67	****
Interaction (X_1, X_2, X_3, X_4)	2335806	6	389301	5.14	**
Quadratic (X_3, X_4)	1467480	2	733740	9.69	***
Residual	1968507	26	75712		
Lack of fit	1957804	22	88991	33.26	**
Pure error	10703	4	2676		

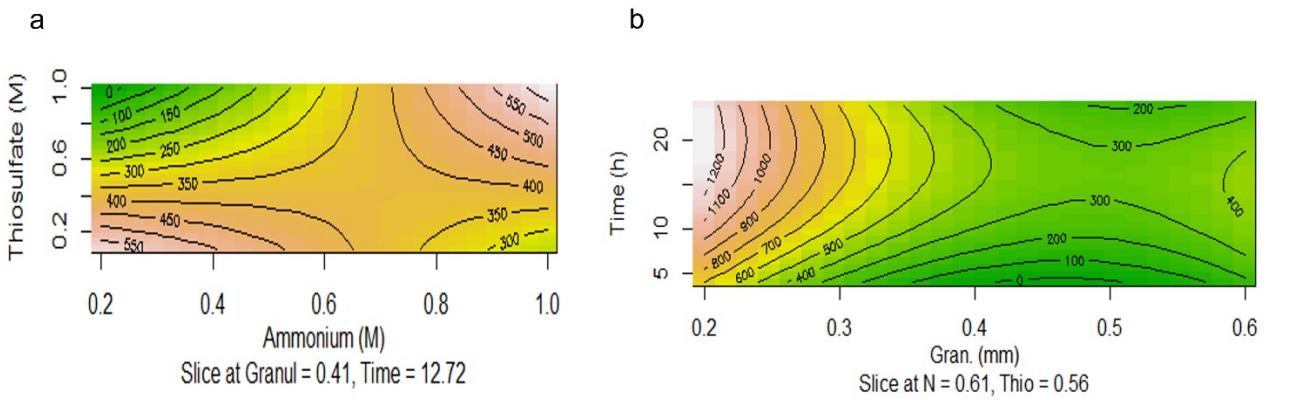

Figure 4. Contours of thiosulfate vs ammonium (a) and time vs granulometry (b) for Au mobilization

3.2 Ag leaching

Figure 5 reports the Ag mobilization profiles in the liquor with three WPCBs particle sizes and different concentration of thiosulfate and ammonia/ammonium. The highest Ag mobilization (around 15 mg/L) was achieved after 8 h with the smallest WPCBs particle size and 0.1 M thiosulfate and 0.2 or 1 M ammonia/ammonium concentration (Figure 5). With the biggest WPCBs particle size Ag dissolution increased during the leaching process with 0.1 M thiosulfate and 0.2 or 1 M ammonia/ammonium, but the values were lower, around 6.5 mg/L, compared to the smallest WPCBs size. The medium size, with the central values of thiosulfate and ammonia/ammonium concentration (0.55 M and 0.6 M, respectively) gave low metal dissolution rate, as well as the big WPCBs particle size with 1 M thiosulfate. This could probably be correlated to the high thiosulfate concentration as the best Ag dissolution was obtained in the experiments with the lowest thiosulfate concentration, or to an unfavourable ratio between thiosulfate and ammonia. The presence of ammonia is fundamental

for the metal mobilization, which occurred when the cupric ions in solution are fully complexed with ammonia, but the concentration was not selective for the metal dissolution. In a previous work was reported that Ag leaching was strictly dependent on pH values, a pH > 9.8 allowed to achieve the maximum cupric-tetraamine concentration, and this resulted in a high Ag dissolution (Ibarra-Galvan et al., 2014).

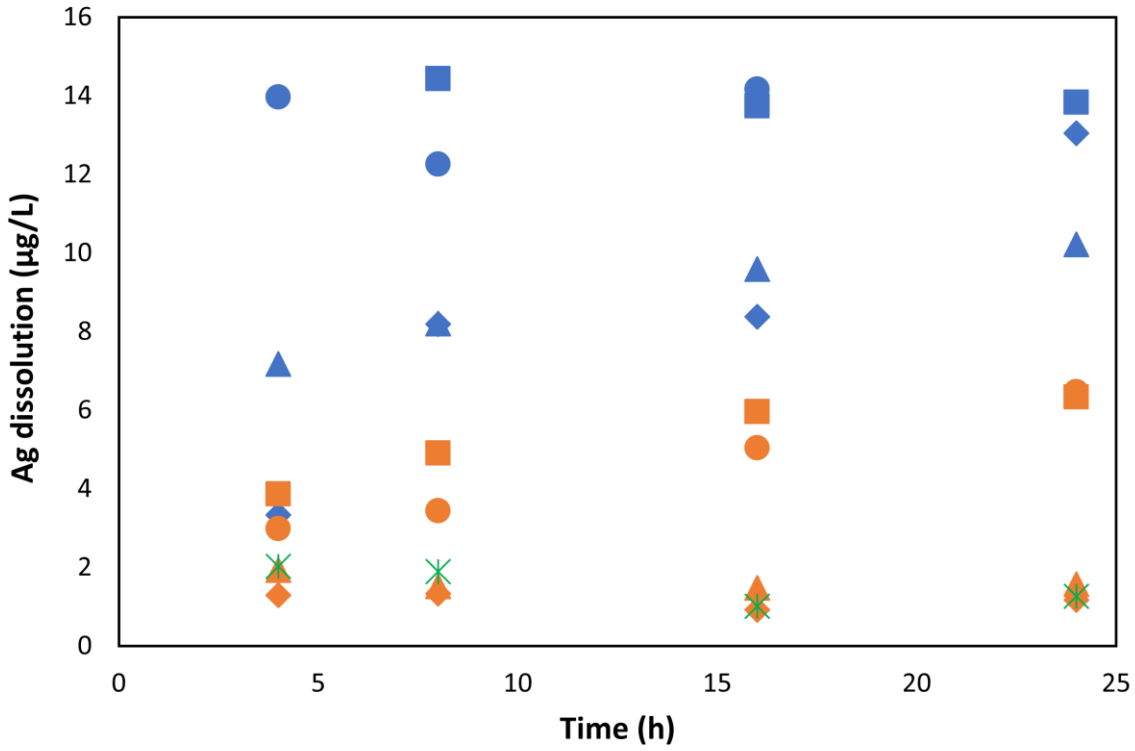

Figure 5. Ag dissolution in the final liquor with small size WPCBs (0.10<Ø<0.25 mm) ● 0.2 M NH_3 0.1 M thiosulfate ■1 M NH_3 0.1 M thiosulfate ♦ 0.2 M NH_3 1 M thiosulfate ▲ 1 M NH_3 1 M thiosulfate, big size WPCBs (0.50<Ø<1.00 mm) ● 0.2 M NH_3 0.1 M thiosulfate ■1 M NH_3 0.1 M thiosulfate ♦ 0.2 M NH_3 1 M thiosulfate ▲ 1 M NH_3 1 M thiosulfate and medium size (0.25<Ø<0.50 mm) ✳ 0.6 M ammonia/ammonium 0.55 M thiosulfate.

The dissolved Ag concentration in the liquor was modelled by statistical analysis as a function of initial ammonia/ammonium concentration, thiosulfate concentration, WPCBs particle size and leaching time. The prediction model for Ag dissolution had a good agreement with the experimental data, with a R^2 of 0.97 (Figure 6). The final predicted equation for Ag dissolution can be expressed as follows (Eq. 5):

$$Ag = 1486.77 + 63.35X_1 - 2083.70X_2 - 3802.32X_3 + 455.44X_4 + 247.27X_1X_2 + 282.45X_1X_3 - 152.46X_1X_4 + 360.95X_2X_3 - 49.28X_2X_4 - 324.05X_3X_4 + 5473.64X_3^2 - 13.77X_4^2 \quad \text{(Eq. 5)}$$

The ANOVA analysis showed that all the factors individually considered influenced the predicted response at a significant confidence level whilst their interaction was not statistically significant (table 3). As can be seen from Figure 7a, ammonia/ammonium concentration had a positive effect in low range (0.1-0.2 M and 0.9-1 M) and a negative effect in the central values with all the thiosulfate concentrations (Figure 7a), granulometries and time tested (data not shown). Concerning the WPCBs particle size only the small size exerted a positive effect on Ag dissolution (Figure 7b).

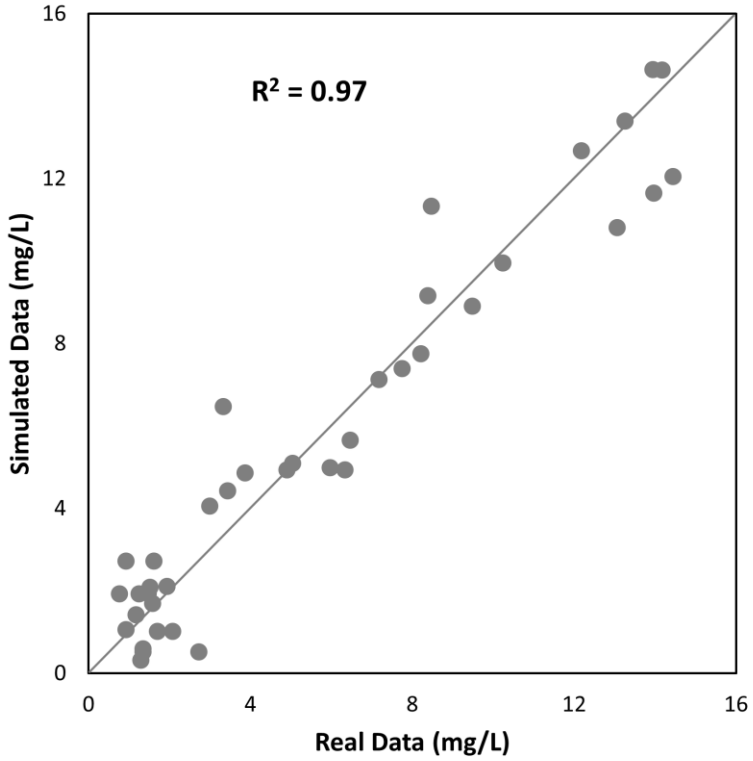

Figure 6. Simulated data vs real data for Ag mobilization.

Table 3. Summary of ANOVA results for RSM for Ag mobilization.

Sources	Sum of squares	df	Mean square	F-value	p-value
Linear (X_1, X_2, X_3, X_4)	551305110	4	137826277	60.98	****
Interaction (X_1, X_2, X_3, X_4)	12257716	6	2042953	0.90	0.51
Quadratic (X_3, X_4)	189728172	2	94864086	41.97	****
Residual	58764834	26	2260186		
Lack of fit	57380976	22	2608226	7.54	*
Pure error	1383858	4	345964		

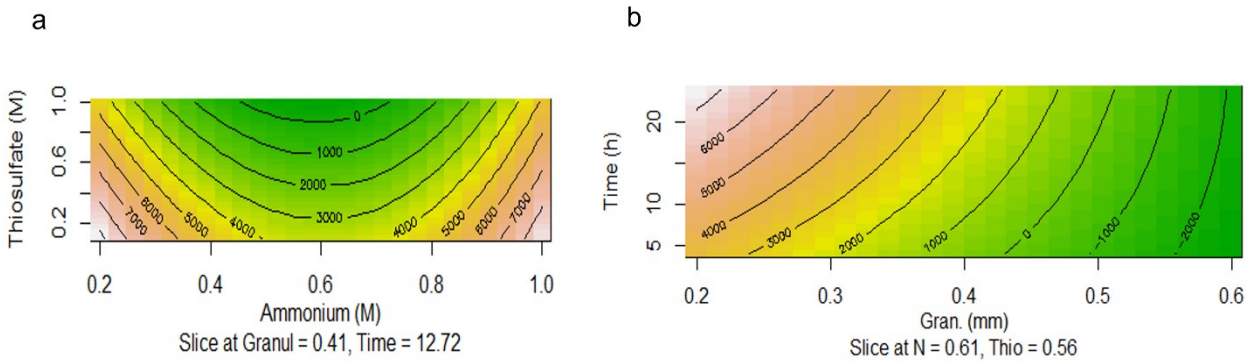

Figure 7. Contours of thiosulfate vs ammonium (a) and time vs granulometry (b) for Ag mobilization.

3.3 Pd leaching

Figure 8 reports the Pd mobilization profiles in the liquor during the leaching process. Pd mobilization was around 300-400 µg/L at all the tested concentrations of thiosulfate and ammonia/ammonium and using both the small and medium WPCBs particle size (Figure 8). Using the biggest WPCBs particle size Pd dissolution was very low (<50 µg/L) in all the tested conditions. As well as Au and Ag, Pd extraction was influenced by particle size dimension. However, Pd mobilization showed a higher rate, compared to Au and Ag, also in the presence of the medium WPCBs particle size, with values comparable to that obtained with the smallest WPCBs size.

It can be seen from Figure 8 that thiosulfate and ammonia/ammonium concentrations were not selective for metal dissolution as there were no big differences in metal dissolution between the various treatments, therefore also in the presence of the central points of the factorial plan the rate of metal dissolution was high. Furthermore, it is possible that the low mobilization of Au and Ag using the medium WPCBs particle size favoured a greater availability of thiosulfate for Pd, thus increasing the dissolution of this metal. Most of the previous works focused on Pd mobilization utilized chloride solution as a leaching reagent (Quinet et al., 2005; Viñals et al., 2006; Behnamfard et al., 2013), and it was reported that Pd leaching increased by increasing hydrogen chloride concentration from 2.5 M to 5 M at 60°C (Behnamfard et al., 2013). Our work demonstrated that the high Pd dissolution rate can be obtain also with thiosulfate, which, compared to chloride, is not toxic or corrosive.

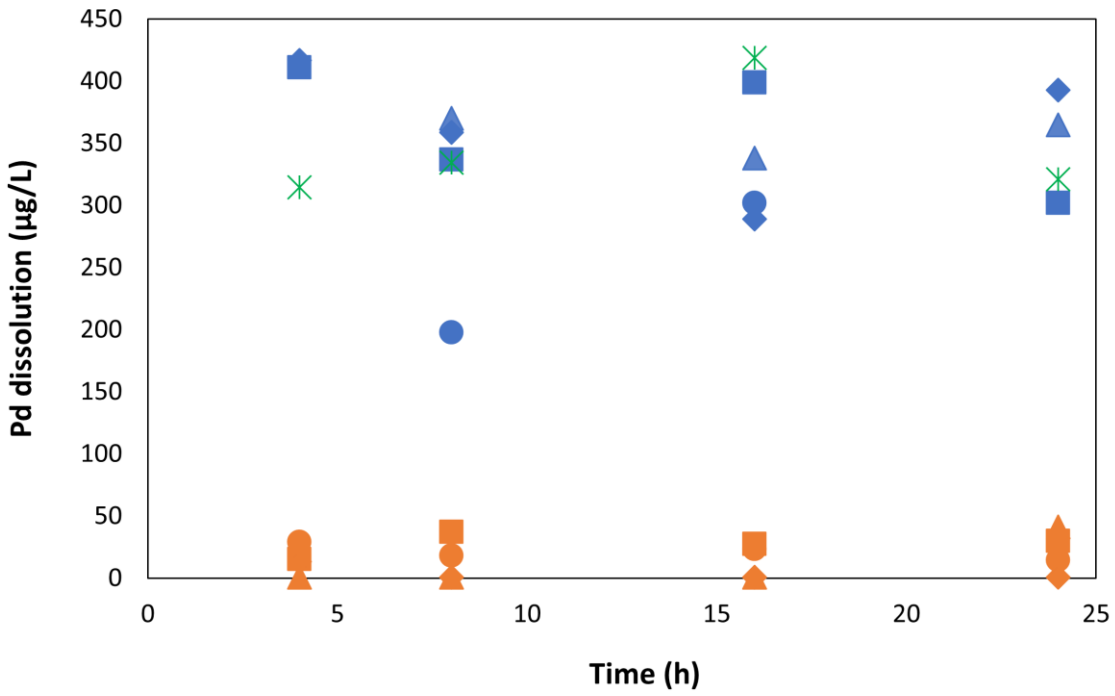

Figure 8. Pd dissolution in the final liquor with small size WPCBs (0.10<Ø<0.25 mm) ● 0.2 M NH_3 0.1 M thiosulfate ■1 M NH_3 0.1 M thiosulfate ♦ 0.2 M NH_3 1 M thiosulfate ▲ 1 M NH_3 1 M thiosulfate, big size WPCBs (0.50<Ø<1.00 mm) ● 0.2 M NH_3 0.1 M thiosulfate ■1 M NH_3 0.1 M thiosulfate ♦ 0.2 M NH_3 1 M thiosulfate ▲ 1 M NH_3 1 M thiosulfate and medium size (0.25<Ø<0.50 mm) ⁎ 0.6 M ammonia/ammonium 0.55 M thiosulfate.

The results were statistically analysed to evaluate the effects of the considered factors on Pd mobilization. Thiosulfate and ammonia/ammonium concentration had a negative effect on Pd concentration in the final liquor, while the interaction between the two factors was not statistically significant, as can be seen from the ANOVA analysis (Table 4). These effects are also expressed by equation 6 (Eq. 6), which describes a quadratic model in good agreement with the experimental data (Figure 9):

$$Pd = 353.22 - 26.54X_1 - 26.50X_2 - 169.88X_3 + 6.33X_4 + 35.72X_1X_2 + 26.24X_1X_3 + 14.33X_1X_4 + 16.64X_2X_3 + 5.74X_2X_4 - 2.83X_3X_4 - 160.64X_3^2 - 2.01X_4^2 \quad \text{(Eq. 6)}$$

As shown in Figure 10, Pd dissolution was comparable to all the tested thiosulfate and ammonia/ammonium concentration, ranging from 320 to 420 µg/L. On the other hand, the granulometry had the most significant effect on Pd mobilization, the statistical analysis confirmed the best Pd dissolution at granulometry between 0.25 and 0.50 mm and the decrease in metal dissolution when the WPCBs particle size increased above 0.50 mm (Figure 10 b).

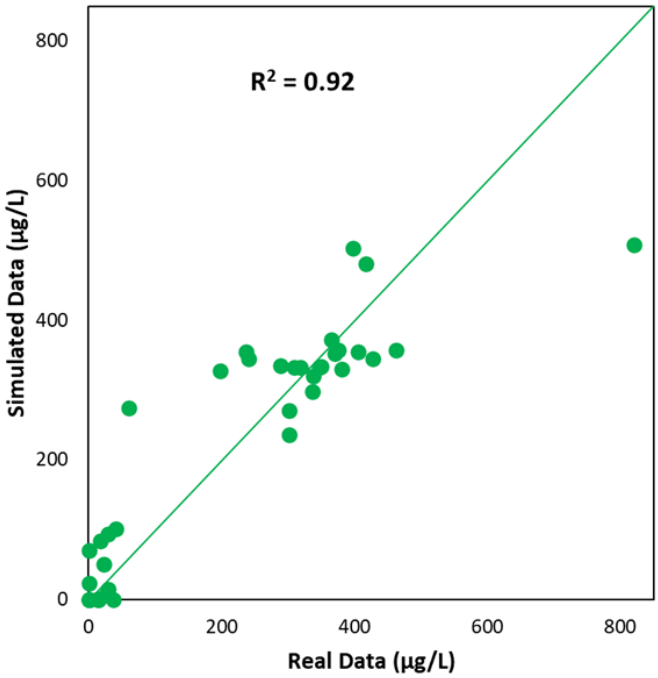

Figure 9. Simulated data vs real data for Pd mobilization.

Table 4. Summary of ANOVA results for RSM for Pd mobilization.

Sources	Sum of squares	df	Mean square	F-value	p-value
Linear (X_1, X_2, X_3, X_4)	919234	4	229809	22.63	****
Interaction (X_1, X_2, X_3, X_4)	103254	6	17209	1.69	0.16
Quadratic (X_3, X_4)	163732	2	81866	8.06	**
Residual	264071	26	10157		
Lack of fit	228562	22	10389	1.17	0.49
Pure error	35509	4	8877		

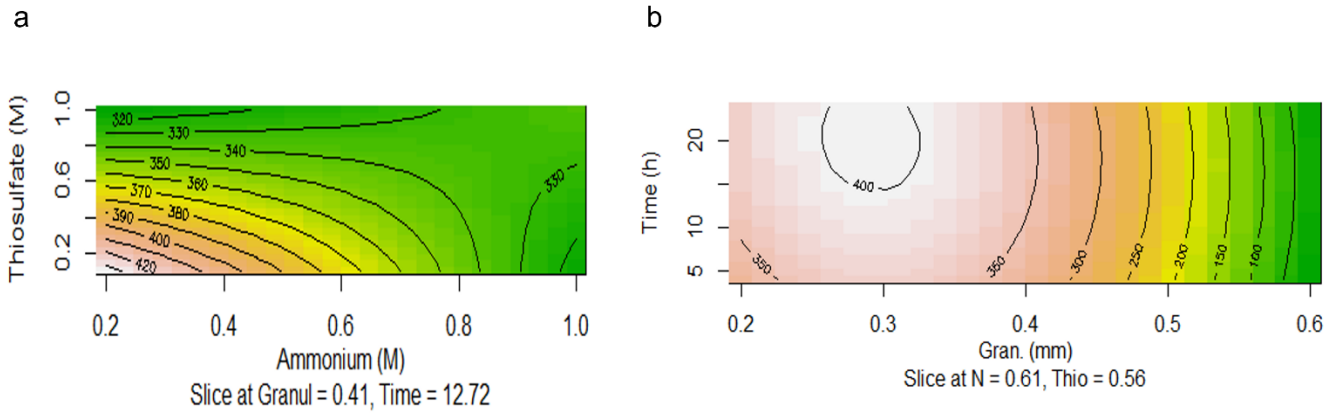

Figure 10. Contours of thiosulfate vs ammonium (a) and time vs granulometry (b) for Pd mobilization.

3.4 PCA analysis

The previous models fit very well with the metal concentrations in the leach liquor. A supplementary statistical analysis was carried out to understand in depth the degree to which each factor affected the leaching efficiencies. In this case the efficiency of the extraction process was considered because the metal concentration in the leach liquor is affected not only by thiosulfate and ammonia starting concentration, but also by the metal concentration in the initial sample. The metal contents were different in the three particle sizes (Table 5). Au showed a concentration 6 times lower in the smallest particle size than in the biggest one. On the other hand, Ag showed an opposite trend, the smallest particle size has an Ag concentration 3 times higher than the biggest one. Conversely, Pd showed a constant concentration in the three particle sizes.

Table 5. Metal concentrations in the different particle sizes.

Metal concentration (mg/kg)	Granulometry					
	0.10<Ø<0.25 mm		0.25<Ø<0.50 mm		0.50<Ø<1.00 mm	
	Mean	St.dev.	Mean	St.dev.	Mean	St.dev.
Au	170	± 30	212	± 6	1100	± 200
Ag	1690	± 50	220	± 3	400	± 100
Pd	42	± 4	32	± 8	30	± 6

Considering the sample heterogeneity, for the PCA the considered variables were the molar ratio between the thiosulfate and the precious metal concentrations in the starting sample, the molar ratio between the ammonia and the precious metal concentrations, the granulometry and the percentage of Au, Ag and Pd extraction. More in detail, the ratio between thiosulfate and precious metal concentrations increased around 15 times increasing the thiosulfate concentration from 0.1 to 1 M with the smallest particle size. On the other hand, when the biggest particle sizes were used, the molar ratio between thiosulfate and precious metal concentrations was quite constant despite of the initial thiosulfate concentration (Figure 11a). Furthermore, the ratio between ammonia and the precious metal concentrations increased 6 times changing the ammonia concentration from 0.2 to 1 M, with the smallest particle size, whilst with the biggest particle size the ammonia concentration ranged between 300 and 30,000 times higher than the precious metal concentrations (Figure 11b). On the contrary, the medium particle size showed a constant thiosulfate and ammonia concentration, around 3,500 times higher than the precious metals with initial concentrations of 0.55 and 0.6 M, respectively (Figure 11). These data confirmed as the ratio between the reagents and the initial metal

concentrations was more fluctuating and these variables are more suitable to understand the chemical leaching mechanism than the initial molar reagent concentrations.

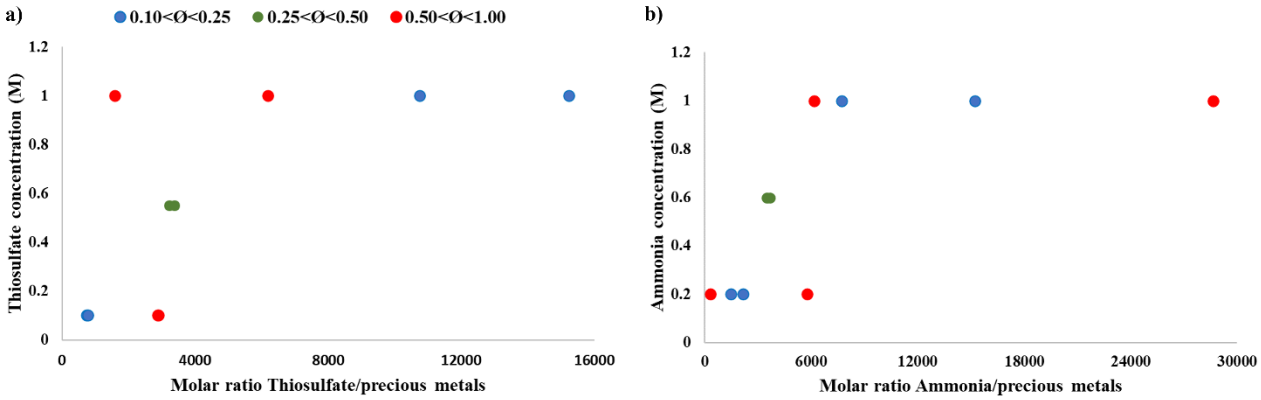

Figure 11. Changing in the molar ratio between thiosulfate (a) or ammonia (b) and the precious metal concentrations in the different experimental plans.

The results of the PCA analysis highlighted as the Au extraction is correlated with the ratio between thiosulfate and the precious metal concentrations, especially for the smallest particle size. The Au mobilization increased by decreasing the particle size from 0.50 to less than 0.25 mm and by increasing the molar ratio between thiosulfate/ammonia and precious metal concentrations. On the contrary, the Ag extraction efficiency was more correlated with the biggest particle size and was not correlated neither with the ratio between thiosulfate and precious metal concentrations, nor to the ratio between the ammonia and the precious metal concentrations (Figure 12). The Ag extraction was between 70 and 90% for all the tested conditions, reaching the highest efficiency with the biggest particle size, irrespective of the ammonia and thiosulfate concentrations. These results are more evident in the Figure 13. The Pd extraction showed the same trend as Au: the efficiency increased by decreasing the particle size, but it was around 100% already with the medium particle size (Figure 13). As Ag, also Pd did not show any correlation with the thiosulfate and ammonia concentration.

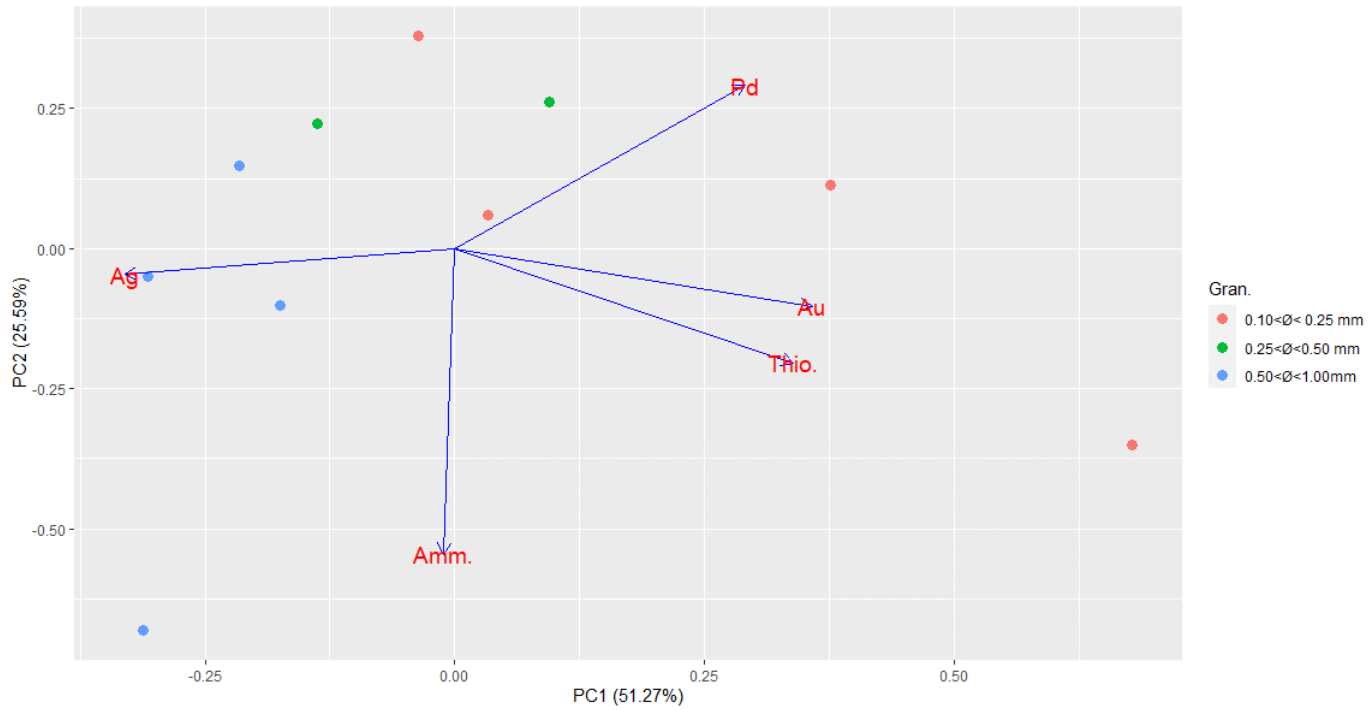

Figure 12. Principal component analysis (PCA) results.

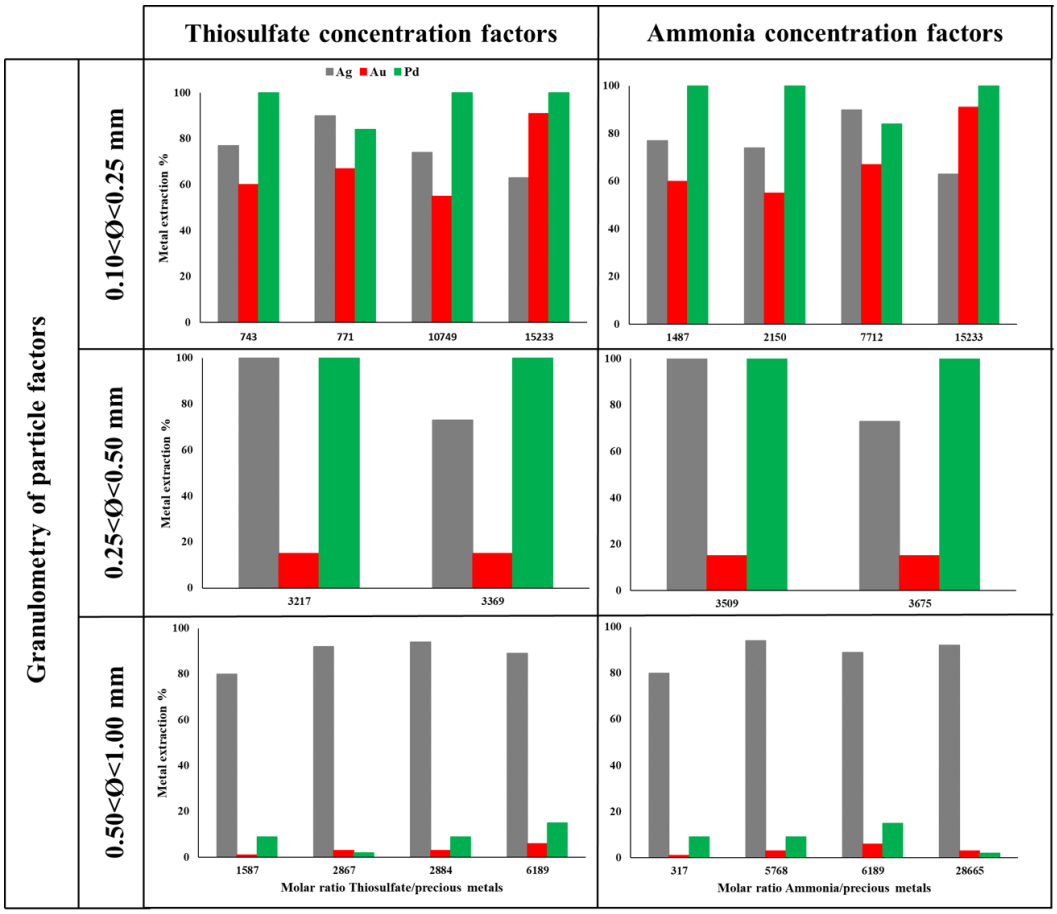

Figure 13. Au, Ag and Pd extraction efficiencies (%) in the different experiments.

4. Conclusion

The results showed as the Au, Ag and Pd leaching is a complex process correlated with thiosulfate, ammonia and precious metal concentrations and the WPCBs particle sizes. The metal concentrations change depending on the granulometry. The Au concentration decreased with decreasing particle size, whilst the Ag increased its concentration in the smallest particle size. On the other hand, the Pd concentration was constant regardless the considered granulometry. The results highlighted as the Au leaching took place only with a granulometry less than 0.25 mm. The percentage of extraction increased by increasing the molar ratio between thiosulfate/ammonia and precious metal concentrations and with a ratio between thiosulfate and ammonia concentration of 1. These results emphasized as the consumption of reagents was very high. The Pd leaching showed the same Au dependence to granulometry, to achieve extraction efficiencies higher than 90% was necessary a granulometry lower than 0.50 mm. Concerning Ag, the extraction efficiencies were constant regardless the considered granulometry. The results also showed as Ag and Pd leaching, unlike Au, depend less on thiosulfate and ammonia concentrations and with the lowest reagent concentrations high percentage of extraction were achieved. These results suggest the possibility of a more selective recovery of precious metals based on the different granulometry of the WPCBs. Furthermore, the increase in selectivity can reduce the reagent consumptions, making the leaching process more sustainable and relevant for the large-scale application.

Further studies are needed to:

- optimize the WPCBs grinding process, as the particle size has proved to be decisive for the leaching of Au but not of the other precious metals, a grinding circuit can be evaluated to decrease costs and increase efficiencies,

- verify the possibility of a leaching process in series. The combination of two chemical processes or a chemical and bacterial leaching can focus more selectively on one single target metal by a better control of all the experimental conditions. After that, a subsequent leaching with the same reagent and different conditions or with a different reagent can improve the extraction of another metal.

The development of innovative and less costly hydrometallurgical techniques leads to a safety e-waste recycling with a decreased environmental pollution.

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Disclosure statement

No potential conflict of interest was reported by the authors.

5. Products

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RESEARCH ARTICLE

Non-toxic, high selectivity process for the extraction of precious metals from waste printed circuit boards

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Figure 14. Article published on Frontieres of Environmental Science and Engineering

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Chapter 5:

PRELIMINARY STUDIES ON PRECIOUS METALS RECOVERY BY RESIN ADSORPTION AND ELECTRODEPOSITION

Abstract

The aim of this work is to assess a preliminary study on the precious metals recovery after a thiosulfate leaching process conducted with a column assay. The evaluated techniques are the resin adsorption and the electrodeposition. The results of the adsorption on the anion resin step showed a good efficiency for all the precious metals but also a co-adsorption of Cu, as the Cu-thiosulfate complexes in the leaching solution compete for the active site of the resin. After the desorption step, the precious metals remained adsorbed on the resin, while Cu was desorbed. This represents a very good result as allowed to separate Cu from precious metals, increasing the purity of the final recovered products. Concerning the electrodeposition process, a recovery of 90% of the precious metals was reported in the first 24 hours, with only a small percentage of Cu. These results suggested as a possible solution to increase the purity of the precious metals recovery to combine the two techniques: employ the anion resin as a first step to remove most of the Cu, improve the precious metals desorption and conduct the electrodeposition process for 24 hours.

Content:

1. Introduction

Thiosulfate is an alternative solution to the conventional leaching based on the utilization of cyanide (Camelino et al., 2015; Ha et al., 2010; Jing-ying et al., 2012; Petter et al., 2014; Senanayake, 2004; Zhang and Senanayake, 2016; Zhang et al., 2012). The dissolution of precious metals in the ammonia thiosulfate solution is an electrochemical reaction catalysed by the presence of cupric ions, which act as catalyst (Cui and Zhang, 2008). The leaching mechanism for Au extraction with a thiosulfate solution in the presence of cupric ions is described by the following reaction (Eq. 1):

$$Au + 5S_2O_3^{2-} + Cu(NH_3)_4^{2+} \rightarrow Au(S_2O_3)_2^{3-} + 4NH_3 + Cu(S_2O_3)_3^{5-} \quad \text{(Eq. 1)}$$

One of the problem associated with thiosulfate leaching is the difficulty in the selective precious metals recovery from the leaching solution (Zhang and Dreisinger, 2004). The most common techniques for precious metals recovery are cementation, activated carbon adsorption, resin adsorption and electrodeposition (Yu et al., 2018).

Cementation is a common technique for precious metals recovery from pregnant thiosulfate solutions mainly with metallic powders such as zinc, iron and aluminium. This technique is not selective and Cu ions in the solution can also precipitate with these metallic powders, competing with the recovery of the precious metals (Dong et al., 2017; Yu et al., 2018). Concerning Au recovery, activated carbon adsorption shows good performance from cyanide solutions because of its high efficiency, moderate costs, and high purity of the product. However, activated carbon has remarkably less affinity for the Au (I) thiosulfate complex, due to the relatively high negative charge of the Au (I) thiosulfate anion, steric limitations or specific interactions between the ligand group and carbon active sites (Aylmore and Muir, 2001; Dong et al., 2017; Xu et al., 2017). Some methods were proposed to improve the recovery technique, such as the addition of a certain amount of cyanide into the pregnant solution to produce a more stable Au-cyanide complex, followed by adsorption on the activated carbon (Xu et al., 2017). Another technique is the activated carbon modification with cyano-cuprous or cupric ferrocyanide (Yu et al., 2018). However, the introduction of any cyanide in the two approaches makes the recovery process not cyanide-free, which thus cannot be considered as a true alternative to cyanide process. Resin adsorption and electrodeposition represent valid alternatives as recovery techniques. Resin adsorption can concentrate the metals with a high loading capacity, fast speed and simultaneous elution and regeneration at ambient temperature (Dong 2017). With electrodeposition the precious metal-thiosulfate anions can migrate to the cathode where they can be reduced to the metallic form. The problem for these approaches is the low selectivity for precious metals in the presence of other anions including copper-thiosulfate, that can deposit at the cathode as well as precious metals, decreasing the purity of the products (Dong et al., 2017).

The aim of this work was to explore the efficiency of resins adsorption for precious metals concentration and the subsequent electrodeposition from the concentrated solution for the recovery of precious metals from a thiosulfate leaching solution.

2. Materials and methods

2.1 Column design and leaching experiment

The precious metal extraction from wasted printed circuit boards (WPCBs) was carried out in a column setup. It was composed by a 100 mL plastic cylinder drilled at the bottom and connected with the starting solution tank by an injection inlet pipe and to a final solution beaker throughout an outlet pipe. The driving force was performed by a peristaltic pump (Figure 1).

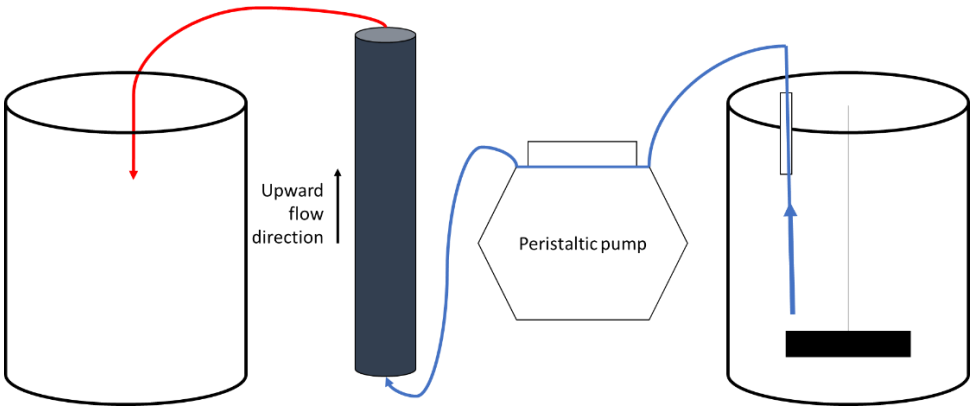

Figure 1. Schematic representation of the column model for metals leaching

The leaching solution was composed of ammonia/ammonium 0.2 M, thiosulfate 0.1 M, and $CuSO_4$ 10 mM at pH 10. The final volume of the solution was 12 L and the leaching time 42 hours. The column was filled with 0.5<Ø<1 mm WPCBs, pretreated with a chemical leaching with 50 g/L Fe^{3+} (Becci et al., 2020, 2019) to reduce Cu content (from 32.65% w/w to 10.5 ppm).

The samples were withdrawn every 30 minutes to analyse the dissolution of the metals. The obtained solution was subjected to recovery tests, and the residue solid samples were analysed for the precious metal content.

2.2 Anion exchange resin column assay

Strong-base anion exchange resin Amberlite IRA-400 was selected from a group of resins that have been investigated previously and adopted to recover precious metals from the leaching solutions. The resin (4.7 g) was transferred to an ion exchange column which was constructed with 8 mL

polypropylene empty column (CHROMABOND Flash DL) and firstly treated with a solution 100 g/L of Na_2SO_4 to substitute chloride (8 mL/h for 2 hours) and washed with deionized water (8 mL/h). After that, the leaching solution was pumped through the column using a peristaltic pump (80 mL/h for 2 hours). Samples of the output current were consecutively taken every 3 minutes to observe the changes in concentration of Cu and precious metals. When the adsorption was finished, the loaded resin was regenerated with the eluant (Na_2SO_4). The flow rate of eluant was 20 BV (bed volume per hour), where 1 BV was the volume of the burette occupied by the resin bed. As well as the service phase, during the elution stage samples of the output current were continuously collected every 3 minutes.

2.3 Electrodeposition

For this study was employed a synthetic solution (250 mL) prepared with deionized water. The synthetic solution was prepared to mimic the metal concentrations supposed to derive from resin ion exchange experiment. Cu, Pd and Au were concentrated 10 times. Au was introduced in the form of $AuCl_4$, as the commercial standard solution (1000 mg/L and 5% HCl), Pd as the commercial standard solution $PdCl_4$ (1000 mg/L and 5% HCl) and Cu as $CuSO_4$. The other chemicals were the same as the real solution (0.1 M thiosulfate and 0.2 M ammonia/ammonium), at pH 10/10.5. The test was executed in a 500 ml beaker, with 250 mL of the synthetic solution. Stainless steel electrodes were connected to the power source and the voltage was regulated at 55 mA. To better control the voltage, an amperemeter was connected in series. During the electrodeposition process the voltage and the pH of the system were recorded. In addition, samples were taken at different time intervals and were analysed by atomic absorption spectrophotometry (AAS) to determine the amount of the metals removed from the solution and deposited on the cathode surface.

3. Results

3.1 Leaching experiments

The metal dissolution in the liquid at the output of the column showed almost the same behaviour for all the precious metals, decreasing during the process (Figure 2). This behaviour could be due to the speed of the input solution, which did not guarantee sufficient contact time between the leaching solution and the solid in the column, as in a previous work we observed that the correct time for a complete leaching of the precious metals was between 16 and 24 hours (Merli et al., 2023). For Ag we observed a sharp increase in the metal concentration followed by an equally rapid decrease in metal concentration in the first 4 hours (Figure 2). This could be due to the fact that the reaction between Ag and thiosulfate is faster compared to Au and Pd reactions. The average concentrations of

the precious metals in the final solution were Ag 1.75 mg/L, Au 481.5 µg/L, and Pd 5 µg/L. Furthermore, also Cu was detected in the final liquor, with a concentration of around 700 mg/L.

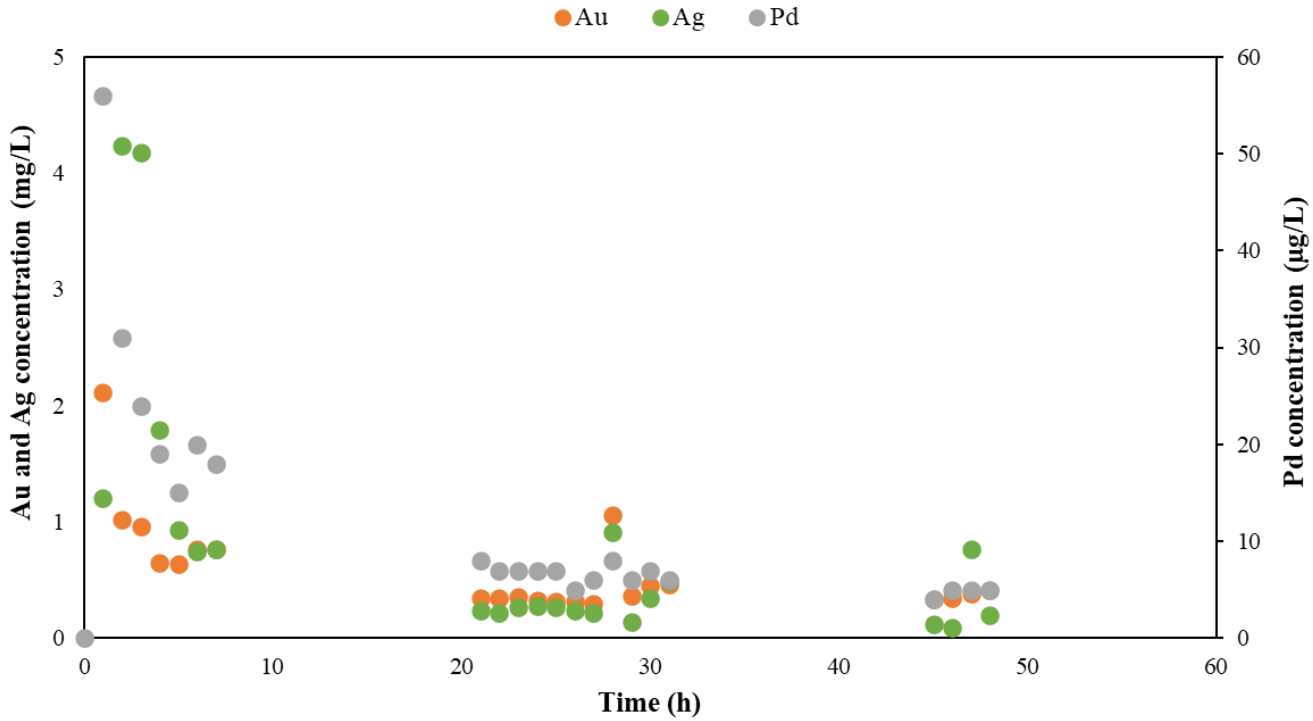

Figure 2. Precious metals dissolution in the solution leaving the column

Anyway, the leaching efficiencies of the column process were estimated analysing the metals concentrations in the initial solid and the final metals concentrations in the solid samples at the top and at the bottom of the column (Figure 3). As expected at the bottom of the column, where the leaching solution input was placed, the leaching efficiency was higher, in particular for Pd which reached around 80% of recovery. The results of the analysis on the initial and final solid samples are reported in Table 1, together with the average leaching efficiencies concerning the total of the column.

Table 1. Precious metals concentration and leaching efficiency before and after the leaching process

	Before leaching (ppm)	After leaching (ppm)	Average leaching efficiency (%)
Au	206.8	110.5	46.6
Ag	84.5	42.8	49.4
Pd	54.1	22.9	57.8

In a previous work conducted in batch conditions, using the same WPCB particle size (0.5<Ø<1 mm) and the same operative conditions (0.1 M thiosulfate, 0.2 M ammonia/ammonium, 10 mM $CuSO_4$) different leaching efficiencies for Au and Pd were obtained, around 4% and 10% respectively (Merli et al., 2023). Thanks to the column design we obtained almost the same leaching efficiency for all the

precious metals, around 50%. The advantage of the column is the easiness of management which exert a better control of the operating conditions, the flexibility, and the lower maintenance costs.

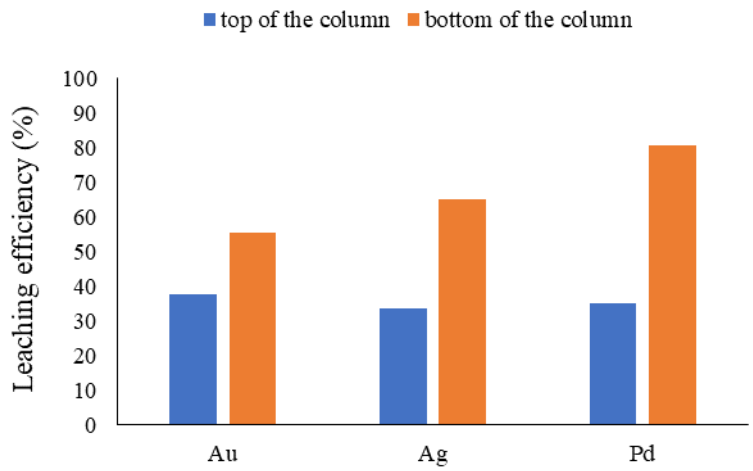

Figure 3. Leaching efficiencies at the top and at the bottom of the column

3.2 Exchange resin column assay

The strong-base anion exchange resin Amberlite IRA-400 was chosen because it demonstrated to be suitable for the adsorption of Au, Ag and Pd in a previous experiment (not reported). In the column assay, the output solution was analysed after the passage into the column with the resin.

The results showed that Ag was totally absorbed on the resin for the first 2 hours, with a recovery efficiency of 100%, and after that it started to be detected in the final solution (Figure 4A). Au and Pd were totally absorbed on the resin, with a 100% recovery, as they were not detected in the output solution. As more solution passed through the column, Ag, Au and Pd loading increased almost linearly and the column was not saturated (Figure 4 A and B). The ion exchange reaction was fast since the precious metals can be rapidly removed from the solution and loaded on the resin in less than 5 minutes.

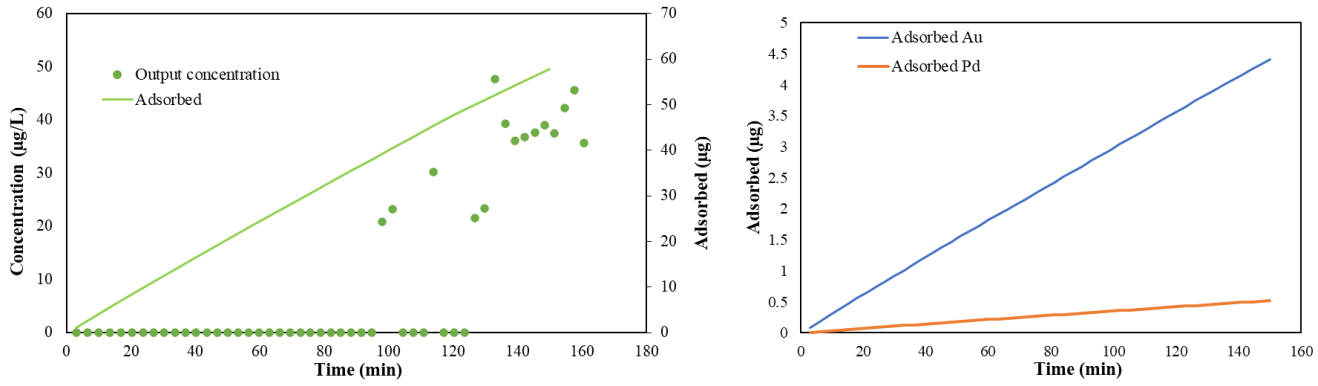

Figure 4. Ag adsorption (A) and Au and Pd retention (B) on the anion resin.

Even if it was not a target metal of our analysis, the adsorption of Cu was analysed, as Cu is one of the most interfering agents with precious metals recovery. The concentration of Cu detected in the

output solution was very low in the first 60 minutes, with a resin adsorption efficiency around 95% (Figure 5A). After that the concentration started to increase in the output solution, as the resin in the column starts to be saturated with the metal, until the Cu concentration in the output solution reached the same concentration of the initial solution, indicating the total saturation of the resin. After a prolonged absorption time the concentration of Cu started to increase in the output solution, indicating that part of this metal was transferred from loaded resin into the leach solution. This could also be due to the increased concentrations of polythionates, like trithionate and tetrathionate, due to unwanted oxidation of thiosulfate in the leach solution, which could adsorb on the resin and compete with Cu thiosulfate complexes for ion-exchange sites (Dong et al., 2019). In another work was reported that as more ammonia thiosulfate solution passed throughout the column, at the optimum pH of 11, Au accumulated on the resin can take the place of Cu as the adsorption of Au on the resin is stronger than Cu and this could favour the selective loading of Au over Cu (Zhang and Dreisinger, 2004).

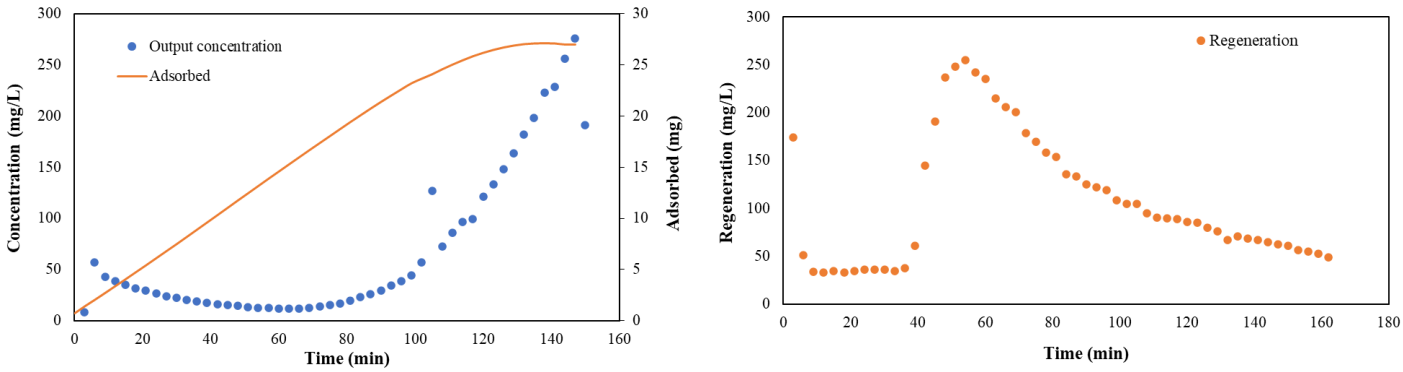

Figure 5. Anionic ion adsorption (A) and elution (B) for Cu

Concerning the regeneration, the profile of the Cu concentration in the outflow from the column showed an unusual behaviour, increasing at 40 minutes after a first decrease (Figure 4B). Cu elution efficiency was around 85% in the first 30 minutes and reached the 100% in the next 100 minutes (Figure 5B).

On the other hand, the precious metals were not detected in the output current during the regeneration operation. This result indicates an excellent starting point for a selective recovery as allowed to separate Cu from precious metals, which remain adsorbed on the resin, increasing the purity of the recovered product. To increase the precious metal elution, after the first Cu removal, it might be useful to increase the reaction time or find a more suitable eluent.

In the work of Gamez et al. (2019) a poor elution was obtained at room temperature with Na_2SO_4 with the addition of ammonia, which regulated the pH of the solution and stabilized the Au-thiosulfate complex (Gámez et al., 2019). They find out that increasing the temperature increased the precious

metals elution, but also Cu dissolution as well and this compromise the purity of the recovered product. Anyway, they reported almost 100% elution of Au and Ag with potassium thiocyanate as eluent at room temperature (Gámez et al., 2019).

In the work of Dong et al. (2019) the elution process was assessed in two times, a first Cu elution with 0.5 M $(NH_4)S_2O_3$ and a subsequent Au elution with a mixed solution of 2 M NaCl and 0.5 M Na_2SO_3. The elution profile was similar to the one we observed for Cu after the first increase-decrease, with a sharp peak followed by a rapid decline. They reported a negligible concentration of Au in the Cu eluate, indicating no Au loss (Dong et al., 2019).

3.3 Electrodeposition

A synthetic solution, which mimic the effect of the resins increasing the metals concentrations, was prepared and utilized for metals recovery, with metals 10 times more concentrated compared to the initial solution. Also in this case Cu behaviour was evaluated.

The evolution of Cu, Au and Pd temporal profiles in the solution during the electrodeposition process are presented in Figure 6. It can be observed that the metals concentrations decreased with time, suggesting the deposition on the cathode. More in detail, Au sharply decreased in the first 24 hours and was not detected at the end of the experiment, demonstrating the total deposition. The same trend was observed for Pd, even if Pd concentration in the starting solution was considerably lower. Cu was the most concentrated metal in the initial solution (around 7 g/L), and a co-deposition of Cu along with Au and Pd was observed. Anyway, in contrast with Au and Pd, the decrease of Cu concentration in the solution started after 24 hours and after 72 hours in the final solution the metal was 100 times less concentrated compared to the initial solution (Figure 6).

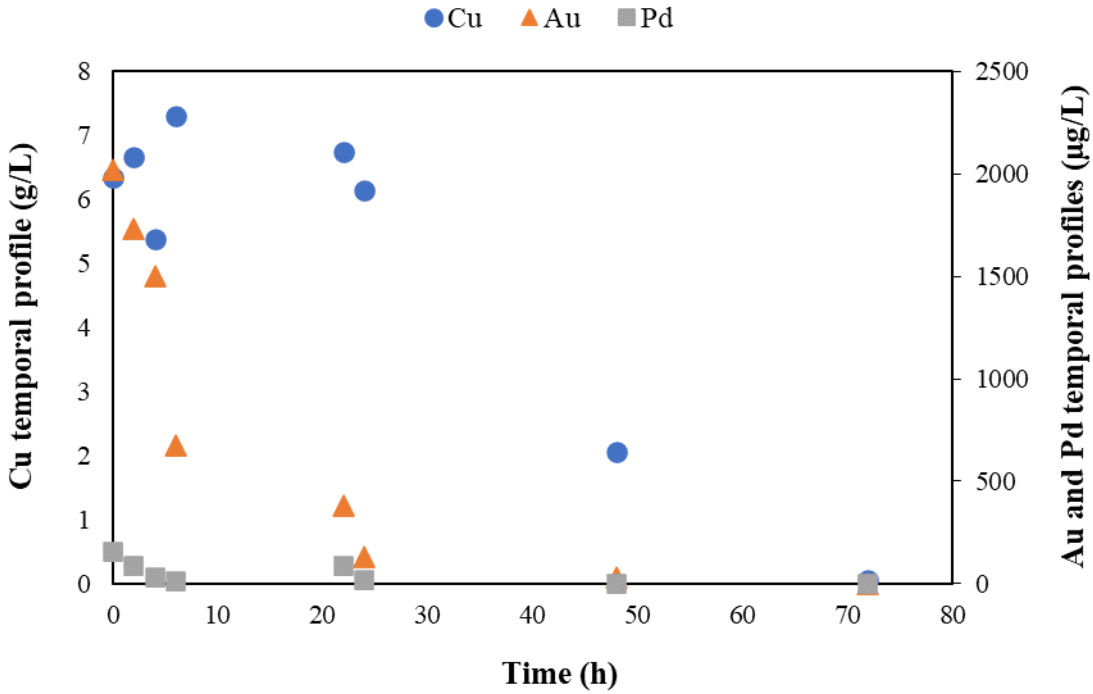

Figure 6. Metals temporal profiles in the leaching solution during the electrodeposition process.

During the experiment a change in the solution colour was observed, from dark blue to a lighter colour, until the end of the experiment when the catholyte was transparent, indicating that the metals were efficiently recovered (Figure 7).

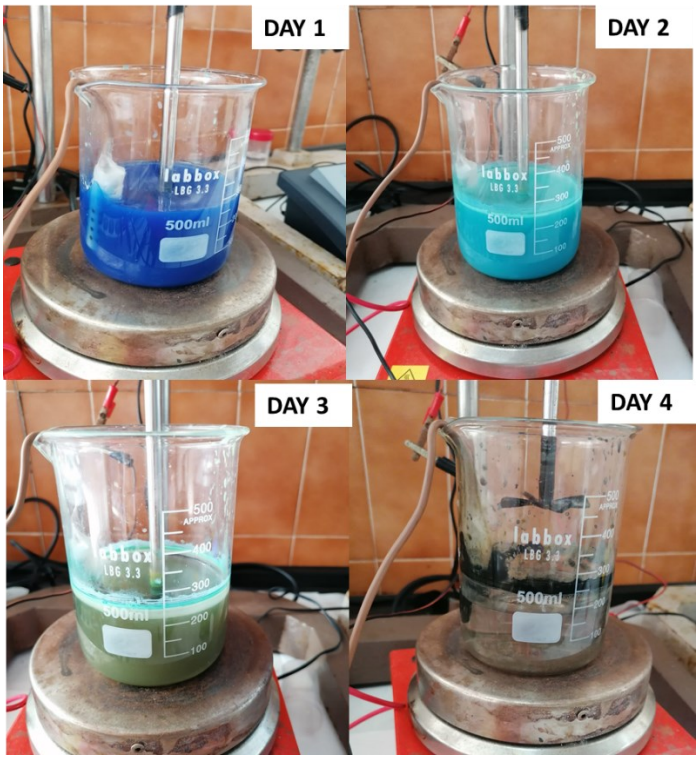

Figure 7. Change in the colour of the solution during the electrodeposition process

The kinetics of electrodeposition for Pd and Au was quick, after 6 hours most of the metal was deposited on the cathode and the metals were recovered with an efficiency of 100% (Figure 8). The same behaviour was reported in another work, which reports an Au recovery efficiency of 75% (Meng et al., 2021). On the contrary, the kinetic of Cu electrodeposition was slower and the metal started to be deposited on the cathode after 24 hours. At the end of the experiment, after 72 hours, Cu was electrodeposited with an efficiency of 99% (Figure 8). The different kinetic between precious metals and Cu electrodeposition is an interesting result in view of the selective recovery of elements, by stopping the reaction at 24 hours a deposition of 90% of the precious metals is obtained, with only a small percentage of Cu.

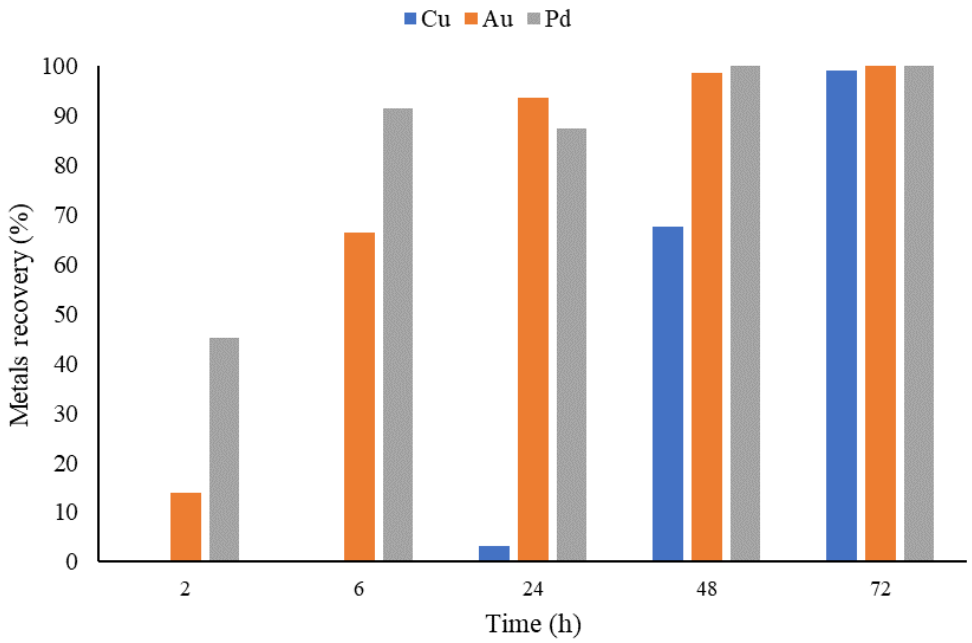

Figure 8. Efficiency of the electrodeposition of Cu, Au and Pd on the cathode (%).

The preliminary results obtained with this experiment are promising for a separation between Cu and precious metals recovery, with the aim of increase the purity of the recovered products. In the work reported by Kasper et al. (2018) it was verified that Cu and Au can be reduced to their metallic state at different values of the potential, and this make possible to selectively recover using suitable applied potential. The results showed that for Au/Cu/thiosulfate solutions, the deposition of Cu occurs at potentials more negative than -600mV$_{Ag/AgCl}$, whereas the deposition of Au occurs in potentials more positive than -600mV$_{Ag/AgCl}$. In this way, it is possible to use electrochemistry for selective recovery of the two metals (Kasper et al., 2018).

Beside, in the real solution there is the competition due to the presence of other metals, like Ag, which can participate in the reduction reaction and compete with Au recovery (Gámez et al., 2019; Meng et al., 2021). In the work of Gamez et al. (2019) it was reported a recovery of 19% for Au and 92% for

Ag, underling as Ag consume part of the energy required for the reduction of Au-thiosulfate complex (Gámez et al., 2019).

4. Conclusions

The objective of this work was a preliminary evaluation of the feasibility of the resin adsorption process followed by an electrodeposition process for the complete recovery of precious metals (Au, Ag and Pd) from a thiosulfate leaching solution.

The leaching process in the column allowed to reach a precious metal recovery of 50%. To enhance the efficiency of this process it could be useful to reduce the speed of the flux throughout the column or to adopt the recirculation of the leaching solution. The strong-base anion exchange resin Amberlite IRA-400 was employed for precious metals adsorption from ammonia-thiosulfate leachate. The adsorption step works well for all the metals but the Cu-thiosulfate complexes, present in the solution, compete for the active site of the ion exchange resin determining adsorption of Cu as well. As regard the elution process, only Cu was desorbed from the resin while the precious metals were not detected in the final solution. This represents a very good result as allowed to separate Cu, adding a pre-elution stage for the first elution of the metal adsorbed on the resin, from precious metals, which remain adsorbed on the resin, increasing the purity of the final recovered products. The precious metals desorption can be enhanced by changing the eluent or by increasing the reaction time.

Electrodeposition test demonstrated to be a suitable process for precious metals recovery, to be used on the solution derived from the resin elution. The results suggest that an electrodeposition time of less than 24 hours is indicated to obtain almost exclusively precious metals deposited on the cathode, with a recovery efficiency of 90%, and only a small percentage of Cu.

The main problem for all the approaches is the presence of Cu, which can complex with thiosulfate and interfere with the leaching, the resin exchange, and the electrodeposition processes. Therefore, the next steps will be addressed to solve this issue and to reduce the competition between Cu and the precious metals, to achieve a suitable precious metals recovery from the leachates. A possible solution could be to combine the resin adsorption and the electrodeposition processes. The anion resin can be employed as a first step to remove most of the Cu, as in the condition of our experiment only Cu was desorbed. In this way, improving the precious metals desorption, it is possible to obtain a pure solution rich in precious metals for the recovery with the electrodeposition process. Further experiments should be done to increase the selectivity of the recovery among precious metals.

Acknowledgment

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Chapter 6:

Environmental sustainability analysis of green technologies for precious metal extractions from end-of-life printed circuit boards

Abstract

In the last years green technologies, such as leaching with thiosulfate and bio-hydrometallurgy, has been increasingly studied as alternative processes to pyrometallurgy or cyanidation for precious metal extraction. The benefits are related to an improvement of the environmental issues of the common techniques, but the problems of these processes are that they require longer time for the extraction and the efficiencies are often lower. In this context, there is the necessity to improve these innovative treatments to increase both the technical and the sustainability levels. The environmental benefits of metals extraction from end-of-life printed circuit boards have been studied using the life cycle assessment (LCA) which is essential for the definition of the most sustainable option and the main criticalities of each scenario. The main objective of this analysis is the quantification of the balance between the impact of the recycling processes and the advantages resulting from the environmental credit for the recovered metals. The comparison of 3 different scenarios for the precious metal extraction, bioleaching (scenario 1), leaching with thiosulfate (scenario 2) and a pyrometallurgical process (scenario 0) showed that the best option was the chemical leaching with thiosulfate. The benefit of the process is highlighted by the positive credits derived from the good metal extraction efficiencies which gave a higher contribution to the total impact than the negative effect of the chemical reagents. Furthermore, LCA analysis has been used to design an additional experimental plan able to combine bioleaching and leaching processes to enhance the efficiency of metals recovery.

Content:

1. Introduction

The electronic waste (e-waste) is one of the fastest growing category of waste of the last century (Işıldar et al., 2018; Z. Li et al., 2019). The frequent changes in the technological industry and consumers behavior along with the shortest life span of the electronic devices has caused a large increase in the generation of e-waste (Amato et al., 2017; Işıldar et al., 2018). The reports predicted that the generation of e-waste will reach 74 Mt globally by 2030 (Forti et al., 2020).

The correct management of e-waste is fundamental due to the high toxicity of its components, such as heavy metals and flame retardants, and also necessary for the conservation of resources, such as valuable metals, since it delays the exhaustion of precious metals from minerals and avoid the environmental problems of mining (Işıldar et al., 2018; Z. Li et al., 2019). In particular the printed circuit boards (PCBs) of mobile phones and personal computers contain proportionately higher amounts of Au and Ag than those present in minerals (Işıldar et al., 2018). The most employed methods for metal recovery from e-waste are pyrometallurgy and hydrometallurgy or a combination of both the processes (Kaya, 2019). Pyrometallurgy includes the use of high temperature furnaces for melting and although it has a high reaction rate and the waste can be easily separated, it requires a high energy demand and large scale facilities (Işıldar et al., 2018; Kaya, 2019). On the other hand, hydrometallurgical processes, such as chemical leaching with thiosulfate or thiourea, are much less energy dependent, more economic and environmentally sustainable (Ghosh et al., 2015; Işıldar et al., 2018). In the last years bio-hydrometallurgy, based on the use of bacteria for the recovery of metals, has been increasingly studied. The process represents an interesting alternative to solve the environmental issues of the most common techniques as it reduces the problem associated with the treatment of waste flows, but it requires longer time for the extraction and the efficiencies are often lower than traditional methods (Kaya, 2019; Merli et al., 2022). Therefore, there is the necessity to improve these innovative treatments to increase both the technical and the sustainability levels. In this context, considering the results obtained from the bioleaching process (Chapter 3) and the thiosulfate leaching (Chapter 4) and the need to develop sustainable processes for precious metals extraction from end-of-life PCBs, the environmental benefit of hydrometallurgical and the bio-hydrometallurgical processes have been studied with the life cycle assessment (LCA). LCA is an environmental impact assessment tool that can be used to quantify the life cycle environmental load of the processes, but also to highlight the weakness of the processes in order to improve it (Amato et al., 2017, 2021). Furthermore, several configurations have been hypothesized and evaluated to identify the best scenario capable of combining efficiency and sustainability.

2. Materials and methods

2.2 Assessment of the carbon footprint

The assessment of the carbon footprint aimed at the comparison among 3 different scenarios for the precious metal extraction from end-of-life PCBs. The different options include the bioleaching process (scenario 1) and the hydrometallurgical process (scenario 2) developed within the present thesis (ref. Chapter 2 and Chapter 4) and a pyrometallurgical process chosen as reference (scenario 0). The impact of the pyrometallurgical process was based on the work of Li et al. (2019). The main objective of this analysis is the identification of the most sustainable option and the quantification of the balance between the impact of the recycling processes (for energy and raw material demand) and the advantage resulting from the environmental credit for the recovered metals. Furthermore, LCA analysis (for the carbon footprint estimation) has been used to support the design of an additional experimental plan able to combine bioleaching and leaching processes to enhance the efficiency of metal recovery. In more details, the theoretical analysis evaluated the carbon footprint of the combination of two bioleaching processes (scenario 3), a bioleaching process followed by a leaching with thiosulfate (scenario 4), two leaching steps with thiosulfate (scenario 5) and a leaching with thiosulfate followed by a bioleaching process (scenario 6), as reported in Figure 1.

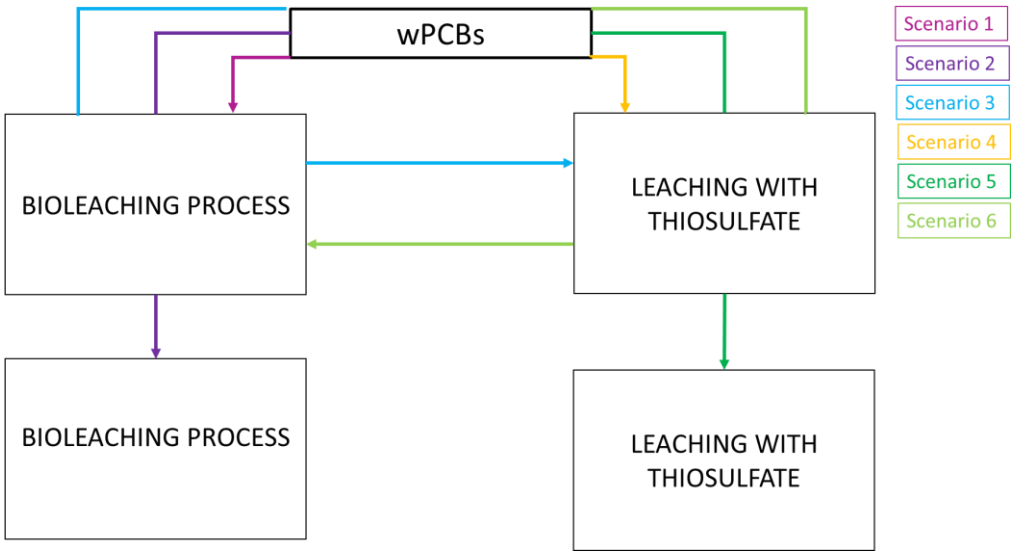

Figure 1. Different scenarios selected for the carbon footprint estimation

The results are essential for the definition of the most sustainable options to pursue also from a technical point of view. The detail of system boundaries considered for scenarios 1 and 2 are reported in Figure 2, the theoretical scenarios (scenario 4-6) are described in Figure 3. In both cases the steps of: PCB production, use, end-use, collection and the preliminary waste treatment are excluded from

the system boundaries since they are common to all options, as well as the recovery step (Figure 2 and Figure 3).

The GaBi software-System and the Database for Life Cycle Engineering (compilation 7.3.3.153; DB version 6.115) were used for the assessment of the carbon footprint related to the scenarios. The method selected for the analysis is EF 3.0, considering only the impact on Climate Change category [kg CO2 eq.] for the estimation of the carbon footprint. The functional unit selected for the analysis is 1 kg of treated wPCBs.

The nutrient medium for the bioleaching process (scenarios 1, 3, 4, 6) contains 5 g/L peptone, 5 g/L NaCl, 1 g/L meat extract and 2 g/L yeast extract. Since some data sets for these ingredients of the medium were not available, literature datawere used. The impact of gelatin has been assumed instead of peptone since it is its precursor (Stelzer et al., 2021). Instead of glycine the impact of methionine was used as this ammino acid was reported to be employed as an enhancer of cyanide synthesis (Li et al., 2020; Ruan et al., 2014). Concerning the thiosulfate leaching the impact of thiosulfate was estimated by the literature (Cairncross, 2020). Some assumptions were necessary to carry out the analysis. The positive effect of the extracted precious metals was quantified as environmental credit (the avoided impact from the primary production for the precious metal mining) and reported as negative value. On the other hand, the solid residues were assumed as zero-impact hypothesizing its further use in material productions (Amato et al., 2019).

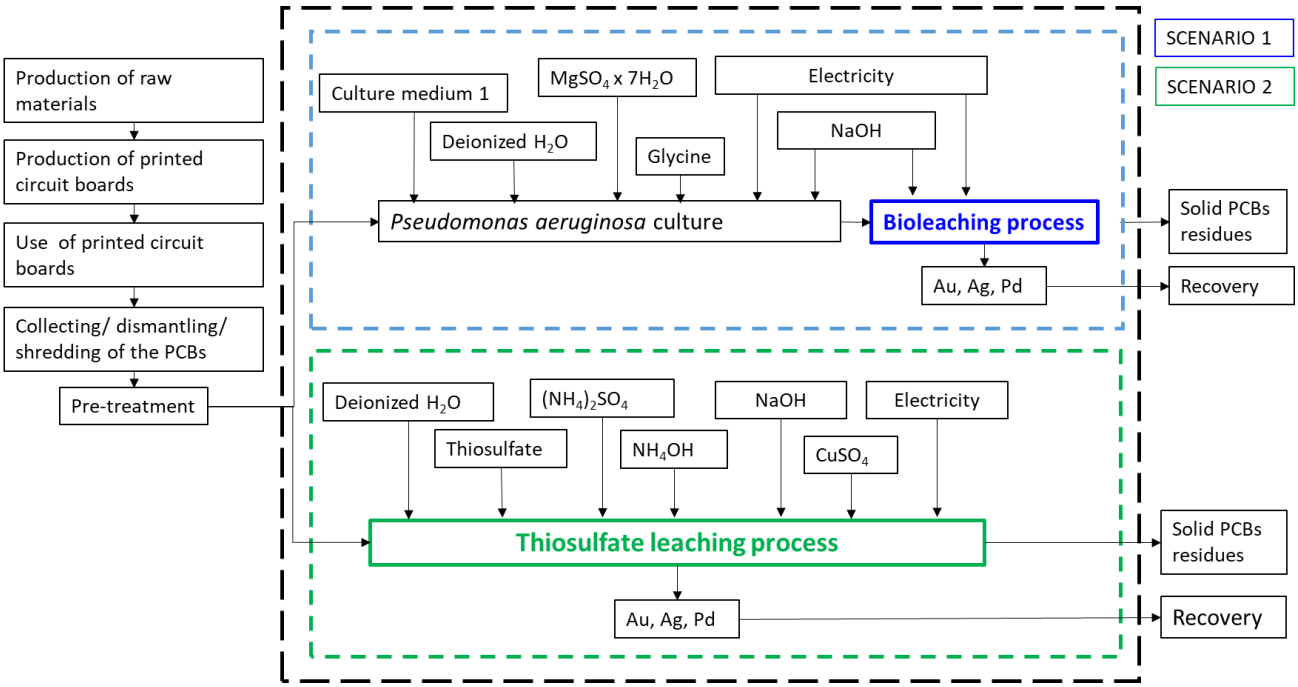

Figure 2. System boundaries for the bioleaching process (scenario 1) and the leaching with thiosulfate (scenario 2).

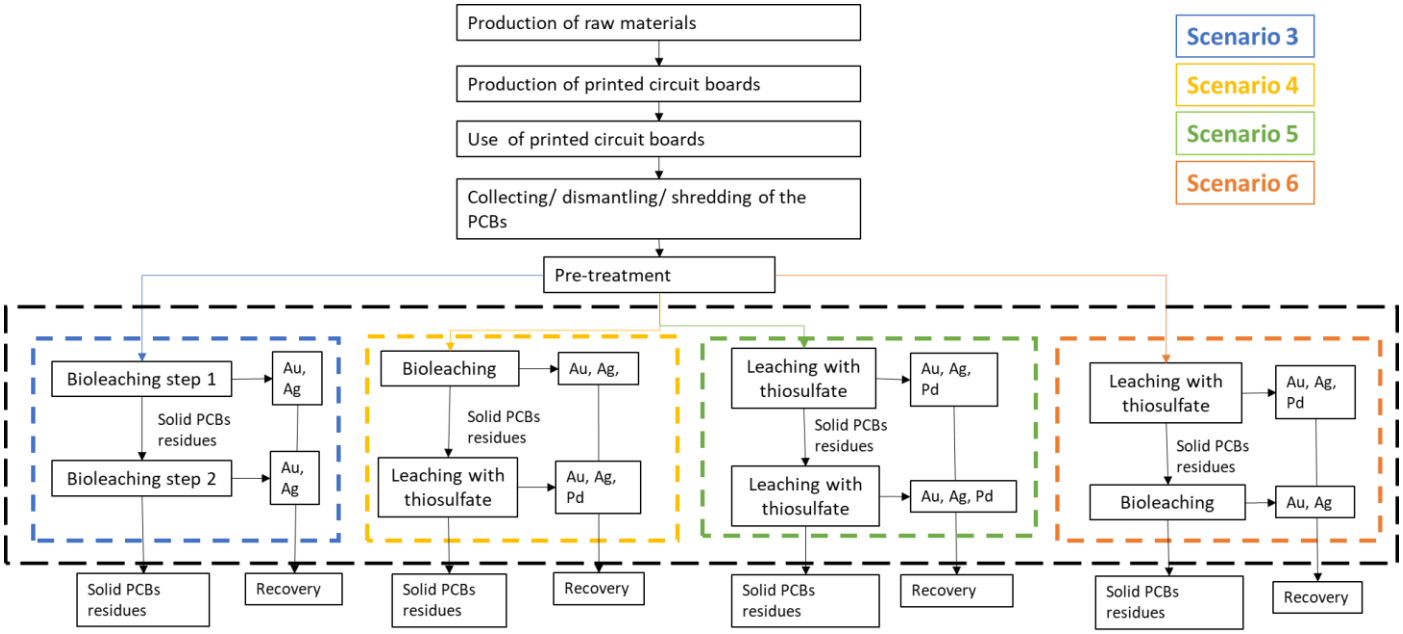

Figure 3. System boundaries for the combined process (scenarios 3 to 6)

2.3 Combined leaching and bioleaching processes

To verify the results of the theoretical LCA analysis, the combined leaching processes of scenarios 3-5 were tested by experimental analysis, while scenario 6 was not tested in this preliminary experimental phase as the theoretical results were comparable to scenario 4.

Two bioleaching treatments in series were performed on the pre-treated WPCBs (scenario 3), a first bioleaching for Ag mobilization followed by a second bioleaching with cyanogenic *P. aeruginosa* for Au extraction. Furthermore, the combination with a thiosulfate leaching process after the first bioleaching was also tested (scenario 4).

In both the bioleaching tests *P. aeruginosa* (10% v/v) was grown in the NB medium enriched with 2.5 g/L of glycine and 0.25 g/L $MgSO_4$ at pH 8. After 26 hours, the pH was raised to 9 by the addition of 2 M NaOH and the wPCBs (1 g/L) were added to the bacterial culture. In the second bioleaching process the added wPCBs derived from the first bioleaching. The tests were performed one after the other and both were conducted for 7 days after the addition of WPCBs. Samples were withdrawn for metals analysis every 24 hours.

The solution for leaching with thiosulfate was composed of 1 M thiosulfate ($Na_2S_2O_3$ anhydrous, 98%), 0.5 M ammonium hydroxide (NH_4OH, 30%), 0.25 M ammonium sulfate ($(NH_4)_2SO_4$, 99%) and 10 mM copper sulfate ($CuSO_4 \cdot 5\ H_2O$). The leaching test was conducted by mixing 10 g/L of WPCBs, deriving from the first bioleaching step, and 100 mL ammonia thiosulfate solution at an agitation speed of 120 rpm, at room temperature, for 24 hours, as reported in Chapter 4.

Two thiosulfate leaching processes were tested in series (scenario 5). The first leaching solution was composed by 0.1 M $Na_2S_2O_3$, 0.5 M NH_4OH, 0.25 M $((NH_4)_2SO_4$ and 10 mM $CuSO_4 \cdot 5\ H_2O$ to enhance Ag extraction. This process was followed by another thiosulfate leaching with 1 M $Na_2S_2O_3$, 0.5 M NH_4OH, 0.25 M $((NH_4)_2SO_4$ and 10 mM $CuSO_4 \cdot 5\ H_2O$ to enhance Au extraction. The pH was kept between 10 and 10.5 throughout the experiments by adding NaOH 10 M. The experiments were performed at an agitation speed of 120 rpm at 25 °C for 24 hours. The samples for chemical analysis were withdrawn every 4, 8, 16 and 24 hours to investigate the effects of the time.

3. Results

3.2 Carbon footprint assessment

The first target of this study was to evaluate the most sustainable option among the precious metal extraction techniques from end-of-life wPCBs. In this regard, the panel of Figure 4 shows that scenario 0 causes the highest environmental impact. Pyrometallurgy is based on incineration at high temperatures which produces a negative effect, with an impact of 30 kg CO_2-eq., due to the emission of gases with a global warming potential. The positive credits, derived from the precious metal extraction, were not enough to balance the overall impact of the process. The benefit achieved by both the green technologies, are highlighted by the comparison with the scenario 0, as both the processes resulted in a net credit for the environment, due to the avoided impact for the primary production of precious metals. The LCA results identified the scenario 2, the chemical leaching with thiosulfate, as the best choice with an overall impact of -22.8 kgCO_2-eq (Figure 4). The positive credits derived from the good metal extraction efficiencies (90% Au, 60% Ag and 99% Pd) gave a higher contribution to the total impact than the negative effect of thiosulfate and ammonia. On the other hand, the bioleaching process (scenario 1) gave a lower impact, mainly due to the energy demand, but also the credits derived from the recovery of the metals were lower (Figure 4).

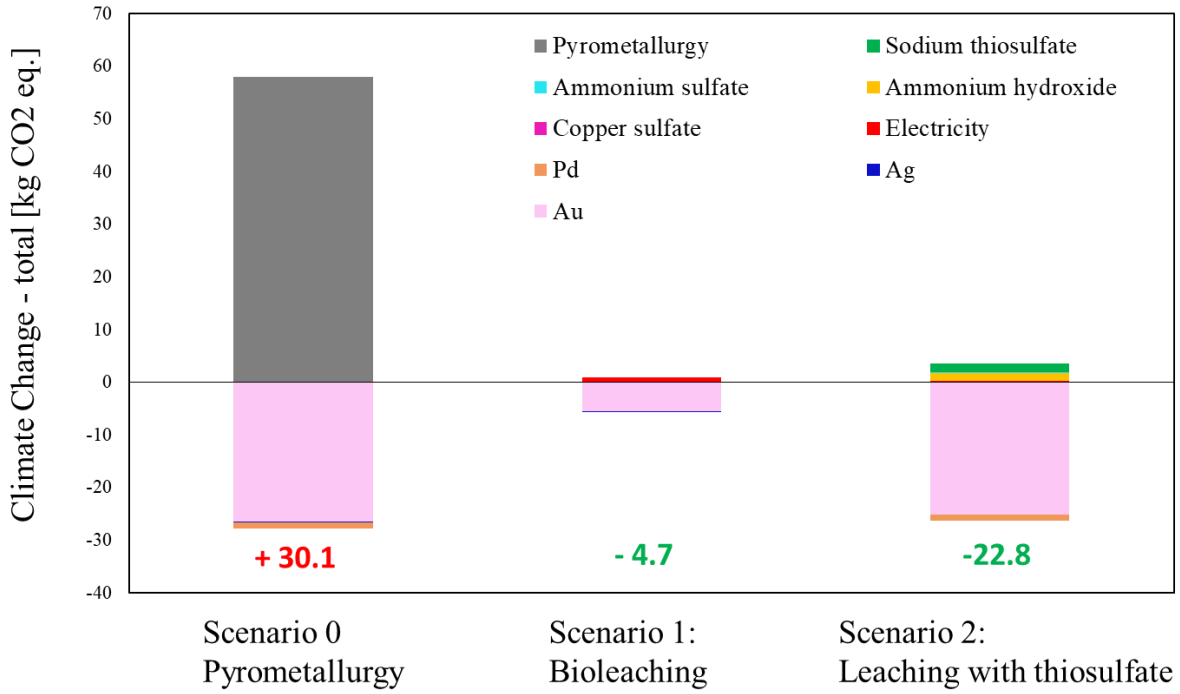

Figure 4. Evaluation of the carbon footprint of the considered scenarios: (1) pyrometallurgy, (2) bioleaching and (3) leaching with thiosulfate (functional unit: 1 kg of PCBs)

The environmental assessment of the theoretical combined processes highlighted as the best scenarios those that included at least one step of thiosulfate leaching. The limit derived from the bioleaching or the double bioleaching processes was the lower metal extraction compared to the combined processes with a chemical leaching step. In more details, the best theoretical scenario resulted to be the scenario 5 (double thiosulfate leaching), where the impact was slightly higher than the single thiosulfate process, but the estimated credits derived from the metals extraction are the highest. The hypothesis, based on previous experiments, is that with a second thiosulfate leaching the extraction of the precious metals is total and complete as changing the ratio between thiosulfate and ammonia concentrations favours the extraction of different metals (ref Chapter 4).

The combined processes including a bioleaching step decreased the negative impact due to thiosulfate and ammonia, as can be seen from Figure 5, but the hypothesis based on the results of the previous works, is that the efficiency of extraction can reach the 90% only for Ag. The extraction of Pd was not previously observed with a bioleaching process, while the maximum experimental Au extraction obtained was 20%, so the extraction can be completed only by the addition of a thiosulfate leaching step. According to the LCA analysis, the lower impact was not enough to make the bioleaching process competitive with the thiosulfate leaching process.

Figure 5. evaluation of the carbon footprint of the considered scenario: (1) bioleaching, (2) leaching with thiosulfate, (3) bioleaching + bioleaching, (4) bioleaching + thiosulfate leaching, (5) thiosulfate + thiosulfate leaching and (6) thiosulfate + bioleaching (functional unit: 1 kg of PCBs)

3.2 Combined leaching processes

The theoretical scenarios produced satisfying results that are worth to be tested and confirmed by experimental analysis, so scenarios 3, 4 and 5 were performed in the laboratory. Scenario 6 was not tested in these preliminary experimental phase as it was observed from the LCA analysis that it gave the same results as the scenario 4.

Concerning the bioleaching, it was reported from the previous experiments that Ag extraction was favoured over Au extraction, so a preliminary bioleaching process was performed to firstly recover Ag. The results of the first bioleaching highlighted a high Ag extraction (around 80%) which was not followed by Au and Pd extraction (Figure 6A). The Ag was not totally removed with the first bioleaching as in the second bioleaching process (Figure 6B) the extraction of Ag was completed. Also in the second bioleaching step, Au and Pd extraction was low, reaching around 10% of extraction (Figure 6B), confirming what suggested by LCA analysis.

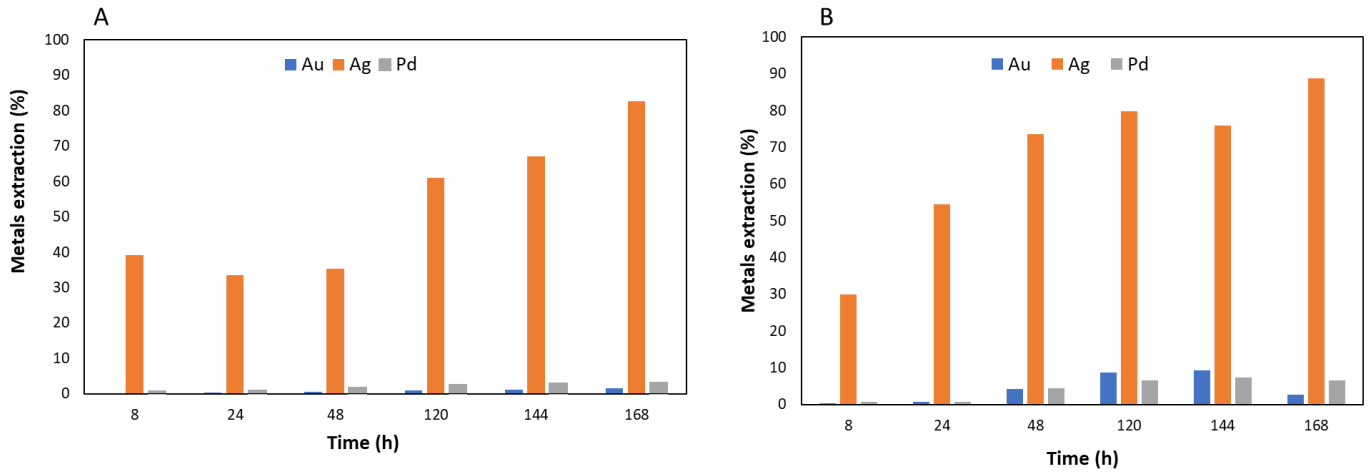

Figure 6. Precious metals extraction efficiencies with the combined bioleaching process

The solid residues derived from the first bioleaching were subject also to a thiosulfate leaching step to combine a first bioleaching and a second thiosulfate leaching processes. The results showed that Ag extraction was completed after 4 hours and Au extraction was higher, around 30 % after 24 hours, confirming a greater effectiveness of a second thiosulfate leaching step to increase the recovery efficiency, compared to another bioleaching process (Figure 7). On the other hand, the experimental analysis did not confirm the same good efficiency for Pd extraction, which was lower than the values hypothesized for the development of LCA (Figure 7).Further experiments will serve to verify and solve this problem.

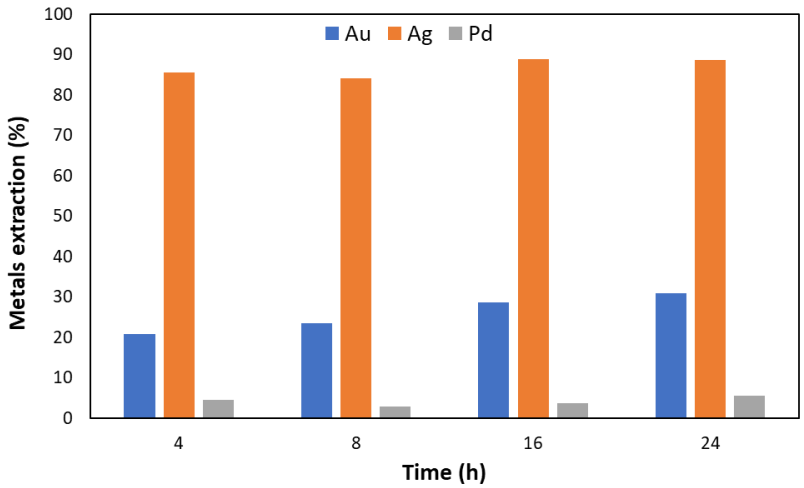

Figure 7. Precious metals extraction efficiencies with the thiosulfate leaching process after the first bioleaching step

The results of the double thiosulfate leaching process (scenario 5) are reported in Figure 8. The first leaching step had as specific target the extraction of Ag and the efficiency was 90% in the first 8 hours. The extraction of Au in the first step was around 50%, while Pd extraction was very low. In the second leaching process the extraction of Ag has been concluded, while Au leaching efficiencies

was around 10%. Even in this case the experimental efficiencies for Au and Pd extraction were lower than the values hypothesized for the development of LCA analysis, but still greater than the processes combined with the bioleaching steps.

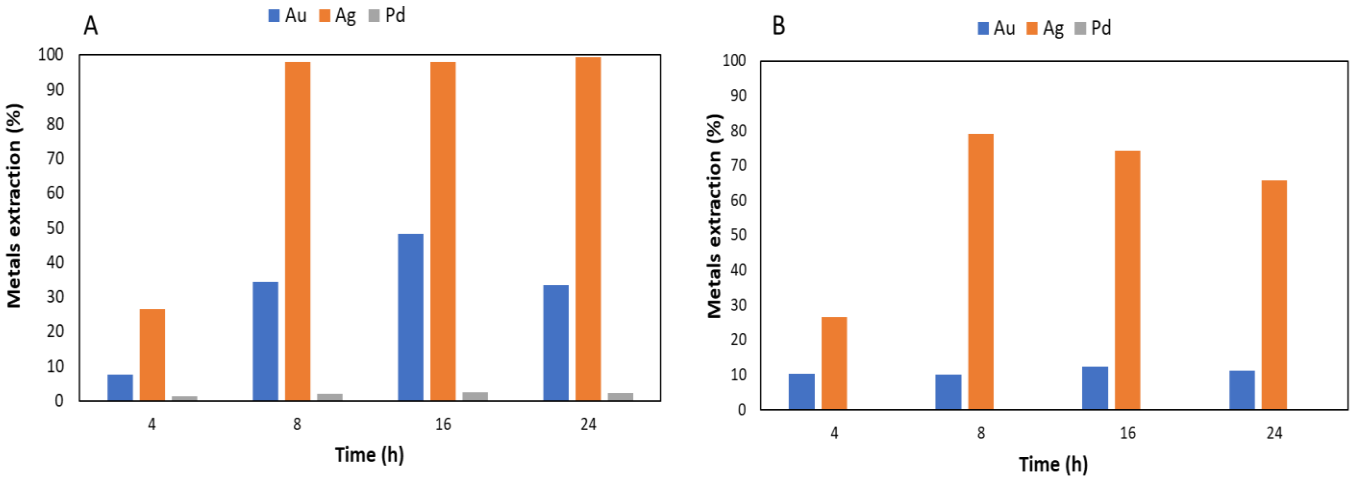

Figure 8. Metals extraction efficiencies with combined thiosulfate leaching processes

4. Conclusions

A LCA comparing the green technologies for precious metals recovery developed within this thesis with a reference pyrometallurgical process was performed. The results showed that pyrometallurgy was the worst scenario in the impact category of climate change even if the recovery of precious metals was total. According to the results of the analysis the best option, considering the impacts and the environmental gains, the thiosulfate leaching process is identified as the most sustainable option (scenario 2). This fact is due to the positive credits deriving from the high recovery efficiency of metals combined with the lowest impacts connected with the employed reagents. The analysis highlights that there is the need of further studies to increase the recovery of Au to make the bioleaching process more competitive. A possibility is the addition of an extra pre-treatment to completely remove the metals which can interfere with Au recovery, such as Cu.

Furthermore, the theoretical LCA analysis together with preliminary experimental tests highlighted as combining two thiosulfate leaching process in series can increase the recovery efficiencies of the metals without increasing the overall impact.

The results of the present study suggested that more effort should be addressed to increase the efficiency of the bioleaching process because by increasing the extraction efficiencies it would become the most environmentally sustainable process for the recovery of metals from end-of-life PCBs.

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