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Éllen Francine Rodrigues

Recovery of copper and gold from computer printed circuit boards by bioleaching with *Aspergillus niger*

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Thesis presented to the Graduate Program in Chemical Engineering of the Federal University of Santa Catarina, as a requirement for obtaining the PhD degree in Chemical Engineering.

Advisor: Prof. Dr. Dachamir Hotza
Co-Advisors: Prof. Dr. Débora de Oliveira
Dr. Alexsandra Valério

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Éllen Francine Rodrigues

Recovery of copper and gold from computer printed circuit boards by bioleaching with *Aspergillus niger*

The present work at doctoral level was evaluated and approved by an examining board composed of the following members:

Prof. Dr. Luciane Maria Colla
University of Passo Fundo (UPF)

Prof. Dr. Cristiano José de Andrade
Federal University of Santa Catarina (UFSC)

Dr. Daniela Bresolin
Federal University of Santa Catarina (UFSC)

We certify that this is the original and final version of the final paper that was considered appropriate to obtain the Title of PhD in the Chemical Engineering Graduate Program.

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Cintia Soares
Data: 29/04/2020 19:15:16-0300
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Prof. Dr. Cíntia Soares
Program Coordinator

Dachamir
Hotza:49523503987
Digitally signed by Dachamir
Hotza:49523503987
Date: 2020.04.29 18:14:05 -03'00'

Prof. Dr. Dachamir Hotza
Advisor

Florianópolis, 2020.

This thesis is dedicated to my parents, Ruth and Niles.

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“The mind that opens to a new idea never comes back to its original size” (Albert Einstein)

ABSTRACT

The recovery of metals from electronic waste and the development of new materials on a micro/nanoscale, using (or from) organic and inorganic compounds, has received prominence in several areas due to the potential for applications. Therefore, chemical and physical procedures have been used to recover metals through the synthesis of metal micro/nanoparticles. However, these methods have some disadvantages, such as the use of toxic or dangerous solvents and the generation of by-products. From this context, the objective of this thesis is to promote the recovery of metals through the biosynthesis of micro/nanoparticles from electronic waste using the fungus *Aspergillus niger*. Firstly, the metal components from waste printed circuit boards (PCBs) have been pre-concentrated using a separation process by an alternative dense medium based on sodium silicate, in comparison with chloroform. The bioleaching was performed in an incubator shaker (160 rpm) at 30 ºC at different PCBs waste concentrations (2.5 to 10 g/L). Three bioleaching approaches were studied: one-step, two-step and spent medium. In the one-step approach, the PCBs are mixed immediately with the *A. niger* in the culture medium at the beginning of the process. In the two-step procedure, PCBs are added after the *A. niger* reaches the maximum growth (logarithmic growth phase). In the spent medium approach, bioleaching experiments are carried out in cell-free acidic filtrates after fungal activation. In the third stage of the work, the microorganism *A. niger* was adapted to the metal fraction of PCBs and used in the process of bioleaching. The results showed that the sodium silicate used in the process of pre-concentration of metals present in PCBs is considered suitable for the process. The sample decanted in sodium silicate solution showed higher content of metallic elements: gold (31 wt.%), silver (21 wt.%), copper (58 wt.%) and palladium (85 wt.%) when compared to the untreated sample (control). The different approaches (one-step, two-step and spent medium) showed that the two-step process is the most suitable for the recovery of metals, being the one with the highest recovery efficiency (100% copper and 42.5% gold). Concerning the particle size distribution, particles with 131 nm were obtained at lower PCBs concentrations (2.5 g/L). The adaptation process of *A. niger* in the metal waste showed higher acid production and efficiency in the bioleaching process when compared to the unadapted *A. niger*. Up to 97.7% copper; 47.1% gold (at 10 g/L metal concentration) and 80.2% copper; 30.9% gold (at 20 g/L metal concentration) were leached from PCBs waste by adapted *A. niger*. From the transmission electron microscopy analysis, it was observed that the leached metallic particles were smaller than 100 nm, thus attesting the production of metallic nanoparticles through the bioleaching process. The results obtained from the analyses carried out confirmed the efficacy of fungal metabolites in leaching the metals present in the computers printed circuit boards and obtaining nanoparticles.

Keywords: *Aspergillus niger*. Bio-hydrometallurgy. Bioleaching. Nanoparticles. Printed circuit boards.

RESUMO

A recuperação de metais a partir de lixo eletrônico e o desenvolvimento de novos materiais em micro/nanoescala, utilizando compostos orgânicos e inorgânicos, têm recebido destaque em diversas áreas devido ao potencial de aplicações. Portanto, processos químicos e físicos têm sido utilizados para recuperar metais através da síntese de micro/nanopartículas metálicas. No entanto, esses métodos apresentam algumas desvantagens, como o uso de solventes tóxicos ou perigosos e a geração de subprodutos. A partir deste contexto, o objetivo desta tese é promover a recuperação de metais através da biossíntese de micro/nanopartículas de resíduos eletrônicos, utilizando o fungo *Aspergillus niger*. Na primeira etapa, os componentes metálicos das placas de circuito impresso de resíduos (PCBs) foram pré-concentrados usando um processo de separação por um meio denso alternativo à base de silicato de sódio, em comparação com o clorofórmio. Na segunda etapa, o processo de biolixiviação foi realizado em um agitador de incubadora (160 rpm) a 30 ºC em diferentes concentrações de resíduos de PCB (2,5 a 10 g/L). Três abordagens de biolixiviação foram estudadas: *one-step*, *two-step* e *spent medium*. Na abordagem *one-step*, as PCBs são adicionadas imediatamente com o *A. niger* no meio de cultura no início do processo. No procedimento *two-step*, as PCBs são adicionadas após o *A. niger* atingir o crescimento máximo (fase de crescimento logarítmico). Na abordagem *spent medium*, a biolixiviação é realizada utilizando os filtrados ácidos sem células após a ativação dos fungos. Na terceira etapa do trabalho, o microrganismo *A. niger* foi adaptado à fração metálica das PCBs e utilizado no processo de biolixiviação. Os resultados mostraram que o silicato de sódio utilizado no processo de pré-concentração de metais presentes nos PCBs é considerado adequado para o processo. A amostra decantada em solução de silicato de sódio apresentou maior teor de elementos metálicos: ouro (31% em peso), prata (21% em peso), cobre (58% em peso) e paládio (85% em peso) quando comparado ao não tratado amostra (controle). As diferentes abordagens (*one-step*, *two-step* e *spent medium*) mostraram que o processo de *two-step* é o mais adequado para a recuperação de metais, sendo o de maior eficiência de recuperação (100% de cobre e 42,5% de ouro). Com relação à distribuição granulométrica, foram obtidas partículas com 131 nm em concentrações mais baixas de PCBs (2,5 g/L). O processo de adaptação de *A. niger* nos resíduos metálicos apresentou maior produção de ácido e eficiência no processo de biolixiviação quando comparado ao *A. niger* não adaptado. Até 97,7% de cobre; 47,1% de ouro (na concentração de 10 g/L de metal) e 80,2% de cobre; 30,9% de ouro (na concentração de 20 g/L de metal) foram lixiviados dos resíduos de PCB por *A. niger* adaptado. A partir da análise por microscopia eletrônica de transmissão, observou-se que as partículas metálicas lixiviadas eram menores que 100 nm, atestando assim a produção de nanopartículas metálicas através do processo de biolixiviação. Os resultados obtidos nas análises realizadas confirmaram a eficácia dos metabólitos fúngicos na lixiviação dos metais presentes nas placas de circuito impresso dos computadores e na obtenção de nanopartículas.

Palavras-chave: *Aspergillus niger*. Bio-hidrometalurgia. Biolixiviação. Nanopartículas. Placas de circuito impresso.

RESUMO ESTENDIDO

Introdução

O aumento do uso de aparelhos eletrônicos como computadores e celulares apresenta novos desafios e impactos ambientais, quando estes são dispostos de maneira inadequada. Esses equipamentos eletrônicos são mencionados como um problema de resíduos emergentes no Brasil, pois a indústria de eletrônicos está crescendo exponencialmente a cada ano e o país está entre os maiores geradores de lixo eletrônico no mundo, totalizando 1,4 milhões de toneladas por ano. De acordo com a Organização das Nações Unidas (ONU), o Brasil é o país no mundo que mais descarta resíduos eletrônicos de forma ineficiente e poluente, pois geralmente estes resíduos são encaminhados para aterros industriais ou incineradoras.

As placas de circuito impresso (PCBs) de computadores são consideradas resíduos eletrônicos de interesse econômico geralmente definido como *urban mine*. As PCBs apresentam em sua composição uma mistura complexa de metais preciosos (Ag, Au, Pd e Pt), metais básicos (Cu, Al, Ni, Si, Zn, Fe), metais tóxicos (Hg, Be, Cd, Cr, Sb e Bi), junto com halogênios e substâncias combustíveis (ZHANG et al., 2016), sendo a concentração destes metais de até 10 vezes maior que nos minérios (VAN EYGEN et al., 2016). A composição complexa e o aumento da geração desses resíduos são motivos importantes para que pesquisas sejam realizadas não só no gerenciamento, mas também na recuperação de metais de interesse.

Ao longo das últimas décadas, o estudo sobre técnicas ambientalmente mais sustentáveis para recuperação de materiais de interesse presentes em resíduos eletrônicos tem sido enfatizado. No entanto, ainda existem obstáculos técnicos que limitam a aplicação industrial desses processos. Atualmente, os processos tradicionais utilizados para a recuperação de metais são a pirometalurgia e a hidrometalurgia. Os processos pirometalúrgicos normalmente estão ligados aos impactos ambientais ocasionados pela geração de gases do efeito estufa e a formação de dioxinas, furanos e gases ácidos gerados na combustão dos materiais poliméricos. Com relação aos processos hidrometalúrgicos, esses podem apresentar impactos ambientais devido à utilização de soluções ácidas e básicas para dissolver o material sólido e à baixa estabilidade química dos resíduos sólidos gerados (OKIBE et al., 2016).

Nesse contexto, surge a bio-hidrometalurgia (biolixiviação), que tem atraído interesse pela recuperação de metais de resíduos eletrônicos devido à sua natureza favorável ao meio ambiente e econômica, e também pela ampla gama de microrganismos capazes de mobilizar metais comuns (Cu, Zn, Fe, Ni) e metais preciosos (Au, Ag, Pd e Pt). A biolixiviação é baseada na conversão de compostos sólidos em formas aquosas solúveis com capacidade de extração usando microrganismos. O processo de biolixiviação se dá através da utilização de produtos metabólicos ou pela redução ou oxidação enzimática dos compostos sólidos (JAAFAR et al., 2016). O *Aspergillus niger* é um fungo eficaz no processo de biolixiviação devido à sua capacidade de produzir ácidos orgânicos e agentes quelantes durante sua fase de crescimento (AKPOR et al., 2015). A baixa toxicidade fez do fungo *A. niger* uma das poucas espécies que receberam o status de GRAS (*Generally Regarded as Safe*) conferido pela *Food and Drug Administration* (FDA) dos Estados Unidos (EUA). Isso faz com que os metabólitos produzidos por essa espécie sejam aceitáveis para o uso em diversas aplicações na indústria (STRICKER; MACH; DE GRAAFF, 2008). Diversos estudos disponíveis na literatura utilizam microrganismos nos processos de biolixiviação (IŞILDAR, 2018b; KIM et al., 2016; KIM; SEO; ROH, 2018; NASERI; BAHALOO-HOREH; MOUSAVI, 2019). Contudo, o uso de fungos filamentosos como *A. niger* para a recuperação de metais presentes em placas de circuito impresso tem sido pouco explorado.

Comparada aos processos físicos e químicos convencionais, a biolixiviação apresenta vantagens ambientais e econômicas, reduzindo impactos ambientais, o consumo de energia e a geração de resíduos. É um processo ecológico seguro e simples (BORJA et al., 2016; PARK; FRAY, 2009).

Objetivos

O objetivo principal do trabalho consiste na recuperação de metais (Cu e Au) presentes em placas de circuito impresso de computadores utilizando *Aspergillus niger*.

Os objetivos específicos foram:

- Caracterizar (física e quimicamente) os PCBs;
- Avaliar dois meios líquidos densos (silicato de sódio e clorofórmio) no processo de separação da fração metálica e não metálica dos PCBs;
- Estudar a influência do tipo de processo de biolixiviação (*one-step*, *two-step* e *spent medium*) e o tempo do processo na recuperação de Cu e Au por *A. niger*;
- Estudar a influência da concentração da fração metálica no processo de biolixiviação;
- Estudar a influência de *A. niger* adaptado no processo de biolixiviação;
- Avaliar o controle e a distribuição de tamanho de partículas metálicas sob diferentes condições de biossíntese.

Metodologia

Para a obtenção da fração metálica presente nas PCBs foi realizado uma etapa de pré-concentração. As placas foram trituradas em moinho de martelo e de facas e os componentes metálicos das PCBs foram pré-concentrados usando um processo de separação em meio denso. A influência de um líquido denso alternativo a base de silicato de sódio em comparação com o clorofórmio (frequentemente utilizado) foi avaliada.

O processo de biolixiviação foi realizado através da inoculação do *A. niger* em meio mineral com diferentes concentrações de fração metálica. Os experimentos foram realizados em *shaker* a 160 rpm e 30 ºC por 24 dias. No Capítulo 3, as variáveis estudadas foram os efeitos da concentração metálica, o tipo do processo de biolixiviação (*one-step*; *two-step* e *spent medium*) e o tempo de processo. No Capítulo 4, avaliou-se o processo de biolixiviação utilizando a cepa de *A. niger* adaptado com PCBs em comparação ao *A. niger* não adaptado. As variáveis estudadas foram: concentração metálica e adaptação do microrganismo.

Para investigar a eficiência do processo de recuperação de metais, foram realizadas análises físicas e químicas. Dentre essas análises, estão a espectrometria de massa com plasma acoplado indutivamente, absorção atômica, distribuição de tamanho de partícula, microscopia eletrônica de transmissão, espectroscopia no infravermelho por transformada de Fourier, entre outras análises específicas para cada capítulo experimental.

Resultados e Discussão

A partir dos resultados obtidos para o processo de pré-concentração dos metais presentes nos PCBs, o silicato de sódio apresentou resultados satisfatórios quando comparados ao clorofórmio. O cobre foi o elemento predominante, apresentando 173 $mg/g_{resíduo}$; com relação às concentrações dos metais preciosos, essas foram de 0,22 $mg/g_{resíduo}$, 7,4 $mg/g_{resíduo}$ e 0,007 $mg/g_{resíduo}$ para ouro, prata e paládio, respectivamente.

As diferentes abordagens estudadas para a recuperação dos metais (*one-step* (100% Cu e 41% Au), *two-step* (100% Cu e 42,5% Au) e *spent medium* (100% Cu e 35,16% Au)) apresentaram resultados satisfatórios de recuperação. No entanto, a abordagem *two-step* foi a mais adequada por apresentar maior eficiência no processo de recuperação. A concentração metálica também influenciou na eficiência do processo de recuperação de metais, indicando que quanto menor a concentração metálica, maior a eficiência de recuperação. A distribuição granulométrica obtida para todos os experimentos variou consideravelmente, partículas menores (131 nm) foram obtidas em concentrações metálicas mais baixas (2,5 g/L).

O processo de adaptação do *A. niger* no resíduo metálico apresentou maior produção de ácidos e eficiência no processo de biolixiviação quando comparado ao *A. niger* não adaptado. A acidez total obtida para *A. niger* adaptado e não adaptado foi de 24% e 19%, respectivamente. Já no processo de biolixiviação, o *A. niger* adaptado apresentou eficiência de recuperação do cobre de 97% (concentração metálica 10 g/L) e 80% (concentração metálica 20 g/L). Com a cepa não adaptada, a taxa de recuperação foi de 90% (concentração metálica 10 g/L) e 62% (concentração metálica 20 g/L). Com relação ao tamanho de partículas obtido nos extratos lixiviados através da análise de MET, estes apresentaram tamanhos menores que 100 nm, indicando assim a obtenção de nanopartículas metálicas.

Considerações finais

As abordagens propostas para o processo de recuperação de metais presentes em placas de circuito impresso de computador foram eficientes. Os resultados obtidos neste estudo mostraram a possibilidade de recuperar metais de resíduos eletrônicos através de um processo de produção mais limpa com custos energéticos e ambientais mais baixos quando comparados aos processos tradicionais de recuperação.

As principais conclusões deste trabalho são:

- O uso de silicato de sódio como uma rota alternativa para pré-concentração de metais de placas de circuito impresso foi eficiente;
- As abordagens propostas apresentaram eficiência de recuperação de cobre acima de 80%;
- *A. niger* adaptado apresentou melhora na produção de ácidos e eficiência no processo de biolixiviação;
- Nanopartículas de Au e Cu foram obtidas através do processo de biolixiviação.

Palavras-chave: Bio-hidrometalurgia. Biolixiviação. *Aspergillus niger*. Placas de circuito impresso.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
CAPES	Coordination Of Superior Level Staff Improvement
CD	Chloroform – Decanted
EDX	Energy Dispersive X-Ray Spectroscopy
EUA	United States of America
FDA	*Food and Drug Administration*
FTIR	Fourier-Transform Infrared Spectroscopy
GRAS	*Generally Regarded As Safe*
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LabMASSA	Laboratory of Mass Transfer
LCME	Central Laboratory of Electronic Microscopy
LCP	Polymer Design and Process Control Laboratory
LEMA	Mass and Atomic Spectrometry Laboratory
LINDEN	Interdisciplinary Laboratory for the Development of Nanostructures
ONU	United Nations
PCB	Printed Circuit Board
SEM	Scanning Electron Microscope
SSD	Sodium Silicate – Decanted
TEM	Transmission Electron Microscopy
UFSC	Federal University of Santa Catarina
UPF	University of Passo Fundo

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CONCEPTUAL DIAGRAM

Recovery of copper and gold from computer printed circuit boards by bioleaching with *Aspergillus niger*

Why?

- In recent years, the increase in the generation and inadequate disposal of electronic wastes has increased significantly in the world;
- The concentration of metals in waste is ~10 times higher than in ores;
- Processes using microorganisms are considered environmentally friendly for particle synthesis.

Who has done it?

- Few studies on the recovery of precious metals using bioprocesses;
- Few studies are found using filamentous fungi in the bioleaching process.

Hypotheses

- Is it possible to recover copper and gold from computer printed circuit boards using biosynthesis?
- Is *A. niger* able to recover metals on a micro/nanometric scale?
- Has the process satisfactory efficiency (>50%)?

Experimental methods

- Preparation and chemical characterization of electronic waste;
- Bioleaching process using fungi for the recovery of copper and gold present in electronic waste;
- Biosynthesis of metallic micro/nanoparticles through metals recovery using *A. niger*;
- Physical, chemical and structural characterization of bioleached extracts.

Answer

- Bioleaching recovery (%) of copper and gold using the *A. niger*;
- Size and shape of the metallic particles obtained in the bioprocess.

1 INTRODUCTION

The electrical and electronic equipment are consumed at high speed in the world, shortening the life cycle of such devices and generating a lot of waste electrical and electronic waste. The e-waste has become the fastest-growing solid waste, which is increasing 3-5% year (ZHANG et al., 2016) and the metal pollution due to the huge electronic waste accumulation is a global problem. The United Nations (UN) estimates that the global waste from electrical and electronic equipment was 24 and 49 million tons in 2002, and 2012, respectively, which is projected to increase to 52.2 million t/year by 2021 (BALDÉ et al., 2017; KAYA, 2016).

The natural sources of metals are limited, and the use of secondary sources as e-waste raw material for resource recovery is fundamental for the conservation of primary ores and decreasing environmental impact due to the reduction of the carbon footprint (PETTER; VEIT; BERNARDES, 2014). Moreover, up to 95 and 85% of energy is saved when recycling aluminum and copper, respectively, compared to their production from primary ores (CUI; FORSSBERG, 2003).

The e-waste, also known as Waste Electric and Electronic Equipment (WEEE), includes a wide range of products with circuitry or electrical components with power or battery supply that have been discarded as waste without the intent of re-use (BALDÉ et al., 2015). Essential constituents of many electronic products are base metals (copper, aluminum, nickel, iron, zinc), precious metals (gold, silver, palladium, and platinum), special metals (indium, selenium, tellurium, tantalum, bismuth, antimony) along with many hazardous materials such as heavy metals, flame retardants, and organic substances.

Among the electronic components, printed circuit boards (PCBs) are the most used components in electronic products, accounting for 3% of the total mass of e-waste. PCB is a generalized term used for the board upon which microelectronic components such as semiconductor chips and capacitors are mounted, and used to support those components (BAZARGAN et al., 2014). PCBs contain 28-32% metal and 68-72% non-metal components (plastics, glass, and ceramics) (BIZZO; FIGUEIREDO; DE ANDRADE, 2014; LIU; LI; GE, 2016; OH et al., 2003). PCBs are a complex mixture of precious metals (Ag, Au, Pd, and Pt), base metals (Cu, Al, Ni, Si, Zn, Fe), and toxic metals (Hg, Be, Cd, Cr, and Sb), also including halogens and combustible substances (CHI et al., 2011; KHALIQ et al., 2014; MENG; GUO; GUO, 2019; ZHANG et al., 2016).

Precious metals are widely applied in the printed circuit board due to their distinct physical and chemical properties, such as good electrical conductivity and corrosion resistance. The concentration of precious metals in PCBs is usually much higher than the concentration of precious metals in ores (CHANCEREL et al., 2009). Precious metals such as gold, palladium, and silver make more than 70% of the value of PCBs that could be recovered whereas copper represents 9.7% of the value (PARK; FRAY, 2009).

This significant amount of valuable materials present in PCBs waste makes them worth to be recycled. Nevertheless, the PCB recycling process is a difficult path due to the complexity of the materials and the presence of toxic substances (KHALIQ et al., 2014). Therefore, it is necessary to develop technologies for recycling PCBs wastes. Metals recovery from e-waste has been a rising area of research in recent years. The choice of appropriate technology to the recovery process is determined by the metal content, the type of accompanying elements and by the character of the material they are embedded in (FICERIOVÁ; BALÁŽ; GOCK, 2011).

The processes of metals recovery of e-waste can be divided into 3 main steps: disassembly, separation or concentration, and refining. Disassembly is the removal of the electronic components. The process of separation and concentration is by grinding, and grinding is of great importance, as it directly interferes with the process of separation of the materials (LUDA, 2011). The separation of metals, polymers, and ceramics is performed based on the size, density and electrical or magnetic properties of the materials to be separated (CUI; FORSSBERG, 2003). The separation obtained in these processes is not sufficient for the materials to be of high purity. Thus, there is a need to employ chemical, thermal and/or biological processes.

The main methods employed in order to recover metals from PCBs are basically mechanical processing technology, pyrometallurgy, and hydrometallurgy, but these processes have high risks of environmental pollution (CUI; FORSSBERG, 2003; KAYA, 2016). Pyrometallurgy is a process that involves high temperatures (800 to 1600 ºC) to obtain and refine materials, which includes incineration, melting, sintering, among other processes (ARGUMEDO-DELIRA; GÓMEZ-MARTÍNEZ; SOTO, 2019; KHALIQ et al., 2014). However, pyrometallurgical processes are usually linked to environmental impacts caused by the generation of greenhouse effect gases and the formation of dioxins, furans and acid gases generated in the combustion of polymeric materials (IANNICELLI-ZUBIANI et al., 2017; R. MANKHAND et al., 2013).

The hydrometallurgical processes leach out the maximum concentration of metals in a rapid and efficient manner. However, the toxicity of chemicals and environmental concerns inhibit their applicability at industrial scale (IANNICELLI-ZUBIANI et al., 2017). The hydrometallurgical process may be better than pyrometallurgy for metals recovery (AKCIL et al., 2015), due to advantages such as easy separation of the main components present in electronic waste and reduction of process costs due to low consumption energy and recycling of chemical reagents used. On the other hand, hydrometallurgy also shows some disadvantages as the requirement of mechanically process the waste to reduce the volume, use of a large number of solutions, and generation of solid waste (SUN; YANG; GUI, 2015; ZHANG; YANG; GUI, 2016).

Some environmentally eco-friendly processes, such as bio-hydrometallurgy are being studied to replace traditional recycling/recovering processes. Bio-hydrometallurgy represents a potential technology to recover precious metals from unconventional sources, including mine tailings and electronic wastes (BORJA et al., 2016). Metal recovery through bio-hydrometallurgy can be divided into two areas: bioleaching and biosorption. Both bioleaching and biosorption processes involve metal leaching through the action of microorganisms. The principle of bioleaching is based on the metals dissolution by oxidizing agents produced by microorganisms, while biosorption includes the adsorption onto the cellular structure.

Usually, bioleaching is carried out under mild temperature, without the addition of toxic chemicals, once the microorganisms can produce organic acids and enzymes used for the metals recovery, as a consequence of metabolic pathway (JOHNSON, 2014). Although the process is simple and easy to operate, a drawback of the process is the long synthesis time (KAKSONEN et al., 2018; SINHA et al., 2018).

The bioleaching process has been seen as a promising technology for the treatment and recovery of precious metals from e-waste, as it is a low energy consumption process and it does not use any toxic reagents. However, the concentration of electronic waste used in the bioleaching process is still low due to the toxicity of some metals against the microorganisms when in excess (TUNCUK et al., 2012). In addition to the waste concentration, other factors should be taken into consideration, such as the presence of toxic substances, particle size and specific surface area of the material to be leached, adaptation of microorganisms, temperature, pH, and nutrient concentration (SILVAS, 2014). The amount of metals recovered by the bioleaching process depends on the microorganism used and the growth conditions applied.

One of the first microorganisms isolated and used in metal recovery studies was *Acidithiobacillus ferrooxidans*, which was able to promote solubilization of copper present in ores (JOHNSON, 2014). *Aspergillus niger* is a filamentous fungus that constitutes a group of aerobic microorganisms with a versatile metabolism, which can grow in liquid and/or solid medium (BRANDL; BOSSHARD; WEGMANN, 2001; HOREH; MOUSAVI; SHOJAOSADATI, 2016). In fact, *A. niger* is one of the most important filamentous fungi species used in biotechnology. In addition, it is important to mention that microorganisms such as fungi *Aspergillus niger* and *Penicillium simplicissimum* can grow in the presence of electronic waste, and the metal bioleaching process depends on the growing conditions. In particular, for *A. niger*, the bioleaching capacity is attributed to the production of organic acids (citric, gluconic, malic, and oxalic acid) (BAHALOO-HOREH; MOUSAVI; BANIASADI, 2018; DÍAZ-MARTÍNEZ et al., 2019; HOREH; MOUSAVI; SHOJAOSADATI, 2016; NARAYANASAMY et al., 2018).

The need to minimize the environmental impacts caused by traditional processes (pyrometallurgical and hydrometallurgical) in the industrial sector, as well as the reduction in the energy consumption of these processes, encourage further studies in the biotechnological area to improve the approach to metal recovery. Bioleaching using *A. niger* is an environmentally friendly approach for the recycling/recovery of metals. Nevertheless, few studies can be found in the literature using filamentous fungi in the bioleaching process. On the other hand, we must emphasize the possibility of recovering large volumes of e-waste. Therefore, for the large-scale implementation of the bioleaching process, it is necessary to optimize the design and performance of the process for the recovery of metals in a biotechnological system that can be useful in obtaining new products.

1.1 OBJECTIVES

1.1.1 General goal

The main purpose of this work was to evaluate metals removal from computers printed circuit boards by bioleaching using *Aspergillus niger*.

1.1.2 Specific objectives

The specific goals were, respectively:

- To characterize (physically and chemically) the PCBs;

- To evaluate two dense liquids medium (sodium silicate and chloroform) in the process of the metal and non-metal fraction separation from PCBs;
- To study the influence of the bioleaching process pathway (one-step, two-step, and spent-medium) and process time on copper and gold recovery by *A. niger*;
- To study the influence of the metal fraction concentration on the bioleaching process of copper and gold;
- To study the influence of adapted *A. niger* on the bioleaching process;
- To evaluate the control and size distribution of metal particles under different biosynthesis conditions.

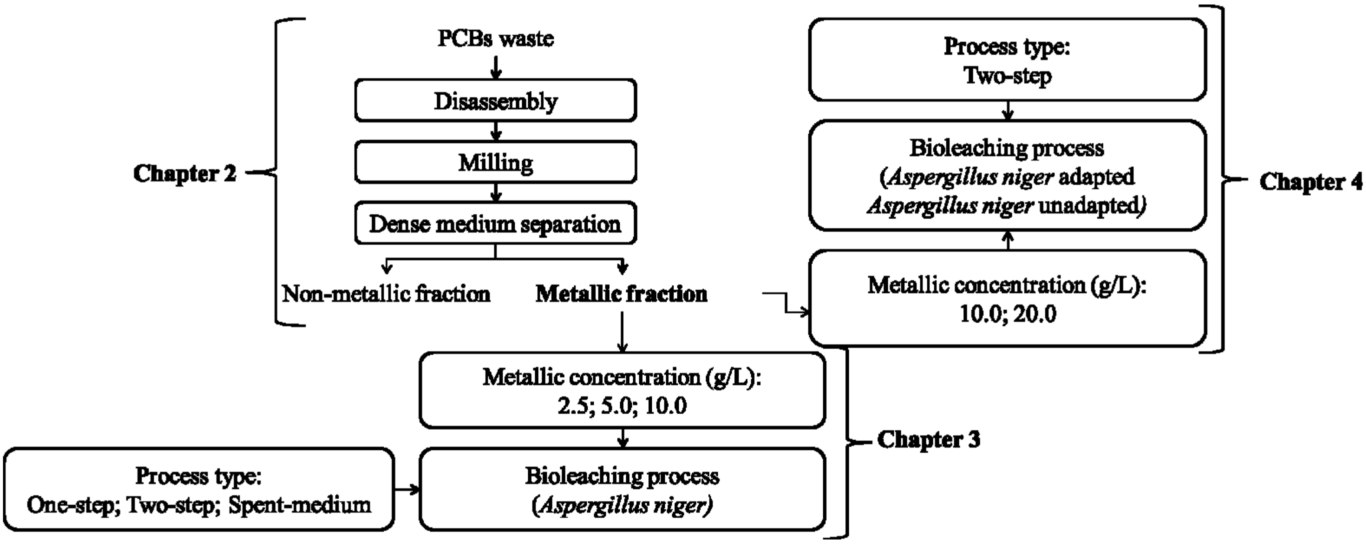
1.2 CONTENT OF THE THESIS

This thesis is divided into 5 chapters:

- Chapter 1 reports a general introduction to the subject discussed in this thesis, the general and the specific objectives;
- Chapter 2 presents an alternative dense medium for pre-concentrating the metal components present in the printed circuit boards by a separation process. Metal and non-metal fractions were obtained, and the results were compared regarding efficiency and environmental aspects;
- In Chapter 3, a biological recovery of copper and gold from discarded computer printed circuit boards was carried out by bioleaching with a fungus, *A. niger*. The influence of process type, process time and metal concentration were studied;
- In Chapter 4, a two-step bioleaching process was performed by adapted *A. niger*;
- Chapter 5 presents the conclusions, as well as some suggestions for future work.

The structure of the experimental section of this thesis is divided into 3 stages (2 to 4 chapters) which are presented in the flowchart in Figure 1.

Figure 1 - Flowchart of the experimental section of the thesis



2 USE OF SODIUM SILICATE AS A DENSE LIQUID FOR METALS PRE-CONCENTRATION FROM PRINTED CIRCUIT BOARD WASTE

2.1 Introduction

The increased use of electronic devices such as computers and cell phones represents new challenges and environmental impacts when they are inappropriately discarded (GOMES et al., 2017). About 20-50 million tons of electronic wastes are produced around the world every year with an annual increase of 3-5% (BALDÉ et al., 2017). These electronic wastes are a complex mixture of precious metals (Ag, Au, Pd and Pt), common metals (Cu, Al, Ni, Si, Zn, and Fe), and toxic metals (Hg, Be, Cd, Cr, Sb, and Bi), along with halogens (Br, Cl) and combustible substances (plastics, additives) (KAYA, 2016; WU et al., 2016).

The printed circuit board is an essential component of e-waste, in charge of 3% of the total mass (HADI et al., 2013). It is possible to find metals in PCBs with concentrations up to 10 times higher than those typically observed in raw minerals (CAYUMIL et al., 2016). The recovery of this type of material from PCB may be referred to as "urban mining". Many studies are found in the literature on the mining of precious metals (Au, Ag, Pd, Pt) and high content metals (Cu, Al, Sn) from waste PCBs by physical, chemical and/or biological methods (JAGANNATH; SHETTY K.; SAIDUTTA, 2017; KIM et al., 2016; LI; EKSTEEN; ORABY, 2018; VEIT; SCHERER, 2014; XIA et al., 2017). On the other hand, there are few studies on pre-treatment of PCB e-waste (BURAT, FIRAT; ÖZER, 2018; FLORES-CAMPOS; ESTRADA-RUIZ; VELARDE-SÁNCHEZ, 2017; MUNSTERMAN; KERSTHOLT, 1996).

The pre-treatment of PCB waste is a fundamental process step for reducing the number of impurities and the consumption of reagents in the leaching process (PRIYA; HAIT, 2018). Pre-treatments include mechanical dismantling (capacitors, resistors, CPU and CPU case, port locations, etc.), and basic physical and/or chemical methods before metal concentration (GOLEV; CORDER, 2017). Some factors such as mineral composition, metals concentration, and particle size distribution are important for choosing the adequate pre-treatment (gravity, separation, and/or dense medium separation) to be used for metals beneficiation from PCB waste.

A dense medium might be used to pre-concentrate minerals from electronic waste. In laboratory, separation media are usually heavy organic liquids, such as chloroform, bromoform, tetrabromoethane, which have densities of 1.49, 2.89, and 2.95 g/cm^3, respectively. Chloroform ($CHCl_3$) is a heavy, volatile and colorless liquid at room temperature. It is usually applied as an intermediate or an extraction solvent in the production of dyes, pesticides, and other substances, and in the water chlorination (BOND; GRAHAM, 2017). Chloroform is volatile (boiling temperature: 61 ºC), and when inhaled can result in effects on the liver (e.g. hepatitis), and central nervous system effects (e.g. depression and irritability) (JOSE; RAMANUJAM; PHILIP, 2019).

Due to the high toxicity and cost of heavy organic liquids, industrial plants avoid their use, so that it is necessary to search for other substances for the same function. An ideal compound to be used in dense medium separation processes would be a liquid having the following characteristics: inexpensive, miscible with water, capable of adjustment over a wide range of density fractions, non-toxic, environmentally friendly, chemically inert, easily separated from products after processing, and stable over the required range of densities (MUNSTERMAN; KERSTHOLT, 1996; SAHIN et al., 2009).

Synthetic alkali silicates are usually manufactured as glasses that dissolve in water to form viscous, alkaline solutions. Sodium silicates contain a desired alkali-to-silica ratio, and variable concentration in aqueous solutions (FALCONE, 2015). They are used as inorganic dispersants in the flotation of minerals, detergency, water treatment, bleach stabilization, enhanced oil recovery, and as a raw material for the syntheses of geopolymers, zeolites, clays, and other siliceous materials (DE ROSSI et al., 2019; STEMPKOWSKA et al., 2017; VIKTOR; GALYNA, 2017; XIE; HAN; WANG, 2018).

Typically, sodium silicate has a density of 1.4 to 2.4 g/cm^3 (PAN et al., 2019), which is adjustable according to the SiO_2:Na_2O ratio and/or concentration in water. Except for the most silicon-rich ones, it is readily soluble in water, being known as water glass. Sodium silicate can be thus an attractive alternative for the replacement of heavy organic liquids in the concentration process.

In this study, metal, polymeric and ceramic materials present in PCBs were separated by sodium silicate, used as an alternative to chloroform as dense liquid medium. The sodium silicate was used as a novel dense liquid medium for the metal pre-concentration process from electronic waste (PCBs). Metal and non-metal fractions were obtained, and the results were compared regarding efficiency and environmental aspects.

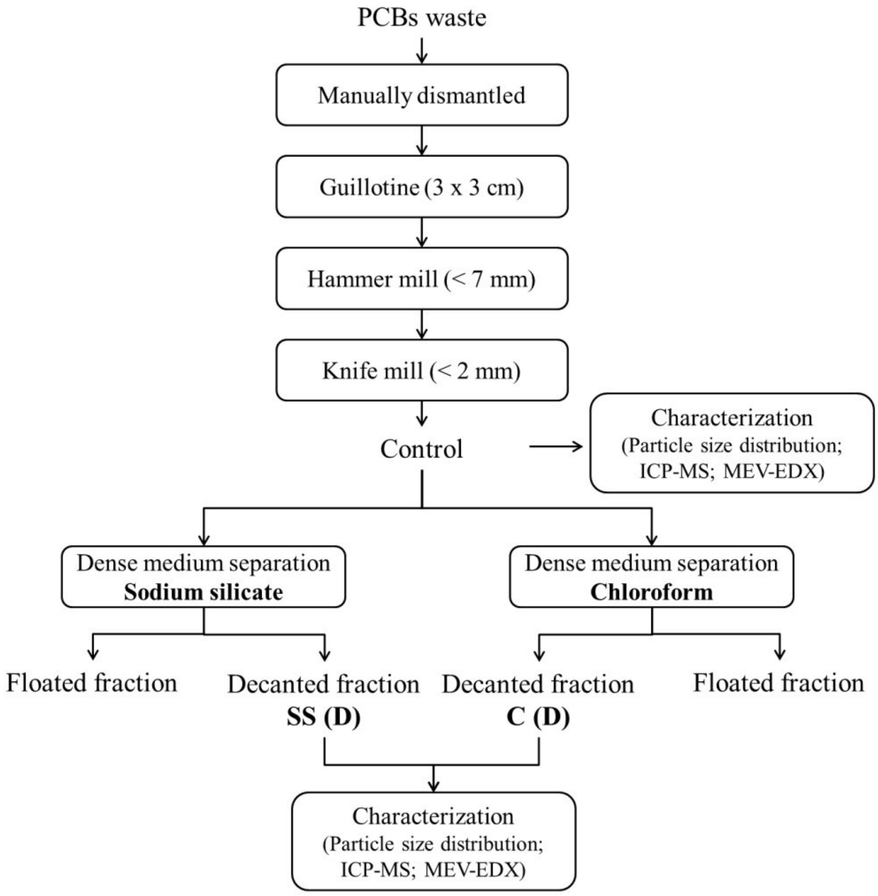
2.2 Material and Methods

2.2.1 Experimental

The PCBs (motherboards) used in the experiments were collected from obsolete desktop computers from different manufacturers. The PCB components including capacitors, resistors, processor carriers, and batteries were manually removed. Only the soldered components were kept for the sequence of experiments and characterization. PCB was cut off using a guillotine into smaller pieces (3 cm × 3 cm), which were then ground using a hammer mill (CT-058 Servitech) until particle sizes of less than 6 mm were obtained. In the sequence, a knife mill (MA 340 Marconi) was applied, so that particle sizes of less than 2 mm could be reached.

Figure 2 shows the flowchart of the experimental procedure employed in this step of the work, which briefly consisted in the following: disassembly, comminution, dense medium separation, and characterization of the metals present in the PCB.

Figure 2 - Flowchart of the experimental procedure.



A commercial sodium silicate concentrated solution in water (9.20 wt.% Na_2O, 29.50 wt.% SiO_2, and 61.30 wt.% H_2O; Quimidrol) and chloroform (99.8 wt.%; Synth) were used as dense liquid media in the separation process. Chloroform, a commonly used medium for gravity separation (GUPTA; YAN, 2006), presents a similar density (1.47 g/cm^3) when compared to that of the sodium silicate water solution (1.42 g/cm^3). The dense medium separation was performed at 25 ºC in a 250 mL graduated cylinder with a 1 to 10 solid:dense liquid medium ratio (wt.%). After the separation process, the fractions obtained with sodium silicate were washed twice with distilled water and those from chloroform were washed twice with ethanol (99.8 wt.%; Neon). The decanted fractions were dried until constant weight at 60 °C. The samples were named as control (without dense medium separation process), SSD (sodium silicate - decanted), and CD (chloroform - decanted).

2.2.2 Characterization of decanted fractions

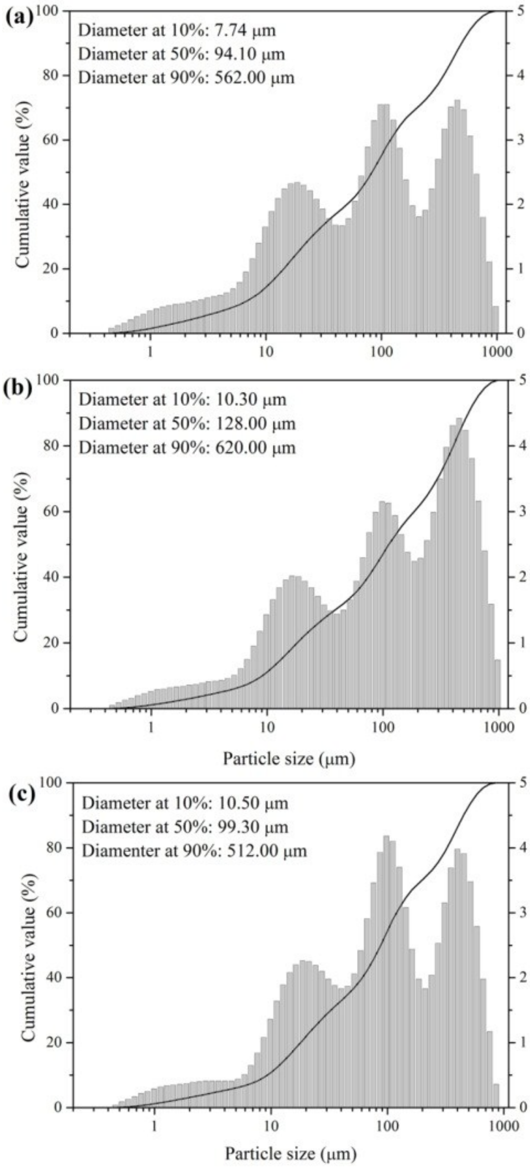
The decanted fractions obtained were analyzed to determine the efficiency of metals separation. Copper, gold, silver, and palladium contents from the control and samples fractions were measured by ICP-MS (NexION 300 D, Perkin Elmer) after digestion in the microwave (MLS 1200, Milestone). The average particle size distribution was determined using a laser scattering particle size analyzer (Mastersizer 3000, Malvern). The morphology and elemental composition were evaluated by scanning electron microscope (SEM) with energy dispersive X-ray spectroscopy (EDX) (TM3030, Hitachi), respectively.

2.3 Results and discussion

Mechanical separation of metal-containing parts in electronic waste by grinding is relatively easy due to the lower interfacial bonds of materials from PCB (ZHANG; FORSSBERG, 1999). Studies related to the release of PCB materials using hammer mill have shown good results in terms of efficiency, even though small losses of fine and light particles during grinding processing been observed (KOYANAKA; ENDOH; OHYA, 2006). The grinding of PCB directly affects the physical operations and the recovery rate of the metals (TAN et al., 2011).

The use of a hammer mill combined with a knife mill showed that was possible to reduce the particle size to ≤0.62 mm (Figure 3). The results also showed a trimodal distribution with a mean particle size (d50) of ~94 μm (control), ~128 μm (SSD), and ~99 μm (CD), respectively.

Figure 3 - Particle size distribution of PCBs from: (a) control; (b) SSD; (c) CD.



The particles size is important once, as reported in the literature (QUAN; LI; GAO, 2012), PCB can be dissociated between metal and nonmetals for particle size ranging from 0.15 to 0.59 mm. The results obtained for the particle size distribution using sodium silicate as dense liquid were similar to the results obtained using chloroform, indicating that sodium silicate does not influence the particle size distribution of the decanted samples.

In thhe analysis of ICP-MS (decanted fractions), it was observed that after dense medium separation, the metals were concentrated for both evaluated methodologies. Among the metals detected in the PBC, copper was the most predominant element (Table 1).

Table 1 - Elemental composition by ICP-MS of metals content in waste printed circuit boards from computers.

	Metals content (wt.%)		
	Control	SSD	CD
Copper (Cu)	7.150 ± 0.020	17.37 ± 0.06	15.96 ± 0.09
Gold (Au)	0.0150 ± 0.0001	0.0220 ± 0.0001	0.0250 ± 0.0001
Silver (Ag)	0.580 ± 0.190	0.740 ± 0.130	0.800 ± 0.001
Palladium (Pd)	0.0001 ± 0.00001	0.0007 ± 0.00001	0.0003 ± 0.00001

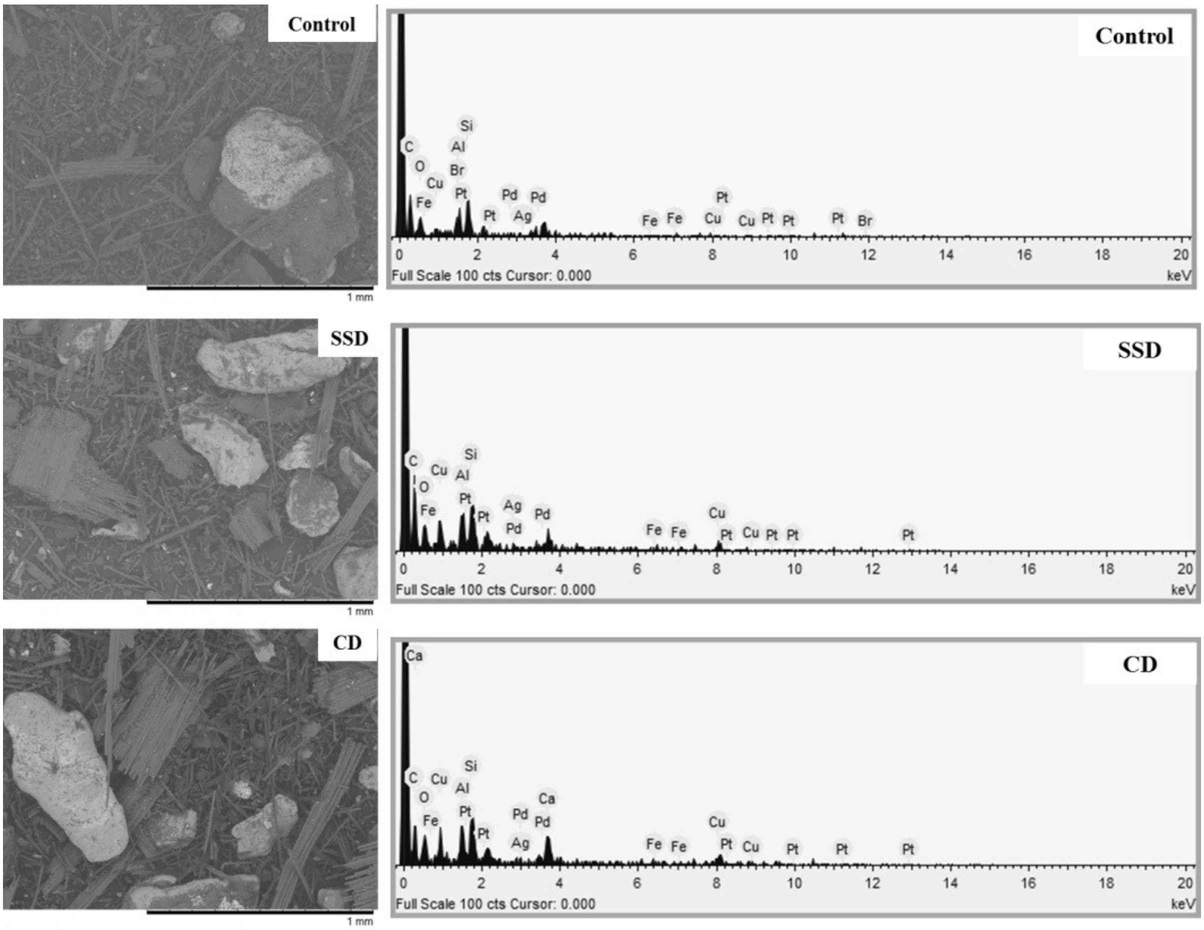
According to Estrada-Ruiz et al. (2016), the e-waste can be classified by the gold content as low (100 g/t Au), medium (100-400 g/t Au), and high (400 g/t Au). The fractions SSD and CD obtained after the dense medium separation process showed a concentration of 220 g/t and 250 g/t Au, respectively, being thus classified as medium grade gold content.

Gold is used in PCB due to the high chemical stability to prevent the oxidation of electrical contacts in computers (GOODMAN, 2002). The total of precious metals (gold, silver, and palladium) showed in Table 1 was less than 1 wt.%, corroborating with the results of Park and Fray (2009).

From the ICP-MS results, it was possible to confirm the efficiency of the methodologies by the close metal content obtained in this work and reported in the literature for gold (0.01 to 0.05 wt%), silver (0.4 to 1.5 wt%), and copper (15 to 25 wt%) (IŞILDAR et al., 2016; NARAYANASAMY et al., 2018; SHIN et al., 2013; VENTURA et al., 2018; YANG et al., 2014; YOO et al., 2009; ZHANG et al., 2016).

EDX analysis (Figure 4) showed several elements including precious metal (Ag, Pt, and Pd), common metals (Cu, Fe, and Al), and other elements (Si, Ca, O, and C) that corresponds to the reinforced fibers and glass fibers materials present in the printed circuit boards. SEM images of the decanted fractions revealed that the PCBs are heterogeneous, with particles with different shapes, textures, and sizes. This heterogeneity is related to the production process, which uses a mixture of materials as of interlaced fibers forming a composite (ESTRADA-RUIZ et al., 2016). The observed brighter points in the micrographs correspond to the metallic particles in the glass fibers used as the base of the PCBs. The metallic particles are not attached to the glass fibers, indicating that the metal material was effectively released after the grinding process.

Figure 4 - SEM with EDX spectrum of PCBs from: control; SSD; CD.



Brome (Br), which is a toxic element as soluble bromide ion (AL-GHOUTI et al., 2017) was observed only in the control sample, showing that the sodium silicate and chloroform as a dense liquid for separation process were able to remove it from in control sample. The presence of Br in the PCB waste can be associated with the polymer base with flame-retardants presence that usually contain Br (ZHANG; FORSSBERG, 1999). Since this kind of substance is a potentially hazardous, some care must be taken during the PCB waste pre-treatment showing the importance of the alternative route for metals pre-concentration as evidenced in this work and other works of literature (ESTRADA-RUIZ et al., 2016; FLORES-CAMPOS; ESTRADA-RUIZ; VELARDE-SÁNCHEZ, 2017; VEIT; SCHERER, 2014; WANG et al., 2018).

2.4 Conclusions

In this chapter, an alternative route for metals pre-concentration from printed circuit boards by dense media separation was established with sodium silicate as dense liquid replacing chloroform. The grinding process (hammer mill and knife mill) was able to reduce the size of the PCBs to <0.62 mm allowing the separation of the metal content from the non-metal fractions. After the dense medium separation process, the SSD sample showed higher content of metal elements: gold (31 wt.%), silver (21 wt.%), copper (58 wt.%), and palladium (85 wt.%), when compared to the control. Thus, the separation using sodium silicate can be considered an eco-friendly and efficient physical beneficiation technique for the subsequent metals recovery from PCBs.

3 RECOVERY OF COPPER AND GOLD FROM COMPUTER PRINTED CIRCUIT BOARDS BY BIOLEACHING WITH *ASPERGILLUS NIGER*

3.1 Introduction

Printed circuit boards (PCBs) are essential components of almost all electronic equipment being approximately 3 wt.% of the total amount of electronic waste (WANG et al., 2017), reaching an annual amount of 44.7 million t/year worldwide in 2016 forecasted to be 52.2 million t/year by 2021 (BALDÉ et al., 2017). As the environmental legislation is not strictly enforced, electronics equipment are often disposed of in landfills or inappropriate places where it can contaminate the soil with heavy metals (CANAL MARQUES; CABRERA; DE FRAGA MALFATTI, 2013).

PCBs waste is a global challenge for the environment and human health because of their complex and hazardous components. PCBs contain metals with purity levels sometimes higher than those of minerals, e.g., copper (12-30 wt.%), nickel (1-5 wt.%), iron (1-3 wt.%), silver (0.05-0.15 wt.%), and gold (0.01-0.07 wt.%) (GHOSH et al., 2015). Nevertheless, hazardous materials and nonmetals such as epoxy resin, glass fibers, ceramics, and other plastics are also present. In addition to the environmental issues related to the reducing the amounts of wastes, the recovery of metals from PCBs waste has also become an economic source due to the metals represent more than 95% of the total value of the board (KAYA, 2016).

Mechanical, pyrometallurgical (WANG et al., 2017), hydrometallurgical (MOOSAKAZEMI; GHASSA; MOHAMMADI, 2019) and biohydrometallurgical processes (KHALIQ et al., 2014; PRIYA; HAIT, 2018) are used for the recovery of metals from PCBs. During the recycling process of electronic wastes, mechanical treatments are employed in the early stage as pre-processing, aiming at separating the valuable and hazardous components and pre-concentrate the metal fraction, which is further routed to end refining processes (CUI; FORSSBERG, 2003).

The pyrometallurgical and hydrometallurgical processes have several impacts on the environment due to the generation of secondary pollutants (such as dioxins, heavy metal pollution, and sludge generation). Thus, there is a requirement to develop eco-friendly treatments, which are intended to increase the sustainability of recycling processes (CANAL MARQUES; CABRERA; DE FRAGA MALFATTI, 2013). Consequently, the research interest is moving towards biohydrometallurgical processes, as a promising technique for metal recovery from PBCs (KHALIQ et al., 2014; PRIYA; HAIT, 2017).

The biohydrometallurgical processes prospect the ability of microorganisms to solubilize metals contained in the solid matrix (bioleaching) or the capacity of microbial biomass to sorb metals from the aqueous solution (biosorption) (KAKSONEN et al., 2018; OH et al., 2003). Bioleaching is performed under mild conditions, usually without the addition of toxic chemicals, since the metabolites produced by the microorganisms are chemical substances, organic acids, polymers, and enzymes that are used for the recovery of metals waste (JOHNSON, 2014).

The amount of recovered metals by the bioleaching process depends on the microorganism used and the growth conditions applied. Fungi have some benefits compared to bacteria in biotechnological processes as wider pH range (1.5-9.8) (XU; LI; LIU, 2016), fast leaching rates, organic acids production, selective metal leaching (FARAJI et al., 2018), and economic advantages (PANT et al., 2012).

Bioleaching of PCBs waste by heterotrophic organisms to produce organic acids, amino acids, and other metabolites has been attracting growing interest (REED et al., 2016). Both fungi and bacteria are able to heterotrophic bioleaching with different high recovery efficiencies. Heterotrophic fungi, including *Aspergillus niger,* have been used effectively to dissolve various metal fractions from electronic wastes (FARAJI et al., 2018; NARAYANASAMY et al., 2018). However, in the current literature, there is not enough information related to the mechanism of action of biocomposites of filamentous fungi in the bioleaching of precious metals, thus requiring further studies on this topic.

Bioleaching can be performed in one-step, in two-steps or with the spent medium. In the one-step method, the PCBs are mixed immediately with the microorganisms in the culture medium. In the two-step procedure, PCBs are added after the microorganisms reach the maximum growth (logarithmic growth phase). In the spent medium approach, bioleaching experiments are carried out in cell-free acidic filtrates after fungal activation (ARGUMEDO-DELIRA; GÓMEZ-MARTÍNEZ; SOTO, 2019; DÍAZ-MARTÍNEZ et al., 2019; FARAJI et al., 2018). In this step of the work, in order to enhance the metal dissolution, three approaches (one-step; two-step and spent medium) were proposed for bioleaching of copper and gold present in computer PCBs using *Aspergillus niger*.

3.2 Materials and Methods

3.2.1 PCBs preparation and metals quantification

PCBs from desktop computers from different manufacturers were manually disassembled to remove capacitors, resistors, processor carriers, and batteries. PCBs waste was cut out using a guillotine into 3×3 cm pieces, and ground using a hammer mill (Servitech CT-058) followed by a knife mill (Marconi MA 340). A dense medium separation process was performed to separate the polymers and ceramic fraction from the metal fraction. A commercial sodium silicate concentrated solution in water (9.20 wt.% Na_2O, 29.50 wt.% SiO_2, and 61.30 wt.% H_2O; Quimidrol) was used as dense liquid media in the separation process. The dense medium separation was performed at 25 ºC in a 250 mL graduated cylinder with a 1 to 10 solid:dense liquid medium ratio (wt.%). After the separation process, the fraction obtained with sodium silicate was washed twice with distilled water. The decanted fraction was dried until constant weight at 60 °C.

Copper (Cu), iron (Fe), aluminum (Al), zinc (Zn), nickel (Ni), silver (Ag), gold (Au) and palladium (Pd) contents from the PCBs were measured by ICP-MS (NexION 300 D, Perkin Elmer) after digestion in the microwave (MLS 1200, Milestone) (Table 2). Microwave digestion was performed using 120 mg of metal fraction, added with 4 ml of nitric acid (HNO_3), 2 ml of hydrochloric acid (HCl) and 1 ml of hydrogen peroxide (H_2O_2) in closed Teflon flasks. The mixture was taken to the microwave oven for 5 min at 250 W, 4 min at 400 W and 3 min at 650 W. After cooling, ultra-pure water was added to the mixture until a final volume of 50 mL. The metal fraction was sterilized at 121 ºC, 101 kPa for 20 min to use in the bioleaching process.

Table 2 - Metal content in PCBs waste.

Metal	Concentration (wt.%)
Cu	15.25±0.35
Fe	1.40±0.06
Al	0.960±0.009
Zn	0.760±0.011
Ni	0.210±0.004
Ag	0.740±0.015
Au	0.02400±0.00001
Pd	0.00100±0.00001

3.2.2 Production and characterization of metabolites

The metabolites produced by *A. niger* were collected every 48 h, and vacuum filtered with Whatman 42 filter paper to determine pH, reducing sugars, total acid, and dry biomass. The dry biomass was determined by mycelium obtained from the vacuum filtration; the mycelium was dried until constant weight at 60 °C. The concentration of reducing sugars was quantified by the DNS method (MILLER, 1959). The total acid was quantified by titration according to APHA-AWWA-WEF (1998). At the end of process (10th day), the sample was analyzed by Fourier Transform Infrared (FTIR) (Shimadzu, Model IRPrestige-21, 8400S) in the diffuse reflectance mode at a resolution of 4 cm^{-1} with scanning in the range of 500-4000 cm^{-1} in KBr pellets, for the determination of active functional groups.

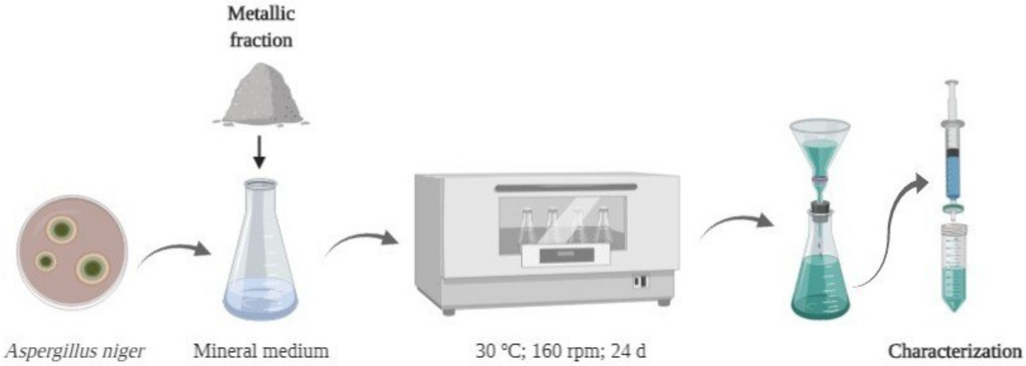
3.2.3 Bioleaching procedure

Aspergillus niger was isolated and identified by Treichel et al. (2016). The microorganism was inoculated in 1 L flasks, containing 100 mL of potato dextrose agar medium (PDA), followed by incubation at 30 ºC for 7 days (RODRIGUES et al., 2017).

One-step, two-step and spent medium bioleaching were performed in 300 mL autoclaved Erlenmeyer flasks with 125 mL mineral medium containing (g/L): $CaCl_2$ (0.1), KH_2PO_4 (0.5), NH_4Cl (1.5), $MgSO_4.7H_2O$ (0.025), glucose (50.0) (MADRIGAL-ARIAS et al., 2015). The pH was set to 4.4 with sulfuric acid 1 N. The medium was sterilized at 121 ºC, 101 kPa for 20 min and the inoculation was performed after cooling the medium to room temperature. Different metal concentrations were used (0.0; 2.5; 5.0; 10.0 g/L) in order to evaluate the influence of the metal concentration on the bioleaching process. The experiments were carried out in duplicate.

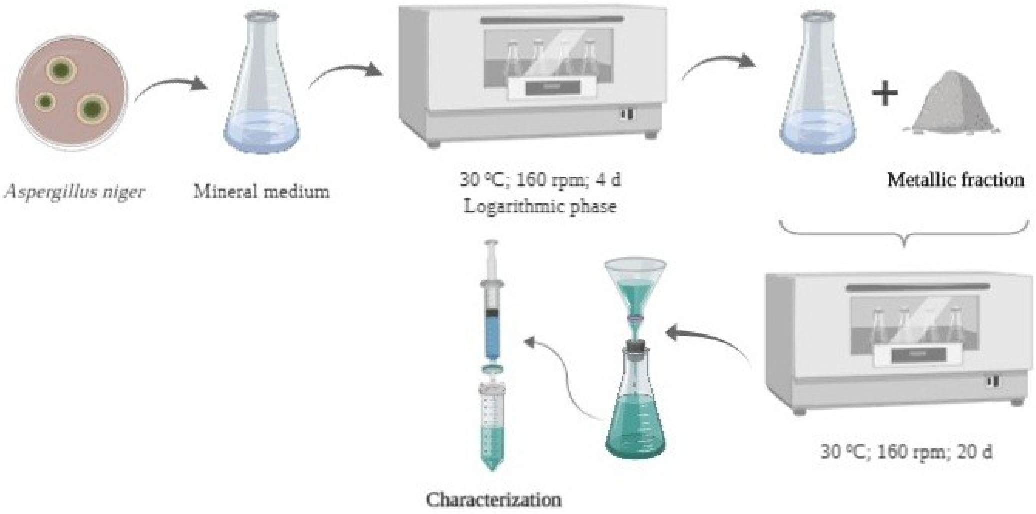
In the one-step bioleaching procedure (Figure 5), the cultures were inoculated with 4×10^{6} spores/mL_{medium} in 125 mL mineral medium containing the metal concentrations and incubated in an orbital shaker-incubator at 30 ºC at 160 rpm for 24 days. Samples were collected in 0, 14 and 24 days.

Figure 5 - Flowchart of the one-step experimental procedure.



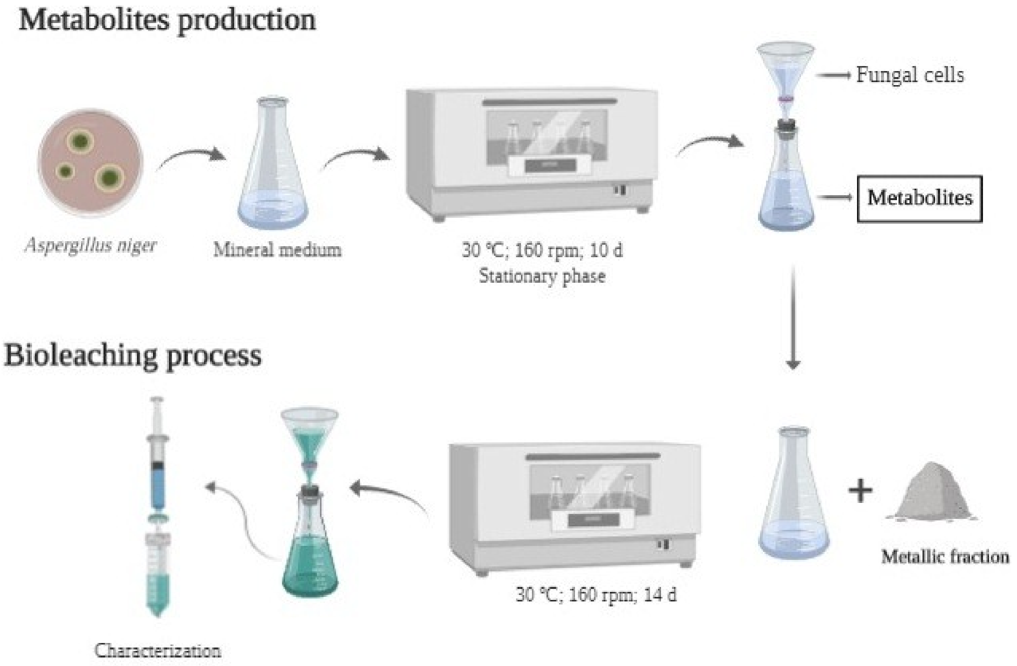
In the two-step procedure (Figure 6), firstly, the *A. niger* was inoculated with 4×10^{6} spores/mL_{medium} in 125 mL mineral medium and no metal fraction was added until the fungi entered the logarithmic phase. The logarithmic phase commences with a sudden reduction in the pH, meaning the beginning of organic acid production. It took 4 days to pH reduction in the *A. niger* growth medium. The autoclaved metal fraction was added to the culture medium and the bioleaching process was carried out in an orbital shaker-incubator at 30 ºC at 160 rpm for 24 days. Samples were collected in 0, 14 and 24 days.

Figure 6 - Flowchart of the two-step experimental procedure.



In the spent medium approach (Fig. 7), the fungi were initially cultured in mineral medium without PCBs until the maximum production of organic acids by *A. niger*, which took 10 days. Then, the suspension of mycelia and liquid medium of each Erlenmeyer flask was filtered through Whatman 42 filter paper, in order to obtain a cell-free spent medium. The metal fraction was added to the filtrate including fungal metabolites (organic acids) and bioleached in a shaker-incubator at 160 rpm, 30 ºC for 14 days. Samples were collected in 0 and 14 days.

Figure 7 - Flowchart of the spent medium experimental procedure.



For all evaluated approaches, the samples were collected on the first day and at the end of the process. After bioleaching, the solution was filtered by a 0.20 mm syringe filter and the filtrate was characterized. The bioleaching recovery was calculated according to Equation 1.

$$\text{Bioleaching recovery (\%)} = \frac{C_1}{C_0} \times 100 \qquad \text{(Eq. 1)}$$

where:

C_0: concentration of metal (Cu or Au) in the solution before bioleaching;
C_1: concentration of metal (Cu or Au) in the solution after bioleaching.

3.2.4 Characterization of bioleached products

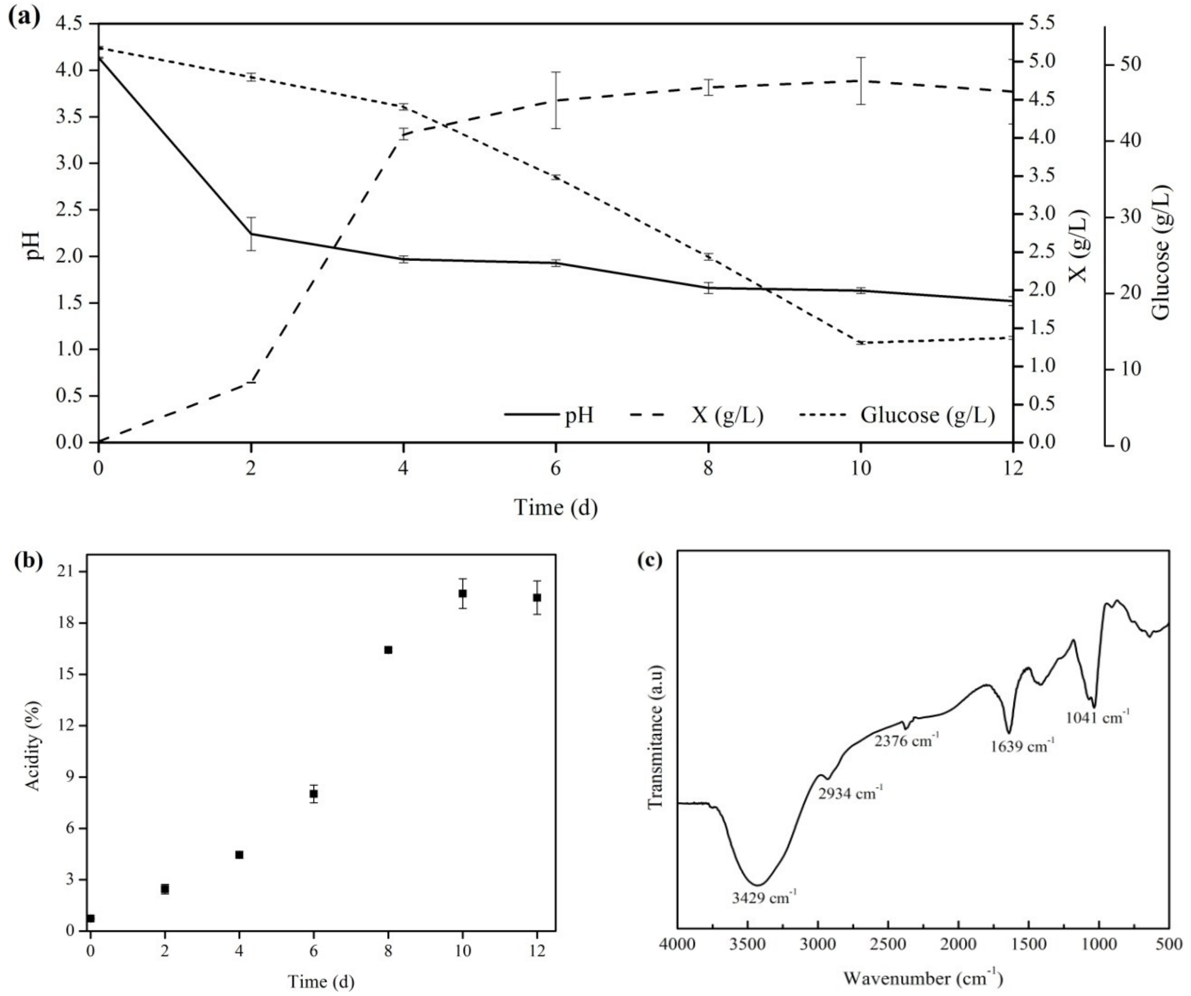
Total copper content was measured by atomic absorption spectrometry (AAS, Shimadzu model AA 6300) using an air-acetylene flame and the gold content were measured by ICP-MS (NexION 300 D, Perkin Elmer). The average particle size distribution was determined using a laser scattering particle size analyzer (Zetasizer Nano ZS, Malvern). The stability of the metallic particles after the bioleaching was evaluated by zeta potential (Stabino Control 2.00.23-Particle Metrix). In order to investigate the morphology of the metallic particles after bioleaching, a transmission electron microscope was used (TEM JEM-1011 JEOL) at 100 kV. A drop of leached solutions was placed on carbon film-coated copper grids (3 mm diameter with 300 mesh) and dried at temperature room for 48 h.

3.3 Results and discussion

3.3.1 Metabolites production by *Aspergillus niger*

For most processes using fungi, glucose is used as a source of carbon and energy and plays an important role in metabolism. The most common commercial organic acids produced by *A. niger* are citric and gluconic acids, both of which are obtained by the fermentation of glucose or sucrose (MAGNUSON; LASURE, 2004). Thus, the effect of initial glucose concentration on substrate consumption and product synthesis during the fermentative process was evaluated (Figure 8). As observed from the results, in the lag phase, low carbon content was consumed for the respiratory chain until the 2nd day. Then, on the 4th day, the glucose was quickly consumed increasing the product synthesis (such as organic acids). *A. niger* consumed up to 72% of the initial glucose supplied at the beginning of the fermentative process.

Figure 8 - *A. niger* growth curve versus pH and glucose reduction in the organics acids production (a), total acidity in the culture medium during the process (b), and FTIR spectra at the end of fermentation process (10 days) (c).



The bioleaching mechanism of *A. niger* is related to the production of low molecular weight metabolites, mainly organic acids as gluconic, citric, oxalic, malic, and succinic, which play important roles in leaching of metal ions (DENG et al., 2013; HOREH; MOUSAVI; SHOJAOSADATI, 2016). The organic acid production (Fig. 8b) indicated an increase in the acidity decreasing the pH throughout the process. On the second day of the process, the pH reduced from 4.13 to 2.24 (Fig 8a), mainly due to the enzymatic action, corresponding to the beginning of organic acid production (Figure 8b) and the dry biomass increased from 0.01 to 2.30 g/L. The pH variation and microbial growth showed reverse behavior, with the pH reduction, the microbial growth increased. As observed, on the 10th day of the process, the microbial growth reached the highest value (4.74 g/L), and then the microbial growth started to decrease due to the inhibition of secreted primary metabolite (especially citric acid) for fungal growth.

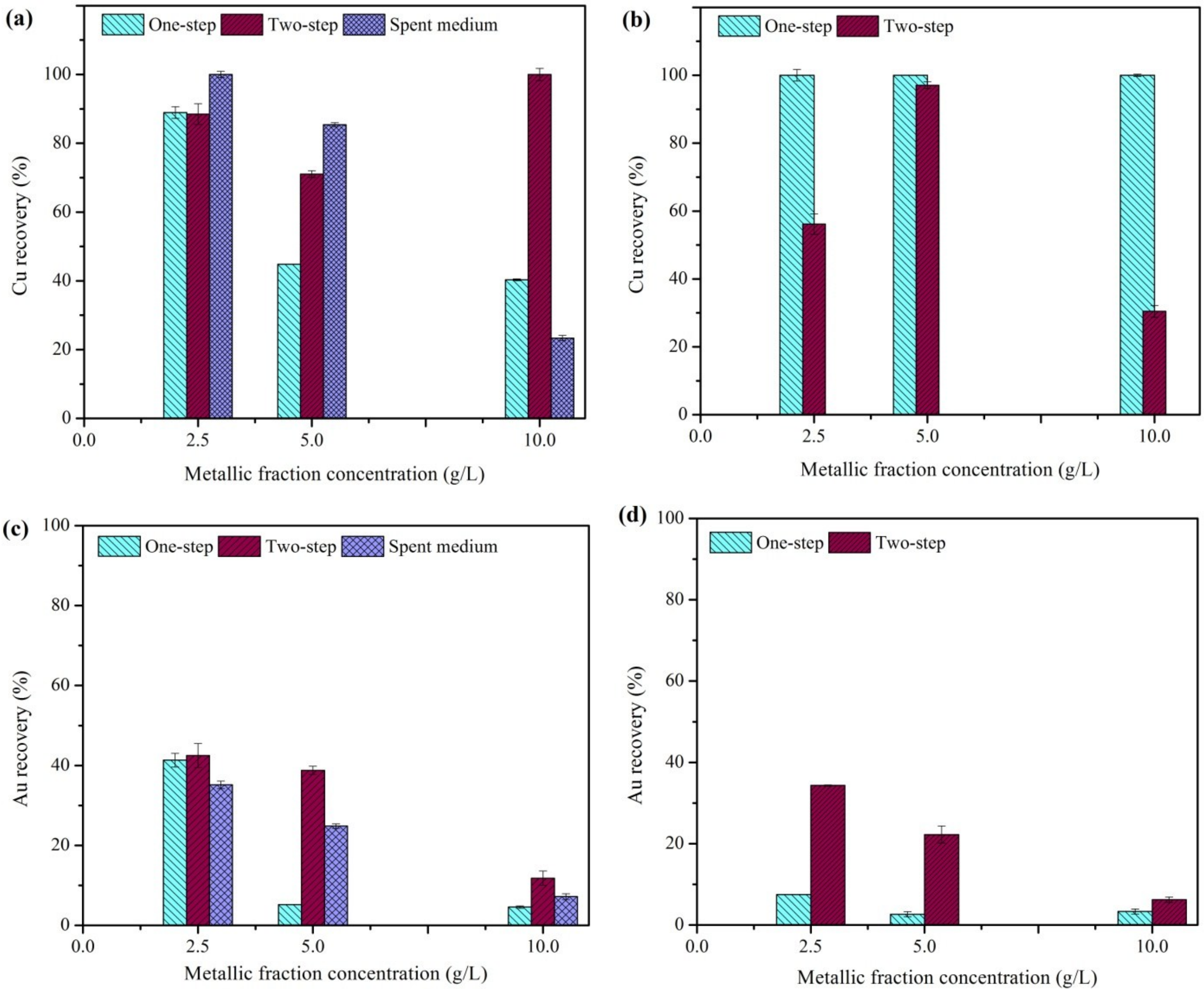
The FTIR spectra at the end of the process (10th day) confirm the organic acid production (Figure 8c). The peak located at 3429 cm^{-1} is assigned to water. Moreover, the small peak observed between 2300 and 2400 cm^{-1} was caused by the presence of CO_2 molecules in the air. The peaks observed at 1639 and 2934 cm^{-1} are associated with stretching vibration C=O groups and O-H stretching. This behavior is characteristic of D-gluconic acid. Malic and citric acids show intense and characteristic bands in the region from 1500 to 900 cm^{-1}. This confirmed the presence of organic acids like oxalic, citric and gluconic ones. Similar results were reported by Bullen et al. (2008); Narayanan and Sakthivel, (2010); Magdum et al. (2014).

3.3.2 Copper and gold bioleaching approach

The chemical composition of PCBs used in this study was analyzed to determine the content of metals present in the waste (Table 2). Cu, Fe, and Al were observed to be the predominant metals in PCBs with the maximum content of 15.25, 1.40 and 0.96 wt.%, respectively. The total metal content for the samples analyzed in this work was ~20 wt.%. Small amounts of precious metals, Au (0.024 wt.%), Ag (0.74 wt.%), and Pd (0.001 wt.%), were also detected. Gold, along with other precious metals, is used as a thin layer contact material in the PCBs due to the chemical stability. The obtained results confirmed a high-grade substrate related to the metallic content. This high content means up to 10-100 times higher Au concentrations than those found in natural ores. Among metals, gold is the main driver for recycling PCBs waste (IŞILDAR, 2018a).

In the literature, it has been reported that *A. niger* is one of the filamentous fungi that presents high percentages of copper bioleaching (>50%) due to the organic acids production that is used to leach metals (DÍAZ-MARTÍNEZ et al., 2019; MADRIGAL-ARIAS et al., 2015). The efficiency of the bioleaching of Cu depends on the concentration of electronic waste and process time. In this study, copper was bioleached from the waste PCBs with efficiency up to 88% (one-step process), 88% (two-step process) and 100% (spent medium process) at 2.5 g/L metallic concentration after 14 processing days (Figure 9a). Higher metallic concentrations showed lower bioleaching efficiency at the one-step and spent medium approach in 14 days. Figure 9b shows that after 24 days, the copper was 100% bioleached using the one-step approach independent of the initial metallic fraction concentration.

Figure 9 - Cu recovery after 14 (a) and 24 days (b) and Au recovery after 14 (c) and 24 (d) days for the different bioleaching approach and initial metallic concentration.



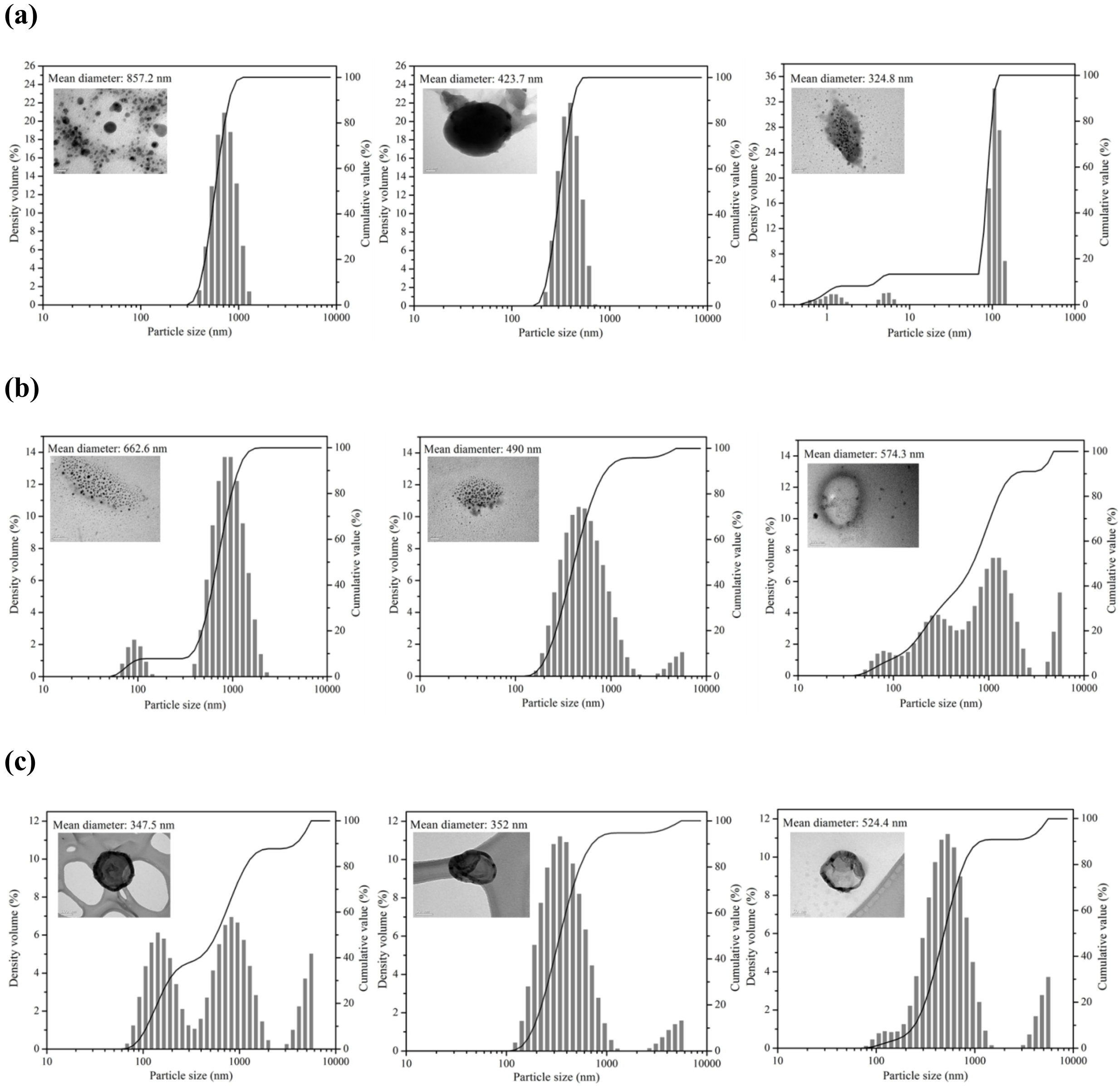
It can be seen that the bioleaching rate in 14 days (Figure 9c and Figure 9d) followed a downward trend in all experiments resulting in a maximum recovery of Au 41%, 42.5%, and 35.1% respectively for the one-step, two-step and spent medium approach at PCBs waste concentration of 2.5 g/L. It is important to note that the recovery rate (recovery as a function of time) of Au using lower PCBs waste concentration (2.5 g/L) was 5 to 10 times higher than that for the most concentrated waste (10 g/L). Increasing waste concentration would be expected to increase the total particle surface area, which would accelerate the leaching process. On the other hand, higher concentrations increase the amount of soluble and dissolved compounds in the medium, which may inhibit microbial activity reducing the Au recovery fraction (XIN et al., 2012).

The highest rates of metal recovery were obtained using the one-step and two-step approaches. However, in the one-step approach, it was possible to observe the growth inhibition of *A. niger* in the experiments with higher metallic fraction concentration (10 g/L). The one-step process is a classical method through exposure to biomass which is conducted via inoculation of spore suspension to the culture medium containing solid waste. In using this approach, the operating and capital costs are reduced because fermentation and leaching processes are done in one step (BAHALOO-HOREH; MOUSAVI; BANIASADI, 2018; BISWAL et al., 2018). In the two-step approach, the fungi grow in the culture medium until the logarithmic growth phase, and later the solid waste is added to the medium. In this approach, the growth of the fungus until the logarithmic phase limits the inhibition effect of the solid waste on microorganism growth, production of organic acids and correspondingly on metal extraction. This approach for an industrial application can be appropriate due to enhanced bioleaching efficiency caused by acidic solution generated before the addition of solid waste to the medium (BISWAL et al., 2018; IŞILDAR, 2018b).

Related to the morphological analysis, the mean particle size distribution obtained at different PCBs concentrations decreased as the PCBs concentration increases. For the one-step approach (Figure 10a) a modal distribution with a mean diameter of 857 nm, and 423 nm, respectively at 2.5, 5 g/L and trimodal distribution with a mean diameter of 324 nm at 10 g/L PCBs waste was obtained. The TEM images of the one-step approach samples showed that the particles were almost spherical with the observation of some agglomeration points. Micro/nanoparticles can have different morphologies, such as prisms, triangular, cylindrical, spherical, cubic, among others (KHAN; SAEED; KHAN, 2017).

The results of the two-step approach (Figure 10b) showed a bimodal distribution with a mean diameter of 662, and 490 nm, respectively for 2.5, 5 g/L and trimodal distribution with a mean diameter of 574 nm for 10 g/L PCBs waste. The mean results of the particle size distribution for the two-step approach showed the same behavior as observed for the one-step approach. Spherical particles were observed with particle size less than 100 nm for all experiments of the two-step approach as observed from the TEM image. The results of the spent medium approach showed a bimodal distribution with a mean diameter of 347, 352, and 524 nm, respectively for 2.5, 5, and 10 g/L PCBs waste. As previously observed, the mean particle size distribution results of the metallic particle at different concentrations of PCBs increased as the PCBs concentration increases. The TEM images of the samples showed that the particles were almost spherical with a slight variation in particle size.

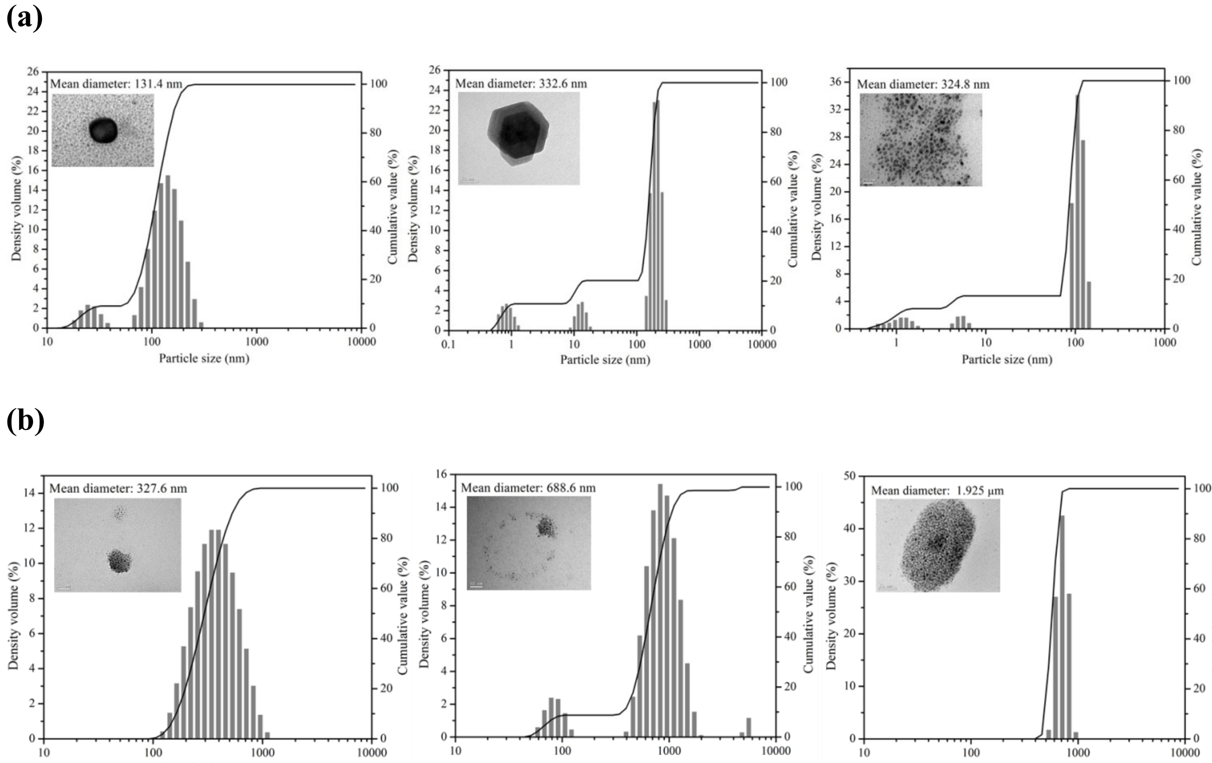
Figure 10 - Particle size distribution and TEM images (upper side detail) after 14 days of one-step (a), two-step (b), and spent medium (c) bioleaching process at 2.5, 5.0 and 10 g/L of PCBs concentration.



The results of the one-step approach (Figure 11a) after 24 days of bioleaching process showed a bimodal distribution with a mean diameter of 131 nm for 2.5, 5 g/L PCB waste and trimodal distribution with a mean diameter of 332, and 324 nm, respectively for 5 and 10 g/L PCBs waste. TEM images showed individual particles as well as a number of aggregates with a majority of spherical nanoparticles. The results of the two-step approach (Figure 11b) showed a modal distribution with a mean diameter of 327 and 1925 nm, respectively for 2.5 and 10 g/L PCB waste and trimodal distribution with a mean diameter of 688 nm for the 5 g/L PCBs waste. The size and shape of metallic particles were evaluated by TEM and the results show that the nanoparticles are spherical, and the average size of metallic nanoparticles is low of 50 nm.

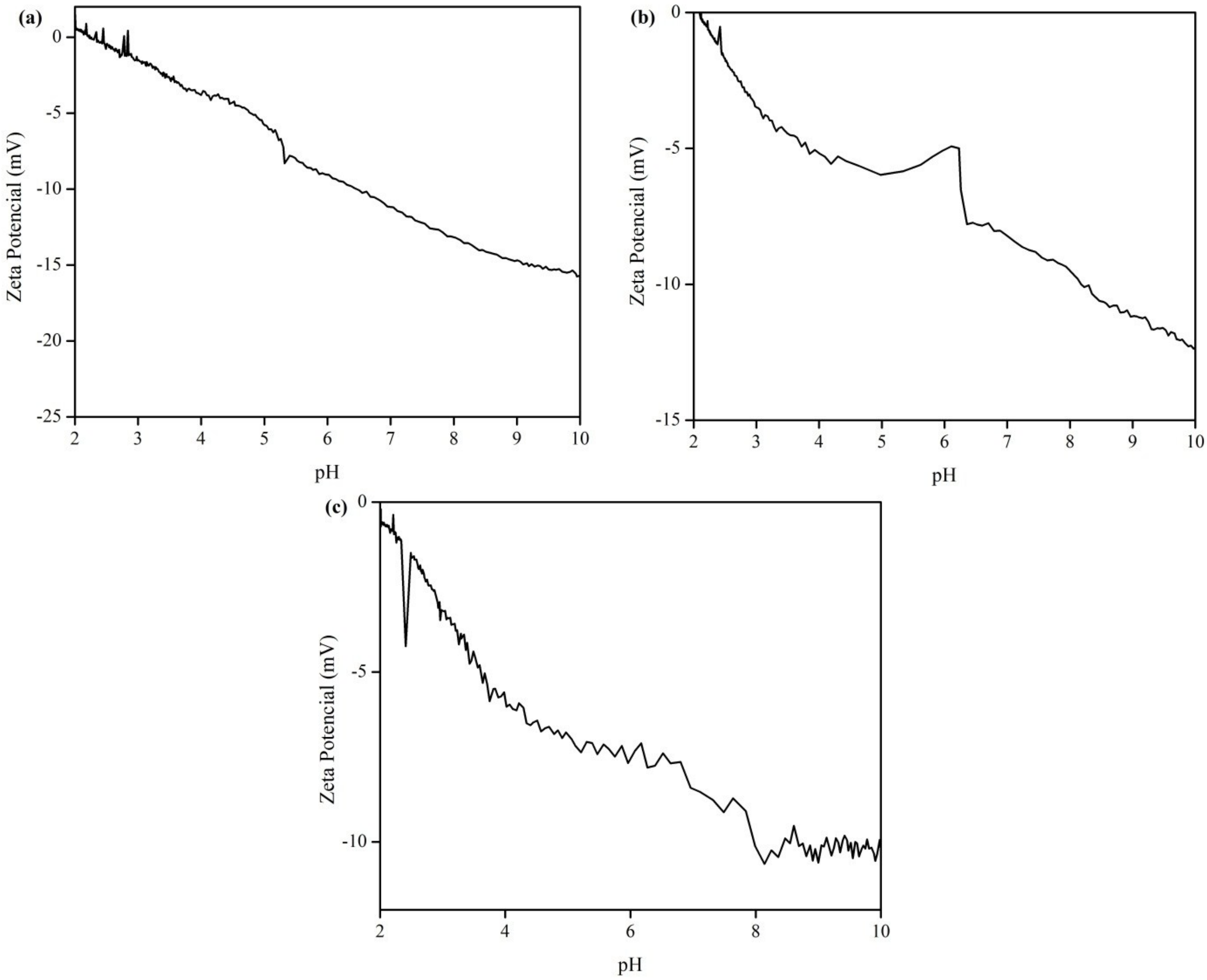
The difference between particle size distributions obtained by dynamic light scattering (DLS) and TEM analysis, might be due to enlarging of the hydrated capping agents by the dynamic light scattering analysis (biomacromolecules, probably protein) or from solvation effects (MUKHERJEE et al., 2008; SRIVASTAVA et al., 2019).

Figure 11 - Particle size distribution and TEM images (upper side detail) after 24 days of one-step (a) and two-step (b) bioleaching process at 2.5, 5.0 and 10 m/L of PCBs concentration.



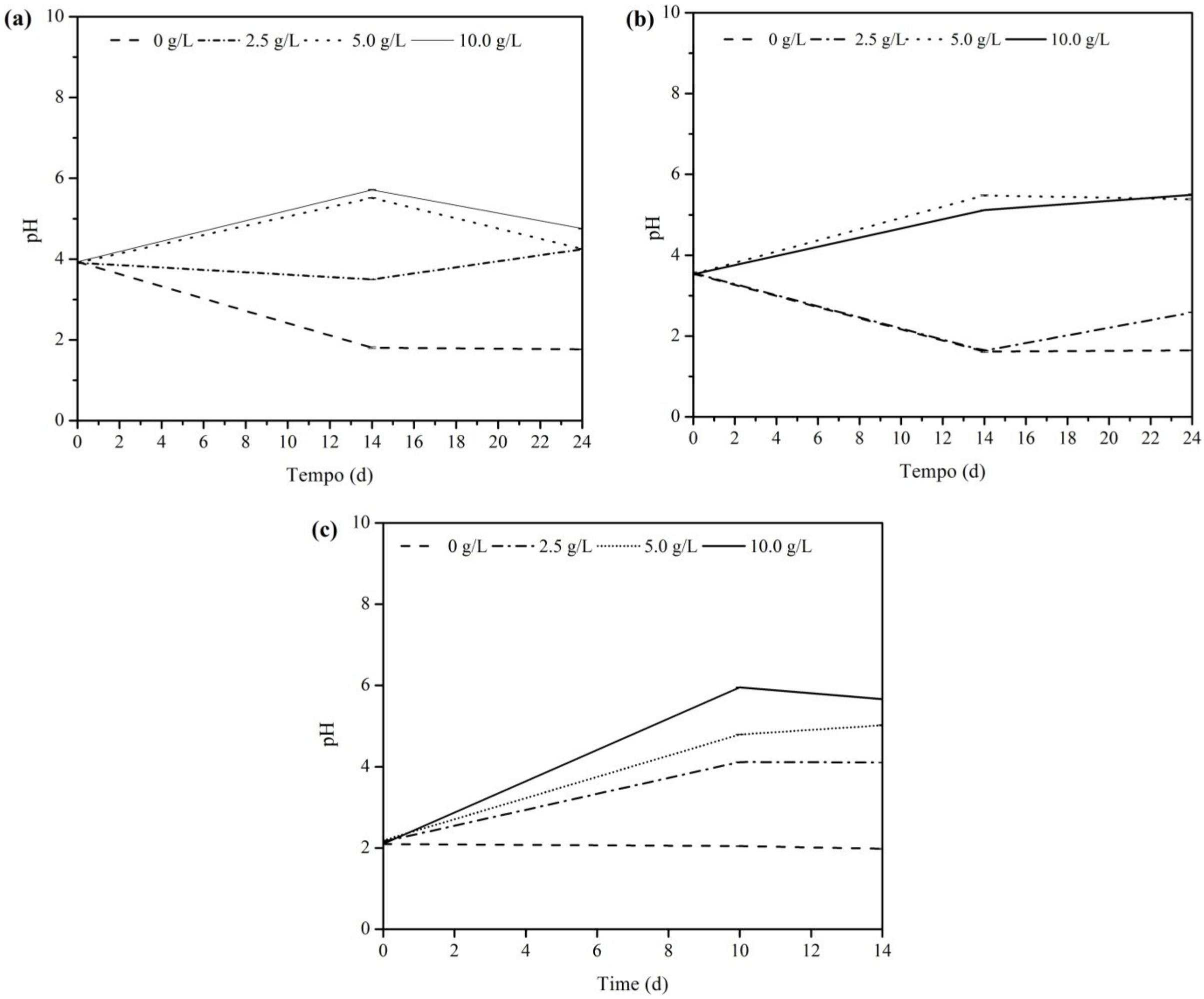
The zeta potential as a function of pH was determined for 3 approaches at a metallic fraction concentration of 2.5 g/L, respectively: (a) one-step, (b) two-step and (3) spent medium (Figure 12). Zeta potential evaluates the magnitude and nature of the surface charge associated with the double layer around the particle. Particles in suspension having zeta potential values over ~25 mV (either positive or negative) are generally regarded as stable, repelling each other and avoiding agglomeration. In all cases (Figure 12 a,b,c) the attraction seems to exceed the repulsion regardless the pH region, and the particles tend to agglomerate, lowering the zeta potential values (KOSMULSKI, 2004; SANKHLA et al., 2016). The isoelectric point (IEP) is around pH 2 for the 3 suspensions analyzed, meaning that at lower pH values the trend to agglomerate is the highest. Thus, the results confirmed high instability, and particle agglomeration was noticed, as previously observed by TEM analysis.

Figure 12 - Zeta potential variation as a function of pH in different bioleaching processes using metallic concentration of 2.5 g/L; 24 days in one-step (a), 14 days in two-step (b) and 14 days in spent-medium (c).



The addition of different PCBs waste concentrations for all experiments in an increased pH level compared to the control as shown in Figure 13 was observed. The pH follows almost the same trend; increases and remains stable until the end of the bioleaching period. The experiments with higher PCB concentrations (10 g/L) showed the highest pH values.

Figure 13 - pH behavior for the evaluated approach one-step (a), two-step (b) and spent-medium (c) in the bioleaching process.



The pH variation can be associated with the consumption of organic acids during the bioleaching process and the presence of metal fractions in the medium (HOREH; MOUSAVI; SHOJAOSADATI, 2016). This effect was also described by Madrigal-Arias et al. (2015) using *A. niger* MXPE6 and *A. niger* MX7 in the presence of PCBs waste. The pH value increases due to the addition of PCBs agrees with the results reported by Brandl et al. (2001), who observed that the pH of the culture medium increases at a high metal concentration in the experiments.

Metal recovery technologies are at the initial stage, and most industrial-scale applications are limited to pyrometallurgical routes applied to a small fraction of metal-rich waste electrical and electronic equipment (WEEE). Currently, biohydrometallurgy is sought to high-grade PCBs with highly valuable metal content. Thus, the selectivity towards individual metals is an important factor in the research strategy, as well as the development of specific technologies required for these processes (IŞILDAR, 2018a). A direct comparison of metals bioleaching studies is unfair since the metal composition of PCBs is heterogeneous and can change with the origin, manufacturer and the acid digestion protocols used by different researchers (NATARAJAN; TING, 2015). Moreover, other factors such as growth medium, leaching period and composition of metal elements in the PCBs can affect the bioprocess and should be considered.

3.4 Conclusions

In this study, *Aspergillus niger* was successfully used to recover copper and gold from PCBs. High recovery efficiency was reached using the one-step (100% Cu and 41% Au) and two-step approaches (100% Cu and 42.5% Au). In the spent medium approach, copper and gold showed a recovery up to 100% and 35.16%, respectively for copper and gold. *A. niger* produced organic acids effective in leaching copper (100% recovery), independent of the approach used. From the results, it was observed that as the concentration of PCBs waste increased, the metal leaching efficiency decreased. Concerning the particle size distribution, particles with 131 nm were obtained at lower PCBs concentrations (2.5 g/L) indicating that the biological leaching of PCBs depends on physicochemical factors. The TEM micrographs revealed the spherical morphology of particles with considerable variation in particle size. In conclusion, bioleaching is an environmental-friendly technology for metal recovery from urban mining.

4 RECOVERY OF COPPER AND GOLD FROM COMPUTER PRINTED CIRCUIT BOARDS BY A TWO-STEP APPROACH USING ADAPTED *ASPERGILLUS NIGER*

4.1 Introduction

The development of new electronics equipment is associated with an increasing demand for precious and rare metals. Precious metals such as silver and gold are in the category of very scarce metals. Metals like copper and nickel are not very scarce, however, their reserves are already at levels of concern (SUN et al., 2017). Currently, electronic waste is one of the fastest-growing solid waste, accumulating a rate of 50 million tons of waste globally with an annual growth rate of 17.6% (SETHURAJAN et al., 2019). Due to the large generation of e-waste, it can be considered as a potential and promising secondary source of metals found at relatively high concentrations (GHOSH et al., 2015).

Electronic residues, such as printed circuit boards (PCBs) from computers, contain significant content of different precious and non-precious metals. PCB is an essential component of almost all electronic and electrical equipment such as computers, televisions, and mobile phones (MANKHAND et al., 2013). The PCBs are generally made up of 40% metals, 30% organic materials, and inorganic oxides (ceramics) (SARVAR; SALARIRAD; SHABANI, 2015). The metal concentration present in computer PCBs can contain 20% copper, 1000 ppm silver, 250 ppm gold, and 110 ppm palladium (WU et al., 2017). In recent years, PCBs recovery has grown rapidly, and the management and recycling are still difficult due to their heterogeneous composition, which includes organic, ceramic, and metallic materials (PIETRELLI; FERRO; VOCCIANTE, 2019).

The recovery of metals from electronic wastes requires disassembly, size reduction and subsequent pyrometallurgical and bio-hydrometallurgical methods. Currently electronic waste is recovered at complex smelters by the pyrometallurgical process. The conventional pyrometallurgical and hydrometallurgical methods are dangerous, non-selective, difficult to control, high-energy and high-capital demanding, and produce secondary pollution as well (TUNCUK et al., 2012).

In order to meet the market need for more eco-friendly processes, it is important to explore other forms of metal recycling and recovery from electronic wastes. Biohydrometallurgical or bioleaching processes are eco-friendly technologies that apply different microorganisms to recover metals from wastes (MARRA et al., 2018; NASERI; BAHALOO-HOREH; MOUSAVI, 2019). The process is economic, quite flexible, and environment friendly carried out at atmospheric pressure and low temperature requiring less energy (GU et al., 2018). Another advantage of this process is that the used reagents for metal solubilization are biologically produced during the bioleaching process (MOUNA; BARAL, 2019).

Aspergillus niger is an effective microorganism in the metal recovery process due to the ability to produce organic acids and chelating agents during its growth phase (AKPOR et al., 2015). One of the limiting factors for the efficiency of the bioleaching using microorganisms is the toxic nature of the metals present in the electronic waste that affects the microorganism growth and the leaching yield during the process. It has been reported that the adaptation process of microorganisms before the bioleaching process can promote the efficiency of the process because the microorganisms develop the ability to survive in the presence of high concentrations of metals through adaptation mutation (HONG et al., 2016). Thus, in this work, to increase the efficiency of the dissolution of copper and gold from computer printed circuit boards, before the bioleaching process, the *A. niger* was adapted to obtain microbial resistance to metals presents in the PCBs. Subsequently, the bioleaching process was performed through the two-step method by adapted *A. niger*.

4.2 Material and Methods

4.2.1 Adaptation process of *A. niger*

The metal fraction of the PCBs used in the bioleaching experiments was obtained as described in the previous chapter. Before the bioleaching process, to increase the tolerance of the fungal strain to the toxicity of the e-waste, the cells were adapted to the metal fraction over a period. At the first step, *Aspergillus niger* was inoculated with 4×10^6 spores/mL_{medium} in 50 mL mineral medium (g/L): $CaCl_2$ (0.1), KH_2PO_4 (0.5), NH_4Cl (1.5), $MgSO_4.7H_2O$ (0.025), glucose (50.0) (MADRIGAL-ARIAS et al., 2015) and 0.5% (w/v) of metal fraction, the medium was incubated in a shaker-incubator at 160 rpm, 30 ºC for 5 days. After the first step, a new fraction of 0.5% (w/v) of the autoclaved metal fraction was added to *A. niger* at the logarithmic phase and incubated in shaker-incubator at 30 ºC, 160 rpm for 15 days. After 15 days, the adapted fungi to 1% (w/v) were cultured on PDA medium (potato dextrose agar) and preserved at 4 ºC for subsequent experiments.

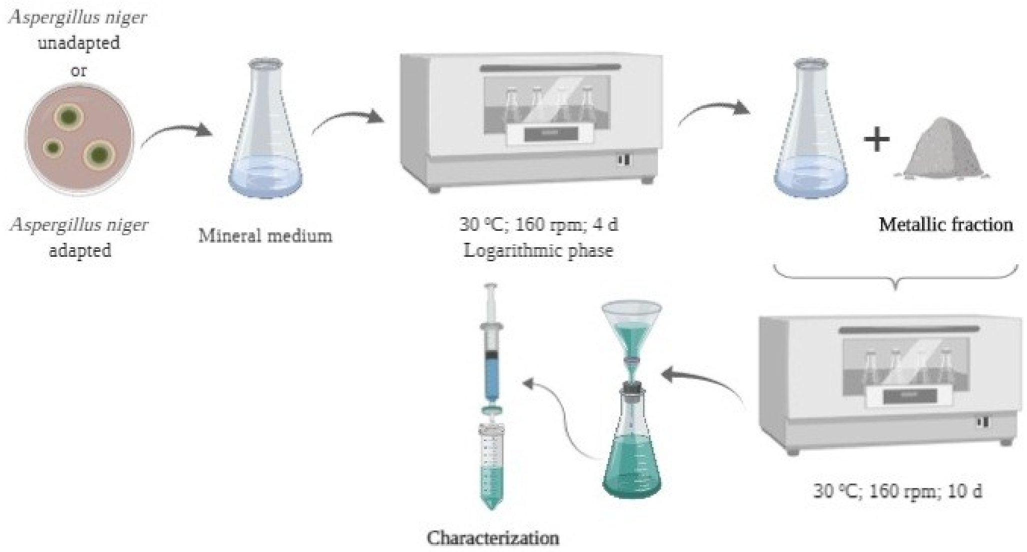
4.2.2 Metabolites production by adapted and unadapted *A. niger*

The metabolites produced by *A. niger* were collected every 48 h, and vacuum filtered with Whatman 42 filter paper to analytical determinations. The biomass dry weight was determined using the mycelium obtained from the vacuum filtration with Whatman 42 filter paper; the mycelium was dried until constant weight at 60 °C. The concentration of reducing sugars was quantified by the DNS method (MILLER, 1959). The total acid was quantified by titration according to APHA-AWWA-WEF (1998). At the end of the process (10th day), the sample was analyzed by Fourier Transform Infrared (FTIR) (Shimadzu, Model IRPrestige-21, 8400S) in the diffuse reflectance mode at a resolution of 4 cm^{-1} with scanning in the range of 500-4000 cm^{-1} in KBr pellets, for the determination of active functional groups.

4.2.3 Bioleaching procedure

The inoculum preparation was carried out by using the *A. niger* before and after adaptation in 1 L flasks with 100 mL of PDA medium (potato dextrose agar) and 1% of the metal fraction, followed by incubation at 30 ºC for 7 days. Then, the two-step bioleaching approach was carried out (Figure 14). The bioleaching process were performed in 300 mL autoclaved Erlenmeyer flasks with 125 mL mineral medium containing (g/L): $CaCl_2$ (0.1), KH_2PO_4 (0.5), NH_4Cl (1.5), $MgSO_4.7H_2O$ (0.025), glucose (50.0) (MADRIGAL-ARIAS et al., 2015). The pH was set to 4.4 with sulfuric acid 1 N. The medium was sterilized at 121 ºC, 101 kPa for 20 min followed by the inoculation at 4×10^6 spores/mL_{medium} in mineral medium with no metallic fraction added until the fungi logarithmic phase (4 days). The metallic concentrations used in the experiments were 10 and 20 g/L added in the logarithmic phase to the culture medium and bioleached in an orbital shaker-incubator at 30 ºC, 160 rpm for 14 days. The experiments were carried out in duplicate. Samples were collected in 0 and 14 days.

Figure 14 - Schematic flowchart of the two-step experimental procedure.



Total copper content from samples was measured by atomic absorption spectrometry (AAS, Shimadzu model AA 6300) using an air-acetylene flame, and gold content was measured by ICP-MS (NexION 300 D, Perkin Elmer). The average particle size distribution was determined by a laser scattering particle size analyzer (Zetasizer Nano ZS, Malvern). In order to evaluate the morphology of the metallic particles after bioleaching, transmission electron microscopy was used (TEM JEM-1011 JEOL) at 100 kV. A drop of leached solutions was placed on carbon film-coated copper grids (3 mm diameter with 300 mesh) and dried at room temperature for 48 h.

4.3 Results and discussion

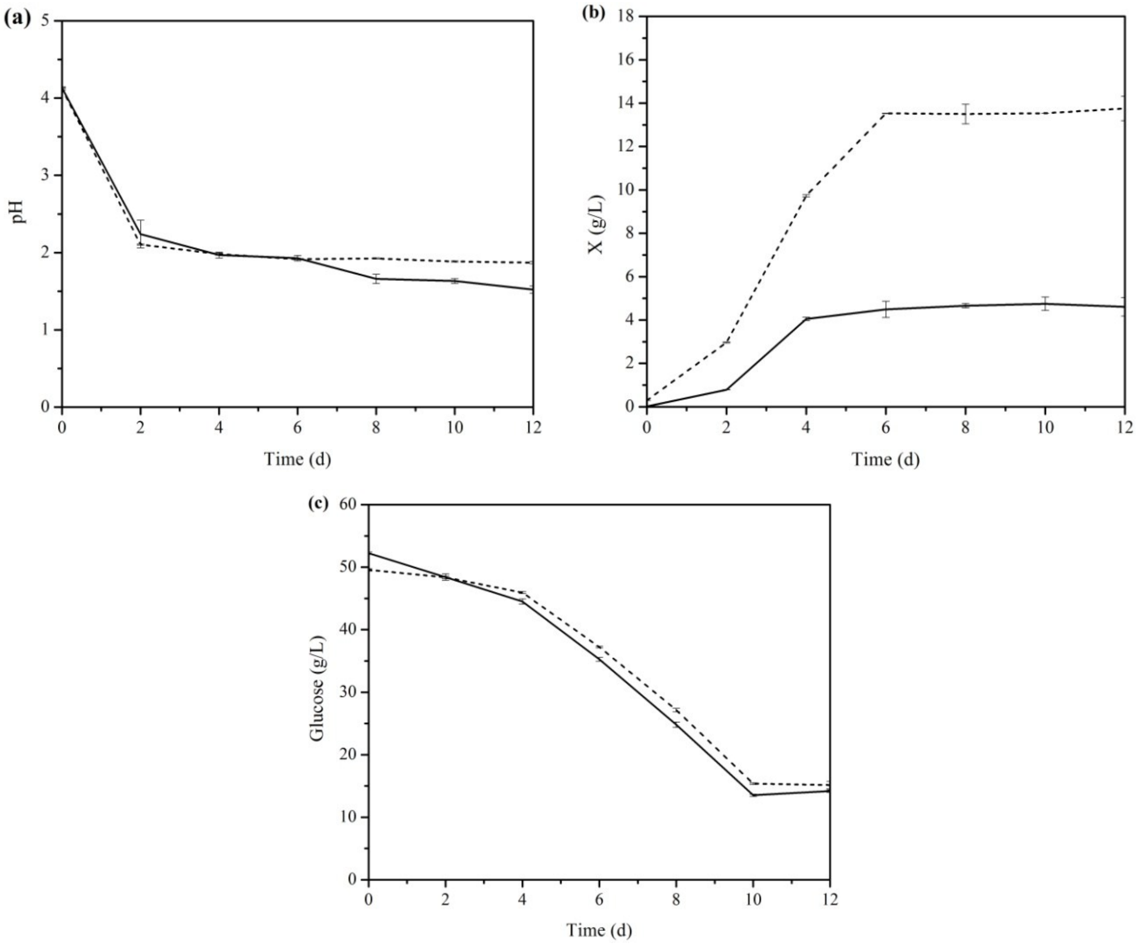
4.3.1 Metabolites production by adapted *A. niger*

The effect of acidity, pH, dry biomass and glucose concentration on substrate consumption of *A. niger* before and after the adaptation is shown in Figure 15 and Figure 16. Fungi as *A. niger* produce organic acids (malic, oxalic, gluconic, citric, lactic) through the Krebs cycle. The rate of organic acid production depends on the rate of microbial growth and utilization of carbon sources (which is glucose in this study).

The observed pH reduction corresponds to the beginning of organic acid production and the beginning of the logarithmic phase. After the 2nd day a pH reduction from 4.1 to 2.2

and 1.98 for *A. niger* was observed before and after the adaptation step, respectively. Along with the pH reduction, the microbial growth increased to 4.05 and 9.70 g/L for *A. niger* before and after the adaptation step, respectively. The maximum microbial growth for both strains occurred on the 10th day, 4.75 g/L and 13.50 g/L, respectively before and after the adaptation step. After the 10th day of the process, a gradual decrease in microbial growth was observed due to the fungi growth inhibition of secreted primary metabolites (as citric acid).

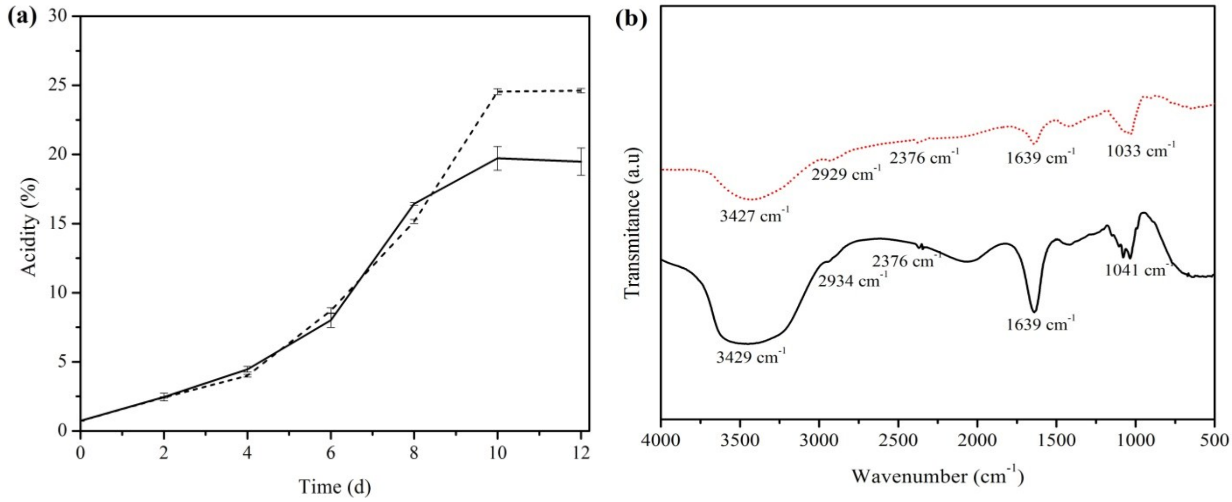
Figure 15 – pH (a), microbial growth (b), and total glucose (c) in the culture medium during the production of organics acids by unadapted (full line) and adapted (dashed line) *A. niger*.



According to the results shown in Figure 15b, adapted *A. niger* showed high growth in the mineral medium compared to the unadapted strain, resulting in a higher organic acid production (Figure 16a). Higher concentrations of organic acid enhance metal immobilization, suggesting an effect of surface complexation reactions as a mechanism of metal release and complexation of metals present in a solution (BAHALOO-HOREH; MOUSAVI;

BANIASADI, 2018). Figure 16b shows the FTIR spectra after 10 days of organic acid production.

Figure 16 - Total acidity in the culture medium during the process (a) and FTIR spectra after 10 days of process (b) using unadapted (full line) and adapted (dashed line) *A. niger*.

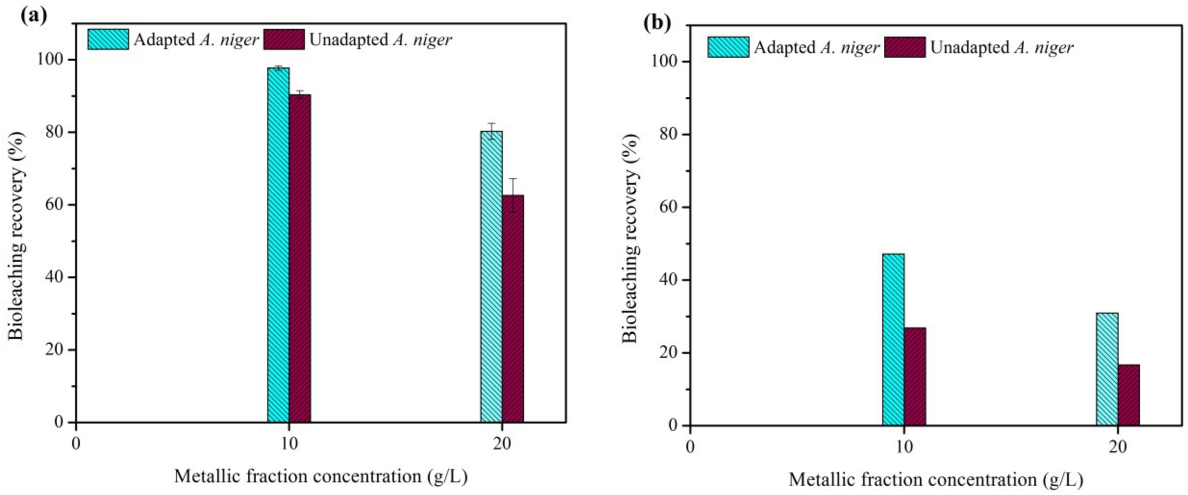


The FTIR spectra obtained for the adapted and unadapted strain showed a similar behavior. The peak located at ~3429 cm^{-1} is related to water. The peaks observed at 1639 and ~2934 cm^{-1} are associated with D-gluconic acid production. Peaks located from 1500 to 900 cm^{-1} are characteristic of malic and citric acids (BULLEN et al., 2008; NARAYANASAMY et al., 2018; PRASHANT A. MAGDUM; et al., 2014) confirming the production of organic acids by adapted *A. niger*.

4.3.2 Copper and gold bioleaching

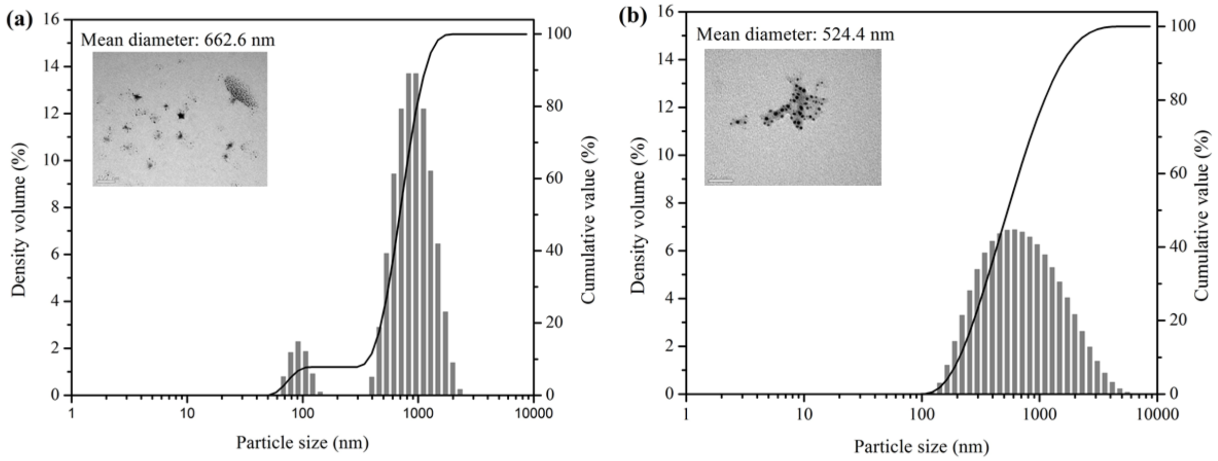
For the copper and gold bioleaching by *A. niger,* before and after adaptation at different metallic concentrations in the (10 to 20 g/L), it was observed that the increase in the metallic concentration from 10 to 20 g/L resulted in a decrease in the metal dissolution (decrease of 17% (copper bioleaching) and 34% (gold bioleaching) for adapted *A. niger*, and decrease of 31% (copper bioleaching) and 37% (gold bioleaching) for unadapted *A. niger*. However, the highest recovery rate was obtained for the adapted *A. niger* (Figure 17). In the bioleaching process, when waste density increases, consequently the metals concentration increase and the inhibitory effects may reduce the microbial and metabolite production, which reduces metal recovery. On the other hand, at low waste density, the process becomes not acceptable from an economical view.

Figure 17 – Recovery of Cu (a) and Au (b) after 14 days at different metallic concentrations by *A. niger* before and after adaptation step.



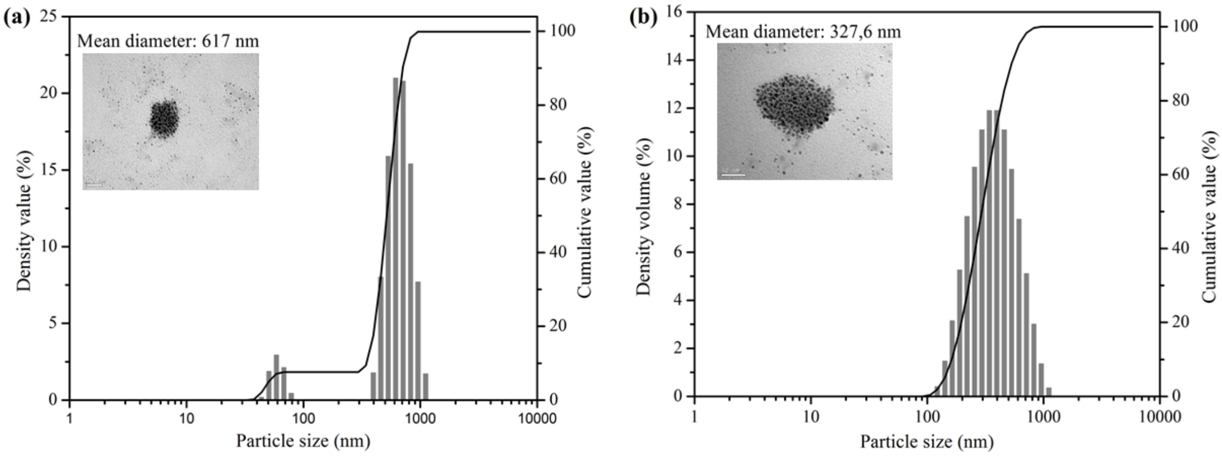
Regarding the particle size distribution after 14 days of the bioleaching process, it was observed that at 10 g/L of metal concentration (Figure 18) a bimodal distribution with a mean diameter of 662 nm for unadapted *A. niger* and a modal distribution with a mean diameter of 524 nm for the adapted *A. niger* were obtained. The TEM images provided insight into the morphology and size details of the metallic particles. From the image, particles can be seen with sizes smaller than 100 nm, confirming the formation of the nanoparticles.

Figure 18 - Particle size distribution and TEM images (upper side detail) after 14 days of two-step bioleaching process at 10 g/L metallic concentration by unadapted (a) and adapted *A. niger* (b).



The results using 20 g/L of metallic fraction concentration by unadapted *A. niger* (Figure 19a) showed particles with bimodal distribution with a mean diameter size of 617 nm and particles with modal distribution with a mean diameter size of 327 nm for adapted *A. niger*. TEM results show that the particles are spherical with an average size low than 50 nm.

Figure 19 - Particle size distribution and TEM images (upper side detail) after 14 days of two-step bioleaching process at 20 g/L metallic concentration by unadapted *A. niger* (a) and adapted *A. niger* (b).



4.4 Conclusions

The sustainable recovery of metals from electronic waste using microorganisms can generate environmental and economic benefits when compared to traditional recovery processes. In this study, up to 97.7% Cu; 47.1% Au (at 10 g/L metallic fraction concentration) and 80.2% Cu; 30.9% Au (at 20 g/L metallic fraction concentration) were successfully leached from PCBs waste by adapted *A. niger*. The adaptation process of *A. niger* in PCBs improved the production of metabolites and the bioleaching efficiency of metals. From the TEM analysis, it was observed that the leached metal particles were smaller than 100 nm showing the presence of nanoparticles.

5 FINAL CONSIDERATIONS

The main findings of this thesis are:

- An alternative route for metals pre-concentration from printed circuit boards by dense media separation was established with sodium silicate as dense liquid replacing chloroform;
- For the one-step, two-step and spent medium approaches, it was observed that as the metal concentration increased, the metal leaching efficiency decreased;
- The two-step approach showed higher bioleaching recovery for copper and gold (100% and 42.5%, respectively);
- *Aspergillus niger* produced metabolites that are effective for leaching copper, regardless the approach used (one-step, two-step or spent medium).
- TEM images revealed the almost spherical morphology of particles with considerable variation in particle size;
- The adaptation process of *A. niger* in PCBs improved the production of metabolites and the leaching efficiency of metals (copper and gold);
- TEM images indicated metal particles smaller than 100 nm.
- Metallic nanoparticles were obtained through the process of bioleaching by *A. niger*.

For future work, some suggestions are proposed:

- Evaluation of the influence of other microorganisms on the bioleaching process;
- Evaluation of the bioleaching recovery for other metals;
- Isolation of microorganisms present on the printed circuit boards;
- Use of printed circuit boards from other electronic equipment;
- Optimization of the scale-up of the bioleaching process.

REFERENCES

AKCIL, A.; ERUST, C.; GAHAN, C. S. EKHA.; OZGUN, M.; SAHIN, M.; TUNCUK, A. Precious metal recovery from waste printed circuit boards using cyanide and non-cyanide lixiviants – A review. **Waste Management**, v. 45, p. 258–271, 2015.

AKPOR, O. B.; MUSA, J. O.; BABALOLA, O. .; ADEJOBI, O. I. Tolerance of *Aspergillus niger* to selected concentrations of metals and sodium chloride. **IOSR Journal of Environmental Science Ver. I**, v. 9, n. 10, p. 2319–2399, 2015.

AL-GHOUTI, M. A.; AL DISI, Z. A.; AL-KAABI, N.; KHRAISHEH, M. Mechanistic insights into the remediation of bromide ions from desalinated water using roasted date pits. **Chemical Engineering Journal**, v. 308, p. 463–475, 2017.

APHA-AWWA-WEF. **Standard Methods fot the Examination of Water and Wastewater**, 1998.

ARGUMEDO-DELIRA, R.; GÓMEZ-MARTÍNEZ, M. J.; SOTO, B. J. Gold bioleaching from printed circuit boards of mobile phones by *Aspergillus niger* in a culture without agitation and with glucose as a carbon source. **Metals**, v. 9, n. 5, p. 521, 2019.

BAHALOO-HOREH, N.; MOUSAVI, S. M.; BANIASADI, M. Use of adapted metal tolerant *Aspergillus niger* to enhance bioleaching ef ficiency of valuable metals from spent lithium-ion mobile phone batteries. **Journal of Cleaner Production**, v. 197, p. 1546–1557, 2018.

BALDÉ, C. P.; FORTI, V.; GRAY, V.; KUEHR, R.; STEGMANN, P. **The Global E-waste Monitor 2017**. [s.l: s.n.].

BALDÉ, C. P.; KUEHR, R.; BLUMENTHAL, K.; FONDEUR GILL, S.; KERN, M.; MICHELI, P.; MAGPANTAY, E.; HUISMAN, J. E-waste Statistics: Guidelines on Classifications, Reporting and Indicators. In: **United Nations University, IAS-SCYCLE**. [s.l: s.n.].

BAZARGAN, A.; BWEGENDAHO, D.; BARFORD, J.; MCKAY, G. Printed circuit board waste as a source for high purity porous silica. **Separation and Purification Technology**, v. 136, p. 88–93, 2014.

BISWAL, B. K.; JADHAV, U. U.; MADHAIYAN, M.; JI, L.; YANG, E.-H.; CAO, B. Biological leaching and chemical precipitation methods for recovery of cCo and Li from spent lithium-ion batteries. **ACS Sustainable Chemistry & Engineering**, v. 6, n. 9, p. 12343–12352, 2018.

BIZZO, W.; FIGUEIREDO, R.; DE ANDRADE, V. Characterization of printed circuit boards for metal and energy recovery after milling and mechanical separation. **Materials**, v. 7, n. 6, p. 4555–4566, 2014.

BOND, T.; GRAHAM, N. Predicting chloroform production from organic precursors. **Water Research**, v. 124, p. 167–176, 2017.

BORJA, D.; NGUYEN, K.; SILVA, R.; PARK, J.; GUPTA, V.; HAN, Y.; LEE, Y.; KIM, H. Experiences and future challenges of bioleaching research in south Korea. **Minerals**, v. 6, n. 4, p. 128, 2016.

BRANDL, H.; BOSSHARD, R.; WEGMANN, M. Computer-munching microbes: metal leaching from electronic scrap by bacteria and fungi. v. 59, p. 319–326, 2001.

BULLEN, H. A.; OEHRLE, S. A.; BENNETT, A. F.; TAYLOR, N. M.; BARTON, H. A. Use of attenuated total reflectance fourier transform infrared spectroscopy to identify microbial metabolic products on carbonate mineral surfaces. **Applied and Environmental Microbiology**, v. 74, n. 14, p. 4553–4559, 2008.

BURAT, FIRAT; ÖZER, M. Physical separation route for printed circuit boards. v. 54, n. 2, p. 554–566, 2018.

CANAL MARQUES, A.; CABRERA, J.; DE FRAGA MALFATTI, C. Printed circuit boards: A review on the perspective of sustainability. **Journal of Environmental Management**, v. 131, p. 298–306, 2013.

CAYUMIL, R.; KHANNA, R.; RAJARAO, R.; MUKHERJEE, P. S.; SAHAJWALLA, V. Concentration of precious metals during their recovery from electronic waste. **Waste Management**, v. 57, p. 121–130, 2016.

CHANCEREL, P.; MESKERS, C. E. M.; HAGELUKEN, C.; ROTTER, V. S. Assessment of precious metal flows during preprocessing of waste electrical and electronic equipment. v. 13, n. 5, 2009.

CHI, T. D.; LEE, J. C.; PANDEY, B. D.; YOO, K.; JEONG, J. Bioleaching of gold and copper from waste mobile phone PCBs by using a cyanogenic bacterium. **Minerals Engineering**, v. 24, n. 11, p. 1219–1222, 2011.

CUI, J.; FORSSBERG, E. Mechanical recycling of waste electric and electronic equipment: a review. **Journal of Hazardous Materials**, v. 99, n. 3, p. 243–263, 2003.

DE ROSSI, A.; SIMÃO, L.; RIBEIRO, M. J.; NOVAIS, R. M.; LABRINCHA, J. A.; HOTZA, D.; MOREIRA, R. F. P. M. In-situ synthesis of zeolites by geopolymerization of biomass fly ash and metakaolin. **Materials Letters**, v. 236, p. 644–648, 2019.

DENG, X.; CHAI, L.; YANG, Z.; TANG, C.; WANG, Y.; SHI, Y. Bioleaching mechanism of heavy metals in the mixture of contaminated soil and slag by using indigenous *Penicillium chrysogenum* strain F1. **Journal of Hazardous Materials**, v. 248–249, n. 1, p. 107–114, 2013.

DÍAZ-MARTÍNEZ, M. E.; ARGUMEDO-DELIRA, R.; SÁNCHEZ-VIVEROS, G.; ALARCÓN, A.; MENDOZA-LÓPEZ, M. R. Microbial bioleaching of Ag, Au and Cu from printed circuit boards of mobile phones. **Current Microbiology**, v. 76, n. 5, p. 536–544, 2019.

ESTRADA-RUIZ, R. H.; FLORES-CAMPOS, R.; GÁMEZ-ALTAMIRANO, H. A.; VELARDE-SÁNCHEZ, E. J. Separation of the metallic and non-metallic fraction from printed circuit boards employing green technology. **Journal of Hazardous Materials**, v. 311, p. 91–99, 2016.

FALCONE, J. S. Silicon compounds: Anthropogenic silicas and silicates. In: **Kirk-Othmer Encyclopedia of Chemical Technology**. Hoboken, NJ, USA: John Wiley & Sons, Inc., 2015. p. 1–32.

FARAJI, F.; GOLMOHAMMADZADEH, R.; RASHCHI, F.; ALIMARDANI, N. Fungal bioleaching of WPCBs using *Aspergillus niger*: Observation, optimization and kinetics. **Journal of Environmental Management**, v. 217, p. 775–787, 2018.

FICERIOVÁ, J.; BALÁŽ, P.; GOCK, E. Leaching of gold, silver and accompanying metals from circuit boards (PCBs) waste. **Acta Montanistica Slovaca**, v. 16, n. 2, p. 128–131, 2011.

FLORES-CAMPOS, R.; ESTRADA-RUIZ, R. H.; VELARDE-SÁNCHEZ, E. J. Study of the physicochemical effects on the separation of the non-metallic fraction from printed circuit boards by inverse flotation. **Waste Management**, v. 69, p. 400–406, 2017.

GHOSH, B.; GHOSH, M. K.; PARHI, P.; MUKHERJEE, P. S.; MISHRA, B. K. Waste Printed Circuit Boards recycling: An extensive assessment of current status. **Journal of Cleaner Production**, v. 94, p. 5–19, 2015.

GOLEV, A.; CORDER, G. D. Quantifying metal values in e-waste in Australia: The value chain perspective. **Minerals Engineering**, v. 107, p. 81–87, 2017.

GOMES, A. S. T.; SOUZA, L. A.; YAMANE, L. H.; SIMAN, R. R. Quantification of E-Waste: A case study in Federal University of Espírito Santo, Brazil. **International Journal of Environmental and Ecological Enginerring**, v. 11, n. 2, p. 195–203, 2017.

GOODMAN, P. Current and future uses of gold in electronics how and where gold is used. **Gold Bulletin**, v. 35, n. 1, p. 21–26, 2002.

GU, T.; RASTEGAR, S. O.; MOUSAVI, S. M.; LI, M.; ZHOU, M. Advances in bioleaching for recovery of metals and bioremediation of fuel ash and sewage sludge. **Bioresource Technology**, v. 261, n. February, p. 428–440, 2018.

GUPTA, A.; YAN, D. S. Gravity Separation. In: **Mineral Processing Design and Operation**. [s.l.] Elsevier, 2006. p. 494–554.

HADI, P.; GAO, P.; BARFORD, J. P.; MCKAY, G. Novel application of the nonmetallic fraction of the recycled printed circuit boards as a toxic heavy metal adsorbent. **Journal of Hazardous Materials**, v. 252–253, p. 166–170, 2013.

HONG, J.; SILVA, R. A.; PARK, J.; LEE, E.; PARK, J.; KIM, H. Adaptation of a mixed culture of acidophiles for a tank biooxidation of refractory gold concentrates containing a high concentration of arsenic. **Journal of Bioscience and Bioengineering**, v. 121, n. 5, p. 536–542, 2016.

HOREH, N. B.; MOUSAVI, S. M.; SHOJAOSADATI, S. A. Bioleaching of valuable metals from spent lithium-ion mobile phone batteries using *Aspergillus niger*. **Journal of Power Sources**, v. 320, p. 257–266, 2016.

IANNICELLI-ZUBIANI, E. M.; GIANI, M. I.; RECANATI, F.; DOTELLI, G.; PURICELLI, S.; CRISTIANI, C. Environmental impacts of a hydrometallurgical process for electronic waste treatment: A life cycle assessment case study. **Journal of Cleaner Production**, v. 140, p. 1204–1216, 2017.

IŞILDAR, A. Biotechnologies for metal recovery from electronic waste and printed circuit boards. In: **Waste Electrical and Electronic Equipment Recycling**. [s.l.] Elsevier, 2018a. p. 241–269.

IŞILDAR, A. **Metal Recovery from Electronic Waste: Biological Versus Chemical Leaching for Recovery of Copper and Gold**. [s.l.] CRC Press, 2018b.

IŞILDAR, A.; VAN DE VOSSENBERG, J.; RENE, E. R.; VAN HULLEBUSCH, E. D.; LENS, P. N. L. Two-step bioleaching of copper and gold from discarded printed circuit boards (PCB). **Waste Management**, v. 57, p. 149–157, nov. 2016.

JAAFAR, R.; ALDOGHACHI, F.; SUHAIMI, N.; MOHAMMED, S. Biosorption of some heavy metals by deinococcus radiodurans isolated from soil in Basra Governorate-Iraq. **Journal of Bioremediation & Biodegradation**, v. 7, n. 2, p. 2–5, 2016.

JAGANNATH, A.; SHETTY K., V.; SAIDUTTA, M. B. Bioleaching of copper from electronic waste using *Acinetobacter* sp. Cr B2 in a pulsed plate column operated in batch and sequential batch mode. **Journal of Environmental Chemical Engineering**, v. 5, n. 2, p. 1599–1607, 2017.

JOHNSON, D. B. Biomining—biotechnologies for extracting and recovering metals from ores and waste materials. **Current Opinion in Biotechnology**, v. 30, p. 24–31, 2014.

JOSE, J.; RAMANUJAM, S.; PHILIP, L. Applicability of pulsed corona discharge treatment for the degradation of chloroform. **Chemical Engineering Journal**, v. 360, p. 1341–1354, 2019.

KAKSONEN, A. H.; BOXALL, N. J.; GUMULYA, Y.; KHALEQUE, H. N.; MORRIS, C.; BOHU, T.; CHENG, K. Y.; USHER, K. M.; LAKANIEMI, A. Recent progress in biohydrometallurgy and microbial characterisation. **Hydrometallurgy**, v. 180, p. 7–25, 2018.

KAYA, M. Recovery of metals and nonmetals from electronic waste by physical and chemical recycling processes. **Waste Management**, v. 57, p. 64–90, 2016.

KHALIQ, A.; RHAMDHANI, M.; BROOKS, G.; MASOOD, S. Metal extraction processes for electronic waste and existing industrial routes: A review and australian perspective. **Resources**, v. 3, n. 1, p. 152–179, 2014.

KHAN, I.; SAEED, K.; KHAN, I. Nanoparticles: Properties, applications and toxicities. **Arabian Journal of Chemistry**, 2017.

KIM, M.-J.; SEO, J.; CHOI, Y.; KIM, G. Bioleaching of spent Zn–Mn or Ni–Cd batteries by *Aspergillus* species. **Waste Management**, v. 51, p. 168–173, 2016.

KIM, Y.; SEO, H.; ROH, Y. Metal recovery from the mobile phone waste by chemical and biological treatments. **Minerals**, v. 8, n. 1, p. 8, 2018.

KOSMULSKI, M. pH-dependent surface charging and points of zero charge II. Update. **Journal of Colloid and Interface Science**, v. 275, n. 1, p. 214–224, 2004.

KOYANAKA, S.; ENDOH, S.; OHYA, H. Effect of impact velocity control on selective grinding of waste printed circuit boards. **Advanced Powder Technology**, v. 17, n. 1, p. 113–126, 2006.

LI, H.; EKSTEEN, J.; ORABY, E. Hydrometallurgical recovery of metals from waste printed circuit boards (WPCBs): Current status and perspectives – A review. **Resources, Conservation and Recycling**, v. 139, p. 122–139, 2018.

LIU, R.; LI, J.; GE, Z. Review on *chromobacterium violaceum* for gold bioleaching from e-waste. **Procedia Environmental Sciences**, v. 31, p. 947–953, 2016.

LUDA, M. P. Recycling of Printed Circuit Boards. In: **Integrated Waste Management - Volume II**. [s.l.] InTech, 2011. p. 285–298.

MADRIGAL-ARIAS, J. E.; ARGUMEDO-DELIRA, R.; ALARCÓN, A.; MENDOZA-LÓPEZ, M. R.; GARCÍA-BARRADAS, O.; CRUZ-SÁNCHEZ, J. S.; FERRERA-CERRATO, R.; JIMÉNEZ-FERNÁNDEZ, M. Bioleaching of gold, copper and nickel from waste cellular phone PCBs and computer gold finger motherboards by two *Aspergillus niger* strains. **Brazilian Journal of Microbiology**, v. 46, n. 3, p. 707–713, 2015.

MAGNUSON, J. K.; LASURE, L. L. Organic acid production by filamentous fungi. In: **Advances in Fungal Biotechnology for Industry,Agriculture, and Medicine**. [s.l: s.n.]. p. 307–340.

MARRA, A.; CESARO, A.; RENE, E. R.; BELGIORNO, V.; LENS, P. N. L. Bioleaching of metals from WEEE shredding dust. **Journal of Environmental Management**, v. 210, p. 180–190, 2018.

MENG, L.; GUO, L.; GUO, Z. Separation of metals from metal-rich particles of crushed waste printed circuit boards by low-pressure filtration. **Waste Management**, v. 84, p. 227–234, 2019.

MILLER, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry**, v. 31, n. 3, p. 426–428, 1959.

MOOSAKAZEMI, F.; GHASSA, S.; MOHAMMADI, M. R. T. Environmentally friendly hydrometallurgical recovery of tin and lead from waste printed circuit boards: Thermodynamic and kinetics studies. **Journal of Cleaner Production**, v. 228, p. 185–196, 2019.

MOUNA, H. M.; BARAL, S. S. A bio-hydrometallurgical approach towards leaching of lanthanum from the spent fluid catalytic cracking catalyst using *Aspergillus niger*. **Hydrometallurgy**, v. 184, n. January, p. 175–182, 2019.

MUKHERJEE, P.; ROY, M.; MANDAL, B. P.; DEY, G. K.; MUKHERJEE, P. K.; GHATAK, J.; TYAGI, A. K.; KALE, S. P. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. **Nanotechnology**, v. 19, n. 7, p. 075103, 2008.

MUNSTERMAN, D.; KERSTHOLT, S. Sodium polytungstate, a new non-toxic alternative to bromoform in heavy liquid separation. **Review of Palaeobotany and Palynology**, v. 91, n. 1–4, p. 417–422, 1996.

NARAYANAN, K. B.; SAKTHIVEL, N. Biological synthesis of metal nanoparticles by microbes. **Advances in Colloid and Interface Science**, v. 156, n. 1–2, p. 1–13, 2010.

NARAYANASAMY, M.; DHANASEKARAN, D.; VINOTHINI, G.; THAJUDDIN, N. Extraction and recovery of precious metals from electronic waste printed circuit boards by bioleaching acidophilic fungi. **International Journal of Environmental Science and Technology**, v. 15, n. 1, p. 119–132, 2018.

NASERI, T.; BAHALOO-HOREH, N.; MOUSAVI, S. M. Bacterial leaching as a green approach for typical metals recovery from end-of-life coin cells batteries. **Journal of Cleaner Production**, v. 220, n. 2019, p. 483–492, 2019.

NATARAJAN, G.; TING, Y. P. Gold biorecovery from e-waste: An improved strategy through spent medium leaching with pH modification. **Chemosphere**, v. 136, p. 232–238, 2015.

OH, C. J.; LEE, S. O.; YANG, H. S.; HA, T. J.; KIM, M. J. Selective leaching of valuable metals from waste printed circuit boards. **Journal of the Air and Waste Management Association**, v. 53, n. 7, p. 897–902, 2003.

OKIBE, N.; GERICKE, M.; HALLBERG, K. B.; JOHNSON, D. B. Enumeration and characterization of acidophilic microorganisms isolated from a pilot plant stirred-tank bioleaching operation. **Applied and Environmental Microbiology**, v. 69, n. 4, p. 1936–1943, 2016.

PAN, W. J.; ZHANG, H. G.; XU, L.; IMAM, H.; SONG, B. T.; YUE, M. Properties of composites with La(Fe, Si)13 hydrides and sodium silicate. **Journal of Magnetism and Magnetic Materials**, v. 476, p. 608–612, 2019.

PANT, D.; JOSHI, D.; UPRETI, M. K.; KOTNALA, R. K. Chemical and biological extraction of metals present in E waste: A hybrid technology. **Waste Management**, v. 32, n. 5, p. 979–990, 2012.

PARK, Y. J.; FRAY, D. J. Recovery of high purity precious metals from printed circuit boards. **Journal of Hazardous Materials**, v. 164, n. 2–3, p. 1152–1158, 2009.

PETTER, P. M. H.; VEIT, H. M.; BERNARDES, A. M. Evaluation of gold and silver leaching from printed circuit board of cellphones. **Waste Management**, v. 34, n. 2, p. 475–482, 2014.

PIETRELLI, L.; FERRO, S.; VOCCIANTE, M. Eco-friendly and cost-effective strategies for metals recovery from printed circuit boards. **Renewable and Sustainable Energy Reviews**, v. 112, p. 317–323, 2019.

PRASHANT A. MAGDUM; BASAVRAJ S. GUNDAPALLI; MARUTHI M. KURUBAR;; NANDIBEWOOR, S. T. Kinetics and mechanistic investigation of oxidation of D-mannitol by periodate in aqueous alkaline medium. **Journal of Applicable Chemistry**, v. 4, p. 1744–1755, 2014.

PRIYA, A.; HAIT, S. Comparative assessment of metallurgical recovery of metals from electronic waste with special emphasis on bioleaching. **Environmental Science and Pollution Research**, v. 24, n. 8, p. 6989–7008, 2017.

PRIYA, A.; HAIT, S. Extraction of metals from high grade waste printed circuit board by conventional and hybrid bioleaching using *Acidithiobacillus ferrooxidans*. **Hydrometallurgy**, v. 177, p. 132–139, 2018.

QUAN, C.; LI, A.; GAO, N. Study on characteristics of printed circuit board liberation and its crushed products. **Waste Management & Research**, v. 30, n. 11, p. 1178–1186, 2012.

R. MANKHAND, T.; K. SINGH, K.; KUMAR GUPTA, S.; DAS, S. Pyrolysis of printed circuit boards. **International Journal of Metallurgical Engineering**, v. 1, n. 6, p. 102–107, 2013.

REED, D. W.; FUJITA, Y.; DAUBARAS, D. L.; JIAO, Y.; THOMPSON, V. S. Bioleaching of rare earth elements from waste phosphors and cracking catalysts. **Hydrometallurgy**, v. 166, p. 34–40, 2016.

RODRIGUES, É. F.; FICANHA, A. M. M.; DALLAGO, R. M.; TREICHEL, H.; REINEHR, C. O.; MACHADO, T. P.; NUNES, G. B.; COLLA, L. M. Production and purification of amylolytic enzymes for saccharification of microalgal biomass. **Bioresource Technology**, v. 225, p. 134–141, 2017.

SAHIN, M.; AYRANCI, K.; KOSUN, E.; AYRANCI, E. Density, sound velocity and viscosity properties of aqueous sodium metatungstate solutions and an application of these solutions in heavy mineral separations. **Chemical Geology**, v. 264, n. 1–4, p. 96–100, 2009.

SANKHLA, A.; SHARMA, R.; YADAV, R. S.; KASHYAP, D.; KOTHARI, S. L.; KACHHWAHA, S. Biosynthesis and characterization of cadmium sulfide nanoparticles – An emphasis of zeta potential behavior due to capping. **Materials Chemistry and Physics**, v. 170, p. 44–51, 2016.

SARVAR, M.; SALARIRAD, M. M.; SHABANI, M. A. Characterization and mechanical separation of metals from computer Printed Circuit Boards (PCBs) based on mineral processing methods. **Waste Management**, v. 45, p. 246–257, 2015.

SETHURAJAN, M.; VAN HULLEBUSCH, E. D.; FONTANA, D.; AKCIL, A.; DEVECI, H.; BATINIC, B.; LEAL, J. P.; GASCHE, T. A.; ALI KUCUKER, M.; KUCHTA, K.; NETO, I. F. F.; SOARES, H. M. V. M.; CHMIELARZ, A. Recent advances on hydrometallurgical recovery of critical and precious elements from end of life electronic wastes - a review. **Critical Reviews in Environmental Science and Technology**, v. 49, n. 3, p. 212–275, 2019.

SHIN, D.; JEONG, J.; LEE, S.; PANDEY, B. D.; LEE, J. Evaluation of bioleaching factors on gold recovery from ore by cyanide-producing bacteria. **Minerals Engineering**, v. 48, p. 20–24, 2013.

SILVAS, F. P. C. **Utilização de hidrometalurgia e biohidrometalurgia para reciclagem de placas de circuito impresso**. [s.l.] Universidade de São Paulo, 2014.

SINHA, R.; CHAUHAN, G.; SINGH, A.; KUMAR, A.; ACHARYA, S. A novel eco-friendly hybrid approach for recovery and reuse of copper from electronic waste. **Journal of Environmental Chemical Engineering**, v. 6, n. 1, p. 1053–1061, 2018.

SRIVASTAVA, S.; BHARGAVA, A.; PATHAK, N.; SRIVASTAVA, P. Production, characterization and antibacterial activity of silver nanoparticles produced by *Fusarium oxysporum* and monitoring of protein-ligand interaction through in-silico approaches. **Microbial Pathogenesis**, v. 129, n. February, p. 136–145, 2019.

STEMPKOWSKA, A.; MASTALSKA-POPŁAWSKA, J.; IZAK, P.; OGŁAZA, L.; TURKOWSKA, M. Stabilization of kaolin clay slurry with sodium silicate of different silicate moduli. **Applied Clay Science**, v. 146, n. February, p. 147–151, 2017.

STRICKER, A. R.; MACH, R. L.; DE GRAAFF, L. H. Regulation of transcription of cellulases- and hemicellulases-encoding genes in *Aspergillus niger* and *Hypocrea jecorina* (*Trichoderma reesei*). **Applied Microbiology and Biotechnology**, v. 78, n. 2, p. 211–220, 2008.

SUN, B.; YANG, C.; GUI, W. A discussion of the control of nonferrous metallurgical processes. **IFAC-PapersOnLine**, v. 48, n. 17, p. 80–85, 2015.

SUN, Z.; CAO, H.; XIAO, Y.; SIETSMA, J.; JIN, W.; AGTERHUIS, H.; YANG, Y. Toward sustainability for recovery of critical metals from electronic waste: The hydrochemistry processes. **ACS Sustainable Chemistry & Engineering**, v. 5, n. 1, p. 21–40, 2017.

TAN, Z.; HE, Y.; XIE, W.; DUAN, C.; ZHOU, E.; YU, Z. Size distribution of wet crushed waste printed circuit boards. **Mining Science and Technology (China)**, v. 21, n. 3, p. 359–363, 2011.

TREICHEL, H.; SBARDELOTTO, M.; VENTURIN, B.; AGNOL, A. D.; MULINARI, J.; GOLUNSKI, S. M.; BALDONI, D. B.; BEVILACQUA, C. B.; SEMINOTI JACQUES, R. J.; VARGAS, G. D. L. P.; MOSSI, A. J. Lipase production from a newly isolated *Aspergillus niger* by solid state fermentation using canola cake as substrate. **Current Biotechnology**, v. 6, n. 4, 2016.

TUNCUK, A.; STAZI, V.; AKCIL, A.; YAZICI, E. Y.; DEVECI, H. Aqueous metal recovery techniques from e-scrap: Hydrometallurgy in recycling. **Minerals Engineering**, v. 25, n. 1, p. 28–37, 2012.

VAN EYGEN, E.; DE MEESTER, S.; TRAN, H. P.; DEWULF, J. Resource savings by urban mining: The case of desktop and laptop computers in Belgium. **Resources, Conservation and Recycling**, v. 107, p. 53–64, 2016.

VEIT, H. M.; SCHERER, J. Use of gravity separation in metals concentration from printed circuit board scraps. **Metallurgy and Materials**. v. 67, n. 1, p. 73–79, 2014.

VENTURA, E.; FUTURO, A.; PINHO, S. C.; ALMEIDA, M. F.; DIAS, J. M. Physical and thermal processing of waste printed circuit boards aiming for the recovery of gold and copper. **Journal of Environmental Management**, v. 223, p. 297–305, 2018.

VIKTOR, S.; GALYNA, K. Effect of water glass on early hardening of portland cement. **Procedia Engineering**, v. 172, p. 977–981, 2017.

WANG, H.; ZHANG, G.; HAO, J.; HE, Y.; ZHANG, T.; YANG, X. Morphology, mineralogy and separation characteristics of nonmetallic fractions from waste printed circuit boards. **Journal of Cleaner Production**, v. 170, p. 1501–1507, 2018.

WANG, H.; ZHANG, S.; LI, B.; PAN, D.; WU, Y.; ZUO, T. Recovery of waste printed circuit boards through pyrometallurgical processing: A review. **Resources, Conservation and Recycling**, v. 126, p. 209–218, 2017.

WU, Y.; LI, Y.; KANG, D.; WANG, J.; ZHANG, Y.; DU, D.; PAN, B.; LIN, Z.; HUANG, C.; DONG, Q. Tetrabromobisphenol A and heavy metal exposure via dust ingestion in an e-waste recycling region in Southeast China. **Science of The Total Environment**, v. 541, p. 356–364, 2016.

WU, Z.; YUAN, W.; LI, J.; WANG, X.; LIU, L.; WANG, J. A critical review on the recycling of copper and precious metals from waste printed circuit boards using hydrometallurgy. **Frontiers of Environmental Science & Engineering**, v. 11, n. 5, p. 8, 2017.

XIA, M. C.; WANG, Y. P.; PENG, T. J.; SHEN, L.; YU, R. L.; LIU, Y. D.; CHEN, M.; LI, J. K.; WU, X. L.; ZENG, W. M. Recycling of metals from pretreated waste printed circuit boards effectively in stirred tank reactor by a moderately thermophilic culture. **Journal of Bioscience and Bioengineering**, v. 123, n. 6, p. 714–721, 2017.

XIE, W.; HAN, Y.; WANG, H. Magnetic Fe3O4/MCM-41 composite-supported sodium silicate as heterogeneous catalysts for biodiesel production. **Renewable Energy**, v. 125, p. 675–681, 2018.

XIN, B.; JIANG, W.; LI, X.; ZHANG, K.; LIU, C.; WANG, R.; WANG, Y. Analysis of reasons for decline of bioleaching efficiency of spent Zn–Mn batteries at high pulp densities and exploration measure for improving performance. **Bioresource Technology**, v. 112, p. 186–192, 2012.

XU, Y.; LI, J.; LIU, L. Current status and future perspective of recycling copper by hydrometallurgy from waste printed circuit boards. **Procedia Environmental Sciences**, v. 31, p. 162–170, 2016.

YANG, Y.; CHEN, S.; LI, S.; CHEN, M.; CHEN, H.; LIU, B. Bioleaching waste printed circuit boards by *Acidithiobacillus ferrooxidans* and its kinetics aspect. **Journal of Biotechnology**, v. 173, n. 1, p. 24–30, 2014.

YOO, J.-M.; JEONG, J.; YOO, K.; LEE, J.; KIM, W. Enrichment of the metallic components from waste printed circuit boards by a mechanical separation process using a stamp mill. **Waste Management**, v. 29, n. 3, p. 1132–1137, 2009.

ZHANG, B.; YANG, C.; GUI, W. Control strategy for hydrometallurgical removal process based on modelling and evaluation. **IFAC-Papers On Line**, v. 49, n. 20, p. 161–166, 2016.

ZHANG, S.; FORSSBERG, E. Intelligent liberation and classification of electronic scrap. **Powder Technology**, v. 105, n. 1–3, p. 295–301, 1999.

ZHANG, W.; REN, J.; LIU, S.; YUAN, Z. Mechanism and clean procedure to extract gold from printed circuit board. **Procedia Environmental Sciences**, v. 31, p. 171–177, 2016.