MULATU YOHANNES NANUSHA

RECOVERY OF Pd AS NANOSIZED PdS BY COMBINING SOLVENT EXTRACTION WITH BIOLOGICAL STRATEGIES BASED ON THE USE OF SULPHATE-REDUCING BACTERIA COMMUNITIES

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Work supervised by:

Prof. Ana Paula Paiva (University of Lisbon)

Prof. Maria Clara Costa (University of Algarve)

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Declaration of Authorship

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred in the text and are listed in the bibliography.

Mulatu Yohannes Nanusha

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I dedicate this thesis
to my wife, and to my children.

Abstract

Platinum group metals (PGMs) are highly demanding metals in current high-techs. However, their supply is limited due to their scarcity in natural resources and expensive mining. Previously, several techniques proposed for the recovery of PGMs from secondary sources are still eco-unfriendly and expensive. In the present study, solvent extraction in combination with cheap, operationally easy and safe techniques involving microbial communities were employed for the recovery of Pd(II) from aqueous media. Accordingly, extraction of Pd(II) by *N,N'*-dimethyl-*N,N'*-dicyclohexylthiodiglycolamide (DMDCHTDGA) in toluene and subsequent stripping of Pd(II) with an acidic thiourea solution were performed, followed by bio-Pd recovery through the use of biogenic sulphide generated by a sulphate-reducing bacteria community. The results revealed that an excellent Pd(II) extraction performance of DMDCHTDGA from binary, tertiary and complex mixtures at 2, 4 and 6 M HCl feed solutions was observed. However, Fe(III) was co-extracted from 4 and 6 M HCl feed solutions, being completely removed with deionized water in the scrubbing stage. Regarding stripping, 94-99% of extracted Pd(II) were stripped using acidic thiourea solution. Concerning palladium bio-recovery, over 99% of stripped Pd(II) were recovered using biogenic sulphide obtained from a bioremediation process and Postgate B medium, in batch assay. Similarly, > 99 % palladium recovery was achieved by directly connecting Pd(II) solution with effluent from bioremediation process. Likewise, 78-99% of scrubbed Fe(III) were bio-recovered. Analysis of the precipitate collected during palladium bio-recovery testified that the precipitate was composed by Pd and S, and consistent with the synthesis of PdS nanoparticles. The elemental analysis of iron precipitate showed the presence of other metals besides Fe and S. Henceforth, the method engaged is environmentally sustainable, safe and cheap, thus attractive to be employed aiming the recovery of Pd or Fe from wastes materials.

Key words: Platinum group metals, solvent extraction, bio-recovery, biogenic sulphide, sulphate reducing bacteria, nanoparticles, bioremediation

Resumo

Os metais do grupo da platina (PGMs) são metais determinantes em aplicações *high-tech* atuais. No entanto, o seu abastecimento é limitado devido à sua escassez em recursos naturais e práticas de mineração dispendiosas. Anteriormente, várias técnicas propostas para a recuperação de PGMs de fontes secundárias são ainda pouco ecológicas e caras. No presente estudo, a extração por solventes em combinação com técnicas baratas, operacionalmente fáceis e seguras envolvendo comunidades microbianas foram empregues para a recuperação de Pd(II) a partir de meios aquosos. Consequentemente, realizou-se a extração de Pd(II) por *N,N'*-dimetil-*N,N'*-diciclo-hexiltiodiglicolamida (DMDCHTDGA) em tolueno e subsequente remoção de Pd(II) com uma solução ácida de tioureia, seguida de recuperação de bio-Pd através do uso de produtos metabólicos (S^{2-}) gerados por uma comunidade de bactérias que reduzem iões sulfato. Observou-se um bom desempenho de extração de Pd(II) pela DMDCHTDGA de misturas binárias, terciárias e complexas a partir de soluções de alimentação de 2, 4 e 6 M HCl. No entanto, o Fe(III) foi co-extraído das soluções 4 e 6 M HCl, sendo completamente removido com água desionizada na etapa de lavagem. Em relação à re-extração, 94-99% de Pd(II) extraído foi removido usando solução ácida de tioureia. No que diz respeito à recuperação biológica de paládio, foram recuperados mais de 99% de Pd(II) utilizando sulfato biologicamente gerado obtido a partir do processo de biorremediação e do meio de Postgate B, respetivamente, no ensaio em lote. Da mesma forma, > 99% de Pd(II) foi recuperado conectando diretamente a solução de Pd(II) com o efluente do processo de biorremediação. Além disso, 78-99% de Fe(III) foram também bio-recuperados. A análise do precipitado recolhido durante a bio-recuperação de Pd(II) mostrou que o precipitado era composto por Pd e S, e consistente com a síntese de nanopartículas de PdS. A análise elementar de precipitados de ferro mostrou a presença de outros metais além de Fe e S. O método agora desenvolvido é ambientalmente sustentável, seguro e barato, e atrativo para ser empregue na recuperação de Pd ou Fe de materiais esgotados ou em fim de vida útil.

Palavras-chave: metais do grupo da platina, extração por solventes, recuperação biológica, sulfato biogénico, bactérias redutoras de sulfato, nanopartículas, biorremediação

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List of Abbreviations

AMD	Acid mine drainage
ANOVA	Analysis of variance
C	Concentration
COD	Chemical oxygen demand
D	Distribution ratio
DMDBTDGA	*N,N'*-dimethyl-*N,N'*-dibutylthiodiglycolamide
DMDCHSA	*N,N'*-dimethyl-*N,N'*-dicyclohexylsuccinamide
DMDCHTDGA	*N,N'*-dimethyl-*N'N'*-dicyclohexylthiodiglycolamide
DMDCHTDMA	*N,N'*-dimethyl-*N,N'*-dicyclohexyltetradecylmalonamide
EDS	Energy dispersive X-ray spectroscopy
E_h	Redox potential
FAAS	Flame atomic absorption spectroscopy
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
MP-AES	Microwave plasma-atomic emission spectroscopy
OD	Optical density
PGMs	Platinum group metals
SRB	Sulphate reducing bacteria
TEM	Transmission electron microscopy
UV-Vis	Ultraviolet – visible spectroscopy
XRD	X-ray diffraction

1. INTRODUCTION

The platinum group metals (PGMs) - platinum, palladium, rhodium, osmium, ruthenium and iridium - are precious metals with economic importance. Among the PGMs, platinum, palladium and rhodium are the most valuable, with more extensive natural resources than the rest. The other PGMs are usually co-extracted with platinum, palladium and rhodium (Elver, 2011; Bernfeld, et al., 1985).

PGMs and their compounds have been increasingly used in current technologies and industries such as pharmaceutical, electronic, petroleum, and chemical, for various purposes (Table 1) (Kramer, et al., 2002; El-Hefny & Daoud, 2013; Cieszynska & Wisniewski, 2010). The wider applications of PGMs in various areas are due to their chemical and physical properties such as exceptional catalytic activity, outstanding resistance to corrosion, excellent mechanical strength, stability to oxidation at high temperature, inertness and excellent colour (Cayumil, et al., 2016; Marinho, et al., 2010). For instance, PGMs are employed as catalysts for reactions in industrial processes, as well as for reducing the emission of harmful gaseous pollutants to the environment, particularly as automotive emission control catalysts (Kramer, et al., 2002). Hence, PGMs can be called as '*Vitamin of modern industry'* and '*First and foremost high technology metal*' (Dong, et al., 2015).

Table 1: Application of PGMs in various areas.

Application	Platinum	Palladium	Rhodium
Electrical	❖ Electrical contacts and electrodes ❖ Hard disc drives	❖ Consumer electronics ❖ Ceramic capacitors ❖ Spark plugs (aircrafts) ❖ Electrical contacts	❖ Thermocouples ❖ Electrical contacts ❖ Spark plugs (aircrafts) ❖ Hard disc drives
Medical	❖ Chemo-therapy ❖ Micro-machine implants		❖ Menthol production
High performance alloys	❖ Glass making ❖ Resistance thermometers	❖ Surgical instruments	❖ Optical instruments ❖ Glass fibre production ❖ Alloying agents
Catalysts	❖ Auto catalysts ❖ Fuel cells ❖ Nitric acid production ❖ Silicon production ❖ Petroleum refining	❖ Auto catalysts ❖ Petroleum refining ❖ Hydrogenation ❖ Hydrogen gas purification	❖ Silicon rubbers ❖ Auto catalysts ❖ Acetic acid production ❖ Menthol production
Others	❖ Jewellery ❖ Investment bars	❖ Jewellery ❖ White gold production ❖ Carbon monoxide detection	❖ Jewellery

Source: (Elver, 2011; Kramer, et al., 2002; El-Hefny & Daoud, 2013; Cieszynska & Wisniewski, 2010; Dong, et al., 2015; Nguyen, et al., 2016; Barakat & Mahmoud, 2004)

1.1 Sources of PGMs

Primarily PGMs are supplied through mining from natural resources. The main suppliers of PGMs around the world are South Africa, Zimbabwe, Canada, Russia, United States of America and others. Among these countries, South Africa is the world top platinum producing country, and holds the largest known reserves of PGMs globally (Elver, 2011; Marinho, et al., 2010; Dong, et al., 2015; Wilburn, 2012), whereas Canada and Russia are the world's major palladium contributors (Bernfeld, et al., 1985).

PGMs are unevenly distributed in natural resources, and are among the least abundant of the Earth's elements. They occur in nature in close association to each other and with other elements such as nickel and copper (Elver, 2011; Bernfeld, et al., 1985; Assunção, et al., 2016a; Matthey, 2016). The PGMs found naturally are not currently matching the demand of the current high-techs and industries. Generally, they are extracted from ores (primary sources). The method of extraction is usually hydrometallurgical, involving the recovery of metals from acidic solutions by precipitation. However, the co-precipitation of transition metals with the PGMs is the main disadvantage of the method, in addition to environmental pollution (Lee, et al., 2010). Hence, separation of PGMs from the other co-precipitated metals is a difficult task. Furthermore, the chemical and physical properties of PGMs are very similar to each other, which makes the separation even more difficult (Nguyen, et al., 2016; Lee, et al., 2010; Raju, et al., 2012; Lee, et al., 2008). Therefore, it is important to discover best and most efficient ways to separate the PGMs from their mixtures and complex matrices (Cieszynska & Wisniewski, 2010; Dong, et al., 2015; Nguyen, et al., 2016).

The supply of platinum from primary sources has increased by 19%, to 6.076 million ounces (oz) in 2015, which is the highest level achieved in the last four years (Matthey, 2016). Likewise, palladium supply for the year 2016 showed a little rise in comparison to 2015. However, the supplies for both metals do not satisfy the gross demand by various sectors (Figure 1) (Matthey, 2016). The highest gross demand and insufficient supply of precious metals, in addition to high production costs and limited abundance, made the recovery of PGMs from secondary sources a viable and cost-effective alternative (Marinho, et al., 2010; Dong, et al., 2015). Environmentally sustainable secondary recovery of PGMs has a significant economic importance, as it minimizes the cost for purchasing new precious metals (Dong, et al., 2015; Raju, et al., 2012; Zhuang, et al., 2015). Moreover, it has also ecological importance, as it reduces contamination of environmental components such as soil and water by PGMs (Dong, et al., 2015; Cayumil, et al., 2016; Nikoloski & Ang, 2014). Furthermore, it also slows

the depletion of resources, requires less energy than production from ore, avoids mining waste, and slows the usage of valuable land for wasteland fills (Crundwell, et al., 2011).

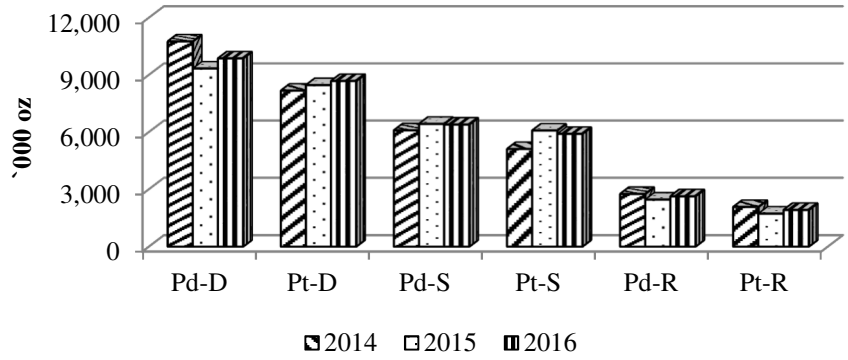


Figure 1: Platinum and palladium demand, supply and recycling (D-Demand, S-Supply and R-Recycling) (Matthey, 2016).

As a result of increase in the demand of PGMs, the traditional methods of recovery do not meet the demand by industries and various sectors (Pana, et al., 2013). The larger proportion of PGMs is still supplied from the primary sources, which needs high labour and costs for extraction and purification (Bernfeld, et al., 1985; Matthey, 2016). In addition to this, a progressive depletion of the PGMs resources made scholars to look for the recovery of PGMs from secondary sources or scraps. The wider application of PGMs contributed for the generation of a considerable amount of wastes. However, liquid and solid wastes, secondary sources, can be recycled and reutilized to meet the increasing demand (Zhuang, et al., 2015). Besides industrial wastes, PGMs can also be recovered from end-of-life products, as well as from by-products and residues created in the primary production (Bernfeld, et al., 1985; Zhuang, et al., 2015). The main secondary sources for PGMs include industrial wastes, domestic wastes, road dust, automobiles, electronics, medical wastes and so on (Elver, 2011; Zhuang, et al., 2015). The levels of precious metals in the secondary sources are greater than those available in the natural ores (Won, et al., 2014).

1.2 Methods for recovery of PGMs

The increasing concerns of scholars are to protect the environment from hazardous chemical substances. Such hazardous substances are emitted to the environment from various anthropogenic sources, such as industry, domestic, mining and others alike (Macaskie, et al., 2010). Environmental legislation supports the protection of environment through controlling the emission of substances, as well as treatments of wastes. The issue of pollution can be

minimized by waste treatment in addition to recycling, recovery, reuse and controlling the amount of pollutants being discharged to the environment (Kislik, 2012).

PGMs are not consumed in their application in various areas of high-techs, but transfer from one manifestation to another (Hageluken, 2012). Thus, they can be recovered using an appropriate technique. The recovery of PGMs has both ecological and economic importance, which perhaps are linked with their depletion in the Earth crust and also with environmentally harmful mining practices (Bunge, et al., 2010). Hence, the traditional methods of mining have to be replaced with more economic and environmentally sustainable techniques, besides the efficient and effective recovery of precious metals from secondary sources (Dong, et al., 2015; Zhuang, et al., 2015).

Spent catalysts are considered as hazardous wastes, possibly contributing for the production of toxic gases (Marinho, et al., 2010). Hence, the recycling of spent catalysts (PGMs) plays an important role in lowering environmental pollution, as well as contributing for PGMs supply in a significant part. Many chemical techniques such as precipitation, ion exchange, solvent extraction, membrane separation and biological techniques (which involve living organisms and their dead cells) can be used for the treatment of wastes (Zhuang, et al., 2015; Macaskie, et al., 2010; Yong, et al., 2002). However, the choice of method depends on a number of factors such as the composition and quality of the waste, complexity of the technique and operation, disposal expertise and, of course, overall economics (Kislik, 2012).

1.2.1 Chemical techniques

PGMs scarcity is related with their low natural abundance and the complexity of their extraction and refining processes (Bernardis, et al., 2005). Hence, the recovery of PGMs from waste materials is a more and more significant research task, and it is also a challenge due to the diversity of matrices involved in waste, which interfere in the process (Dong, et al., 2015; Raju, et al., 2012). However, knowing the increasing demand for these precious metals, it is important to look for the efficient ways to recover these metals from secondary sources (Nguyen, et al., 2016). With this regard, and when the hydrometallurgical option is considered, various methods such as ion exchange (Nikoloski & Ang, 2014), solvent extraction (Paiva, et al., 2015b; Paiva, et al., 2014b), precipitation (Lee, et al., 2010; Schreier & Edtmaier, 2003) were revealed by scholars for the extraction of PGMs. Among these methods, solvent extraction has got a wider application, as it shows high extraction efficiencies (Nguyen, et al., 2016; Bernardis, et al., 2005). In liquid-liquid (or solvent) extraction the separation is based on

the distribution ratios, varying from element to element, which can be modified by chemical methods to achieve favourable conditions for the efficient recovery of PGMs (Elver, 2011). It involves two immiscible phases, allowing the capturing of metal ions from one phase to the other (Assunção, et al., 2016a). However, as a result of formation of many chemical species in the extraction media, the separation and purification of PGMs is not an easy task. Furthermore, the most challenging job is to find environmentally friendly and safe solvents taking the environmental and human points of view into account, and also finding solvents with high selectivity and extraction ability towards PGMs in the presence of complex matrices.

Literature survey publicized that the extraction and stripping of PGMs from chloride media, particularly palladium and platinum, were extensively investigated by researchers. Recently, compounds such as *N,N'*-tetrasubstituted succinamides (Costa, et al., 2016), *N,N'*-dimethyl-*N,N'*-dicyclohexyltetradecylmalonamide (DMDCHTDMA) (Assunção, et al., 2016a), *N,N'*-dimethyl-*N,N'*-dicyclohexylsuccinamide (DMDCHSA) (Assunção, et al., 2016a), *N,N'*-dimethyl-*N,N'*-dibutylthiodiglycolamide (DMDBTDGA) (Paiva, et al., 2015b) and *N,N'*-dimethyl-*N'N'*-dicyclohexylthiodiglycolamide (DMDCHTDGA) (Paiva, et al., 2014b) were revealed for the extraction of PGMs from aqueous chloride media (Figure 2).

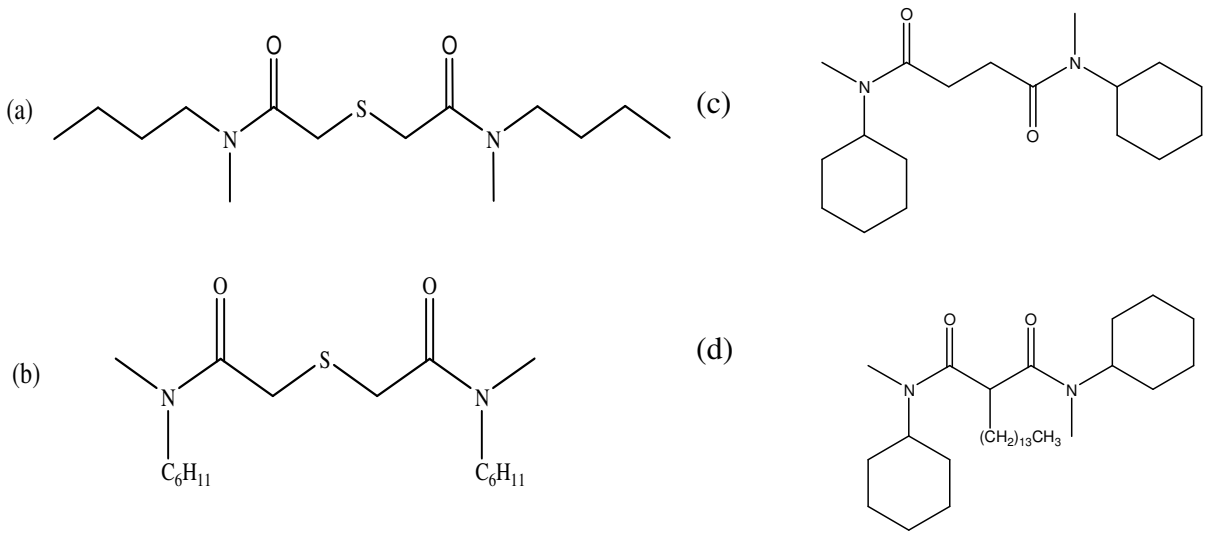


Figure 2: Structure of organic extractants. (a) *N,N'*-dimethyl-*N,N'*-dibutylthiodiglycolamide (DMDBTDGA), (b) *N,N'*-dimethyl-*N,N'*-dicyclohexylthiodiglycolamide (DMDCHTDGA), (c) *N,N'*-dimethyl-*N,N'*-dicyclohexylsuccinamide (DMDCHSA) and (d) *N,N'*-dimethyl-*N,N'*-dicyclohexyltetradecylmalonamide (DMDCHTDMA).

DMDBTDGA in toluene (Paiva, et al., 2015b; Ortet & Paiva, 2015) and DMDCHTDGA in 1,2-dichloroethane (Paiva, et al., 2014b) were investigated for the extraction performance towards Pt(IV) and Pd(II) from aqueous HCl media. Accordingly, DMDBTDGA in toluene

showed appreciable capacity to extract Pd(II) over Pt(IV) and Rh(III) (Paiva, et al., 2015b). Similarly, DMDCHTDGA in 1,2-dichloroethane exhibited an excellent extraction performance for Pd(II) regardless of HCl concentration. Pt(IV) extraction using DMDCHTDGA in 1,2-dichloroethane showed that the extraction performance increases with increasing HCl concentration (Paiva, et al., 2014b).

1.2.2 Biological techniques

Besides the traditional cyanidation, amalgamation, zinc cementation and precipitation processes, a number of methods (hydro- and pyro- metallurgical) are used for the recovery of PGMs (Nikoloski & Ang, 2014; Shen & Xue, 2007). However, these methods have general disadvantages: they are not cost effective, exhibit poor selectivity (Nikoloski & Ang, 2014) and release secondary waste to the environment (Dong, et al., 2015; Pat-Espadas, et al., 2016; Corte, et al., 2012). Moreover, the methods need substantial investment, labour and time (Pat-Espadas, et al., 2016). In consequence, solvent extraction techniques with the combination of an eco-friendly and cost-effective approach, a biological method, have been recently introduced (Ju, et al., 2016). This approach involves the interaction between PGMs bearing aqueous solutions and the metabolic products from bacterial communities, used to remove or recover the metals (Assunção, et al., 2016a).

Microbial mediated bio-recovery of precious metals involves the reduction or precipitation of metal ions through binding on highly active surface bacterial cells and interaction with their metabolic products (Windt, et al., 2005; Assunção, et al., 2016b; Assunção, et al., 2016a). These bio-recovery methods using bacterial communities and their metabolic products exhibit economic benefits (Ju, et al., 2016; Windt, et al., 2005), and are environmentally friendly (Konishi, et al., 2007), as they can be carried out at ambient temperature and at about neutral pH, with minimal consumption of energy and being environmentally safe (Konishi, et al., 2007). Precious metals such as palladium, platinum, rhodium, silver and gold have been recovered through biological reduction to insoluble metals or through precipitation from their aqueous solutions (Konishi, et al., 2007; Assunção, et al., 2016a). This is a promising alternative technique for the recovery of precious metals over the chemical methods (Corte, et al., 2012). Studies indicated that the biological methods are efficient and effective for the recycling/removal of PGMs from aqueous media (Assunção, et al., 2016a; Zhuang, et al., 2015; Won, et al., 2014; Bunge, et al., 2010). Furthermore, bio-reductively recovered PGMs such as

Pd showed a better catalytic activity than those chemically synthesized for the catalysis of a number of reactions (Bunge, et al., 2010).

Recently, another work revealed that combining liquid-liquid extraction with a bacterial community showed potential for the recovery/removal of PGMs from aqueous media (Assunção, et al., 2016a). Researchers are diverting to a biological facilitated reduction and recovery of PGMs, as it is an operation with less aggressive conditions, economic, easy handling, applicable for low metal concentrations and avoiding the production of toxic by-products (Assunção, et al., 2016a; Macaskie, et al., 2010; Pat-Espadas, et al., 2016). In this regard, the bacterial strains and communities such as *Desulfovibrio desulfuricans* (Yong, et al., 2002 ; Macaskie, et al., 2010), *Shewanella oneidensis* (Windt, et al., 2005), *Shewanella algae* (Konishi, et al., 2007), *Cyanobacteria* (Brayner, et al., 2007), *Cupriavidus necator* (Bunge, et al., 2010), *Pseudomonas putida* (Bunge, et al., 2010), *Paracoccus denitrificans* (Bunge, et al., 2010) and *Desulfovibrio sp*. (Rashamuse & Whiteley, 2007) were used for the bio-recovery of PGMs.

1.3 Biogenic sulphide for the recovery of metals

Several conventional techniques such as precipitation, ion-exchange, and absorption have been used for the recovery and removal of metals from aqueous solutions. Due to its ease and fast operation, metal ion recovery through precipitation was widely employed. For effective precipitation of metals, sulphide is of the most preferred ion species, as it can form insoluble metal sulphides regardless of the composition of the aqueous solutions. However, chemically synthesized sulphide salts are impractical to use from toxicological and environmental points of view (Azabou, et al., 2007; Cao, et al., 2009; Lewis, 2010). Thus, immobilization of metal ions in the form of insoluble metal sulphides through microbial mediated sulphur compounds reduction and precipitation is one of the most widely studied alternative biological techniques over physical-chemical methods. Ordinarily, in waste water treatment plant (WWTP), sulphate reducing bacteria (SRB) communities are responsible for the generation of H_2S which causes huge problems due to its toxic and volatile nature (Costa, et al., 2012; Costa, et al., 2013; Webb, et al., 1998). Consequently, its removal is a demanding issue needing to be considered as a result of excessive generation during bioremediation processes. However, this biologically generated sulphide plays a significant role for the removal/recovery of metals from aqueous solutions, even at smaller concentrations, in the form of insoluble metal sulphide (Costa, et al., 2012; Azabou, et al., 2007). Moreover, the invention of metal sulphide nanoparticles using

biologic sulphide has immense relevance from environmental points of view (Webb, et al., 1998). Thus, SRB has an ecologic, environmental and economic importance (Jain, 1995).

Mining, mineral and metallurgical industries have shown tremendous growth all over the world for the provision of metals and mineral commodities. Hence, generating wastes causes huge environmental concerns, as they contain various hazardous metals. The biogenic sulphides can be engaged for the removal/recovery of those metals from the metal rich effluents, such as metallurgical, industrial, domestic as well as acid mine drainage (Costa, et al., 2013). The use of biogenic sulphide for the recovery or removal of metal ions resolves the issue related with disposal, conversely by synthesising high value materials. Obligate anaerobes, SRB play a significant role for the generation of sulphide through conversion of sulphate to sulphide, with simultaneous oxidation of organic substrates (Postgate, 1984). Equation (1) and (2) represents the general reaction for SRB, which employs simple organic compounds as electron donors and sulphate as the external electron acceptor, with subsequent generation of sulphide and formation of insoluble metal sulphide precipitates (Postgate, 1984; Webb, et al., 1998; Cao, et al., 2009; Jong & Parry, 2003; Rashamuse & Whiteley, 2007).

$$\text{Organic Matter (C, H, O)} + SO_4^{2-} \xrightarrow{\text{SRB}} HS^- + HCO_3^- \quad (1)$$

$$M^{2+} + HS^- \rightarrow MS + H^+ \quad (M^{2+} \text{ represents divalent metal ions}) \quad (2)$$

Precipitation of metal ions using biogenic sulphides is the most widely used technique for the removal or recovery of metal ions from waste materials. It is serving as the best alternative for the recovery of metal ions from aqueous solutions. It has the advantage of potential selectivity, fast reaction rate, better settling property and reuse of sulphide precipitates (Bhagat, et al., 2004). However, the difficulty in dosing of sulphide, associated with its volatility, makes the technique to be less practical (Lewis, 2010).

1.4 Environmental sustainability and metal bio-recovery

A number of techniques such as high gravity, microwave irradiation, solventless thermolysis, chemical micelle, chemical vapour deposition, electrodeposition, etc., has been employed for the synthesis of metal, metal oxide and metal sulphide nanoparticle (Xin, et al., 2008; Costa, et al., 2012; Costa, et al., 2013; Jose & Jagirdar, 2010). However, the above mentioned physical-chemical techniques are eco-unfriendly and expensive, as some of them involve high temperature-pressure operational conditions, complicated operation, toxic substances and require expensive raw materials (Costa, et al., 2013; Xin, et al., 2008). In this regard, the

increasing pressure around environmental sustainability of a nanometal synthesis (recovery) process stimulates researchers to develop new techniques, which should be greener, eco-friendly and guarantee the use of nontoxic or less toxic substances. Henceforth, the researchers turned their face to biological techniques (Makarov, et al., 2014). Accordingly, biological techniques are getting wider application in metallurgy as they are environmentally sustainable and eco-friendly methods for the recovery or synthesis of metals based nanoparticles from metal bearing solutions (Xin, et al., 2008).

Generating sulphides biologically from waste materials and employing them for the recovery or synthesis of nanosized particles are well documented (Hulkoti & Taranath, 2014; Costa, et al., 2012; Costa, et al., 2013; Assunção, et al., 2016a; Assunção, et al., 2016b). The sulphides generated in WWTP by SRB are used as precipitating or reducing agents, especially for treatment of metal contaminated water. Therefore, applying these biologically generated sulphides for the synthesis of metal nanosized particles has environmental and economic advantages, as the sulphide is naturally generated by SRB from wastes in the environment (Xin, et al., 2008; Costa, et al., 2013). This process is simple and cheap because it uses waste as raw materials for the generation of sulphide.

1.5 Determination of the amount of metal ions extracted and recovered

The determination of the concentrations of metal ions was carried out using flame atomic absorption spectroscopy (FAAS), microwave plasma-atomic emission spectroscopy (MP-AES) and/or inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The contents of metal ions in organic phases after equilibration were determined by mass balance. The metal ions extraction efficiency (percentage of extraction) to the organic phases and the percentage recovered by stripping reagents were determined using the mathematical equations (3) to (7).

- The concentration (C) of metal ions extracted by organic phase

$$= C_{\text{Metal in initial aqueous phase}} - C_{\text{Metal left in aqueous phase after extraction}} \quad (3)$$

- Percentage of metal ions extracted by organic phase

$$= \frac{C_{\text{Metal extracted by organic phase}}}{C_{\text{Metal in initial aqueous phase}}} \times 100 \quad (4)$$

- Distribution ratio (D) $= \dfrac{C_{\text{Metal extracted by organic phase}}}{C_{\text{Metal in aqueous phase after extraction}}}$ (5)

❖ Percentage of stripping $= \frac{C_{\text{Metal stripped}}}{C_{\text{Metal in organic phase}}} \times 100$ (6)

❖ Percentage of recovery $= \frac{C_{\text{Metal bio-recovered}}}{C_{\text{Metal before bio-recovery}}} \times 100$ (7)

1.6 Scope of the present study

The present study employed solvent extraction techniques in combination with the use of bacterial communities envisaging the extraction and recovery of palladium and platinum from secondary sources. The extraction of PGMs from single, binary, tertiary and complex metal solutions was carried out using DMDCHTDGA or DMDBTDGA in toluene as organic phases. Various reagents were evaluated for their selectivity and efficiency to strip PGMs from loaded organic media. The metabolic product containing biogenic sulphide generated biologically was employed for the recovery of metal ions from the solutions resulting from the scrubbing and stripping stages of the solvent extraction process. The recovered metals in the form of precipitates were characterized using suitable analytical tools.

1.7 Significance of the study

There are a number of methods that have been employed for the recovery of PGMs from the postconsumer scraps (secondary sources), to increase their supply. But still the inflation of gross demands for PGMs as the result of their increasing utilization in current technologies is unsatisfied. On the other hand, the methods for the recycling of PGMs have to be environmentally friendly and cost effective, besides the efficient and selective recovery of PGMs. This study contributes for finding environmentally friendly and cost-effective ways to recover PGMs from secondary sources. It may provide alternative ways for recovery of PGMs not only from scraps but also from ores, for those who are engaged in these activities.

1.8 Objective of the study

In this study, chemical and biological technologies were combined and used for the extraction, separation and recovery of PGMs from aqueous media. Hence, Pt(IV) and Pd(II) were extracted by DMDCHTDGA and DMDBTDGA in toluene from aqueous HCl media. Then, after stripping of metal ions with adequate reagents, the final aqueous phases were exposed to the metabolic products (MPs) from the Postgate B nutrient growth medium and effluent from the bioreactor for the bioremediation of acid mine drainage (AMD) aiming the bio-recovery of Pt(IV) and Pd(II). Thus, the objective of the present study was (i) to investigate the extraction potential of DMDCHTDGA and DMDBTDGA toward Pd(II) and Pt(IV) contained in acidic

aqueous feed media, (ii) to assess promising stripping agents able to remove the metal ions from the loaded organic phases, (iii) to investigate the bio-recovery of PGMs employing biogenic sulphides from both Postgate B nutrient growth medium and effluent from a bioreactor of a bioremediation process for AMD treatment and (iv) characterize the collected precipitates to determine their size, morphology, nature and elemental composition.

2. EXPERIMENTAL PART

2.1 Reagents and equipments

For preparation of the metal aqueous feed solutions in HCl media, atomic absorption spectroscopy standards of platinum (1001 mg/L ± 4 mg/L, Fluka) and palladium (1003 mg/L ± 4 mg/L, Fluka) in 5 % HCl solution, and chloride salts of Fe(III) (96 %, British Drug Houses), Cu(II) (97 %, Aldrich Chemical Company), Zn(II) (99.5 %, E. Merck, Darmstadt), Ni(II) (98 %, Anala R®, BDH Limited Poole) and La(III) (99 %, Anala R®, BDH Limited Poole) were used. Likewise, stripping reagents were prepared from HCl (37%, Analytical reagent grade, Fisher Scientific), HNO_3 (65%, Merck Kommanditgesellschaft auf Aktien), H_2SO_4 (96%, Pronalab), NH_4Cl (Sigma-Aldrich), NH_3 (25%, Sigma-Aldrich), KSCN (Sigma-Aldrich), $Na_2S_2O_3$ (Panreac Quimica SAU), Na_2SO_3 (Merck), NaCl (Panreac) and thiourea (Sigma-Aldrich). Deionized and seawater were also used to strip the metal ions. The extractant organic phases were prepared from previously synthesized organic compounds, DMDCHTDGA (Paiva, et al., 2015b) and DMDBTDGA (Paiva, et al., 2014b), dissolved in toluene (ABSOLVE, José Manuel Gomes dos Santos, Lda.). For the preparation of Postgate B nutrient medium KH_2PO_4 (AnalaR NORMAPUR), NH_4Cl (Panreac), $FeSO_4.7H_2O$ (Panreac), $MgSO_4.7H_2O$ (Panreac), $CaSO_4.2H_2O$ (Panreac), Yeast extract (HIMEDIA), Ascorbic acid (VWR Chemicals), rezazurin ($C_{12}H_7NO_4$), thioglycolic acid (VWR Chemicals) and paraffin oil (Vencilab) were used.

A magnetic stirrer (AGIMATIC-N) was employed for the extraction and stripping experiments. A flame atomic absorption spectrophotometer (FAAS, novAA 350, Analytik Jena, Jena, Germany), microwave plasma-atomic emission spectroscopy (MP-AES, Agilent Technologies, 4200) and an inductively coupled plasma-atomic emission spectroscopy apparatus (ICP-AES, Horiba Jobin-Yvon, Ultima) were used to determine the metal ion concentrations in the aqueous phases before and after extraction, as well as in the stripping solutions. Ultraviolet-Visible spectroscopy (UV-Vis, DR 2800, Hach-Lange) were employed for the sulphide, sulphate and optical density determinations. Glass pH electrode (VWR, SJ 223) and a Pt electrode coupled with a reference saturated calomel electrode (SCE, CRISON, Codigo 5261) were used for pH and redox potential measurements, respectively. A centrifuge (Hettich ROTOFIX 32A) was used for separation of the precipitates from the supernatant. A sterile syringe filter (VWR) was used for filtering metabolic products. X-ray Diffraction (XRD, PANalytical X`Pert Pro powder diffractometer) and transmission electron microscopy (TEM,

Hitachi model H8100 with a LaB6 filament) coupled with energy dispersive X-ray spectroscopy (EDS or EDX, ThermoNoran) were used for precipitate characterization.

2.2 Solvent extraction of metals

2.2.1 Preparation of solutions

Aqueous feed solutions containing about 100 mg/L Pt(IV) and 100 mg/L Pd(II) were prepared in separate volumetric flasks**.** Binary feed solutions of Pd(II)-Fe(III) and Pd(II)-Cu(II) containing 100 mg/L of each metal ion were also prepared, separately. Likewise, tertiary feed solutions containing concentrations (mg/L) of 100 Pd(II), 500 Fe(III) and 500 Cu(II) were prepared as well. Other feed solutions containing six metal ions with concentrations (mg/L) of 100 Pd(II) and La(III), and 500 Fe(III), Cu(II), Zn(II) and Ni(II) were also arranged. All feed aqueous solutions were prepared in HCl media. Organic solutions of 0.02 M DMDCHTDGA and 0.02 M DMDBTDGA in toluene were prepared, separately. Regarding the stripping reagents, aqueous solutions of 0.1 M thiourea in 1 M HCl, 1 M HCl, 1 M HNO_3, 1 M H_2SO_4, 0.05 M NaCl, 8.2 mM $Na_2S_2O_3$, 8.2 mM KSCN, 8.2 mM Na_2SO_3, and a mixture of 1 M NH_3 and 0.1 M NH_4Cl were prepared in distinct flasks. Deionized and seawater were also evaluated for the stripping of the metal ions.

2.2.2 Extraction of the metal ions

The extraction of the metal ion(s) from each feed aqueous phase(s) was performed by mixing equal volumes of the organic phase (DMDCHTDGA or DMDBTDGA in toluene) with the feed aqueous solution. The mixtures were stirred with a magnetic stirrer, with a rotation speed of about 1000 rpm for 15 minutes at room temperature (Paiva, et al., 2014b; Paiva, et al., 2015b). Then, after transferring each mixture into a separatory funnel, the raffinate was filtered to ensure no entrance of solid particles and mutual entrainments. Both organic and aqueous phases were collected in sample flasks for stripping of metal ions from loaded organic phases, and for determination of the metal ion(s) contents in the aqueous phase using FAAS, ICP-AES or MP-AES, respectively.

2.2.3 Stripping of the metal ion(s)

For stripping of the metal ion(s) from the organic phases, equal volumes of metal ion(s) loaded organic solutions (DMDCHTDGA or DMDBTDGA) and stripping agents were mixed in 50 mL Erlenmeyer flasks. The mixtures were stirred for 30 minutes, with a rotation speed of 1000 rpm at room temperature (Paiva, et al., 2015b; Ortet & Paiva, 2015). The aqueous and organic

phases were separated. The aqueous phase was filtered and the concentrations of metal ion(s) were determined using FAAS, ICP-AES or MP-AES.

2.3 Biological recovery of metals

For the bio-recovery of palladium and iron, solutions resulting from the stripping and scrubbing of loaded organic phase previously equilibrated with feed solution containing Pd(II), Fe(III), Ni(II), Zn(II), La(III) and Cu(II), were used. The biologically generated sulphides by SRB were engaged for the bio-recovery of metals.

2.3.1 Microbial growth and re-inoculation

A mixed culture containing SRB, obtained from sludge of the WWTP of Montenegro, located near the city of Faro, Algarve, South Portugal, was employed in this study. The enrichment culture medium used was Postgate B nutrient medium (Postgate, 1984) with the following chemical composition (in g/L): 0.5 KH_2PO_4, 1 NH_4Cl, 0.5 $FeSO_4.7H_2O$, 2 $MgSO_4.7H_2O$, 1 $CaSO_4.2H_2O$, 3.5 sodium lactate, 0.1 ascorbic acid, 1 yeast extract, 0.1 thioglycollic acid, and 0.01 rezazurin ($C_{12}H_7NO_4$) as redox indicator. The compounds were mixed (dissolved) in a glass culture bottle to a final volume of 400 mL with tap water at room temperature. The pH of the medium was adjusted to 7.03 ± 0.03 using NaOH solution. Then, the nutrient medium was sterilized by autoclaving. In the beginning of the experiment the Postgate's B medium was aerobic and thus, presented a pink colour due to the presence of oxidized resazurin.

After autoclaving, 10% (v/v) sludge from the WWTP was added to the medium followed by the addition of 10 mL paraffin oil. The bottle was sealed with butyl rubber stoppers and aluminium seals, followed by incubation at room temperature. The culture was sub-cultured using 10% (v/v) of SRB inoculum. Re-inoculation of the bacteria was performed by adopting the same Postgate B medium. From the previous enrichment, the bacteria were harvested and washed with the new growth medium. Then, the collected bacteria were transferred to a new medium for re-inoculation. The pH of the growth medium was neutralized. The nutrient growth medium was kept at room temperature to allow the growth of bacteria. Growth parameters, namely, the pH, redox potential (E_h), optical density (OD at 600 nm), sulphide and sulphate ion (SO_4^{2-}) concentrations were measured weekly (Jain, 1995; Costa, et al., 2012).

2.3.1.1 Recovery of metals using biogenic sulphide from Postgate B medium

Recovery of palladium from the aqueous solution resulting in the stripping stage of solvent extraction was performed using biogenic sulphide generated in batch by the SRB community

in Postgate B medium (experiment A – Figure 3). This stage of experiment was performed after ensuring the achievement of sufficient amount of sulphide in the nutrient medium to guarantee an eventual complete precipitation of Pd(II) as PdS. Henceforth, the biogenic sulphides were used for Pd(II) precipitation (experiment A1). Pd(II) solution was taken in a sample bottle tapped with butyl rubber and aluminium seal, followed by addition of biogenic sulphide solution to achieve sulphide to Pd(II) molar ratio of about 2:1. For the introduction of the biogenic sulphide solution, a sterile syringe coupled with 0.2 µm filter was used to ensure no entrance of contaminant (solid particles and bacteria) to the reaction bottle. The operation was performed slowly drop by drop, using a gas tight syringe fitted with a hypodermic needle under continuous stirring of the metal solution with a magnetic stirrer (Figure 3). Then, the reaction bottle was stored at room temperature for Pd(II) to be precipitated. Samples were collected frequently to determine the progress of palladium recovery. The precipitates were separated from supernatant for further analysis. Each step was repeated for the recovery of iron (experiment A2) from the solution resulting in the scrubbing stage of solvent extraction, except sulphide to Fe(III) ratio which was changed to 2.5:1. The experiments were conducted in triplicates and carried out under sterile conditions.

Experiment A – Batch assay using biogenic sulphide from Postgate B medium after 28 days of incubation

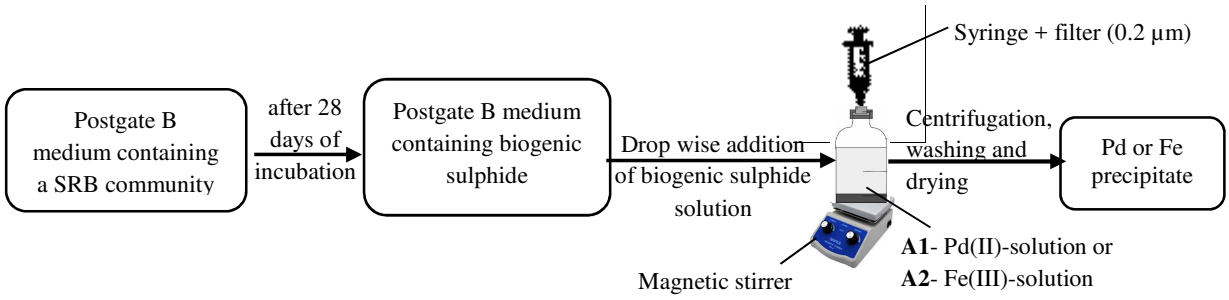


Figure 3: Schematic diagram of the batch assays for the recovery of palladium (experiment A1) or iron (experiment A2) from aqueous solutions resulting from stripping and scrubbing stage, respectively, of solvent extraction, using biogenic sulphide from Postgate B medium after 28 days of incubation.

2.3.2 Recovery of metals using an effluent from a SRB bioremediation process

The effluent of a laboratory scale bioremediation process for AMD treatment, based on the use of SRB, was employed for the recovery of palladium or iron from aqueous solutions. The bioremediation system was previously set-up as indicated in Figure 4. The bioremediation system has two basic components: a neutralization tank and an up-flow anaerobic packed bed bioreactor containing the SRB inoculum and coarse sand as a support matrix (Figure 4). The SRB present in the bioreactor were feed with winery waste as carbon and electron source

(Quinta do Barranco Longo, Lda., located in Algoz, Portugal) and AMD (Mina de São Domingos, Portugal), as a sulphate source, in the ratio that resulted in the best sulphate removal efficiency (Vitor, et al., 2015). Through this way, the system converts sulphate to sulphide by employing SRB communities. Thus, the recovery of palladium or iron were carried out in batch and in continuous using the effluent containing biogenic sulphide from the bioremediation system.

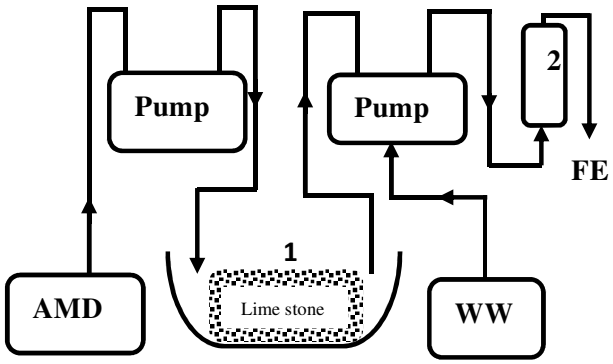


Figure 4: Schematic representation of the bioremediation process for AMD treatment in continuous flow (AMD – Acid Mine Drainage; WW – Winery Waste; FE – Final Effluent; 1 - Neutralization Tank; 2 – Up-flow Anaerobic Packed Bed bioreactor).

2.3.2.1 Batch method

The recovery of palladium or iron was performed using the effluent collected from the bioremediation process described above using a procedure similar to that one described in Figure 3. The parameters of the effluent such as sulphide, sulphate, pH, redox potential and optical density were measured to characterize it and to assure the use of a volume of effluent containing sufficient amount of biogenic sulphide for complete metal precipitation. Figure 5 displays the schematic flow diagram used for recovery of palladium (experiment B1) or iron (experiment B2) in batch using the effluent collected from the AMD bioremediation process.

Experiment B – Batch assay using effluent from the bioremediation process for AMD treatment

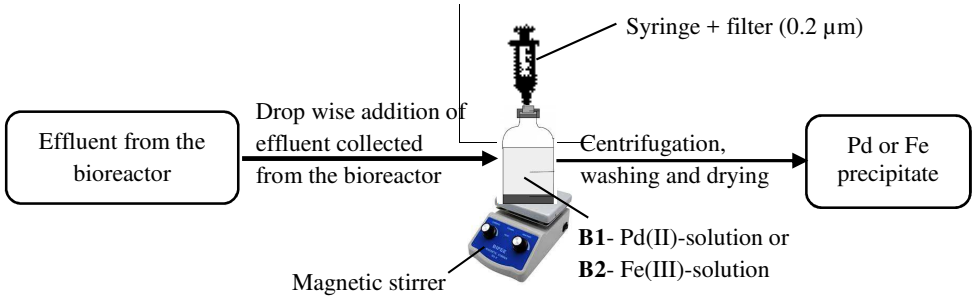


Figure 5: Schematic diagram of the batch assay for the recovery of palladium (experiment B1) or iron (experiment B2) from aqueous solutions resulting from the stripping or scrubbing stages, respectively, of solvent extraction, using effluent collected from the bioremediation process for AMD treatment.

2.3.2.2 Continuous – flow method

A continuous flow assay was also employed aiming the recovery of palladium or iron from aqueous solutions resulting from the stripping and scrubbing stage, respectively, of the solvent extraction process (experiment C– Figure 6). Accordingly, the effluent from the bioremediation process for AMD treatment introduced directly to the bottle containing 50 mL Pd(II) solution (experiment C1). To prevent possible contamination of the synthesised precipitate by bacteria and/or other solid particles, a 0.2 µm filter was attached at the effluent entrance to the bottle. The concentration of sulphide and the flow rate of the effluent with and without filter were pre-determined. While introducing the effluent, in a continuous mode, Pd(II) solution in the bottle was under continuous stirring using a magnetic stirrer for the entire period. Periodically samples were taken to monitor the progress of palladium recovery. A similar continuous procedure to that one employed for palladium was followed for the bio-recovery of iron from 10 mL Fe(III) solution resulting from the scrubbing stage of solvent extraction (experiment C2) solution was used. It was impossible to perform these experiments in triplicate at the same time due to the fact that the bioreactor has only one effluent exit. However, the analysis to monitor the recovery of metal was performed in triplicate by withdrawing three samples from the reaction bottle.

Experiment C – Continuous flow assay using effluent from the bioremediation process for AMD treatment

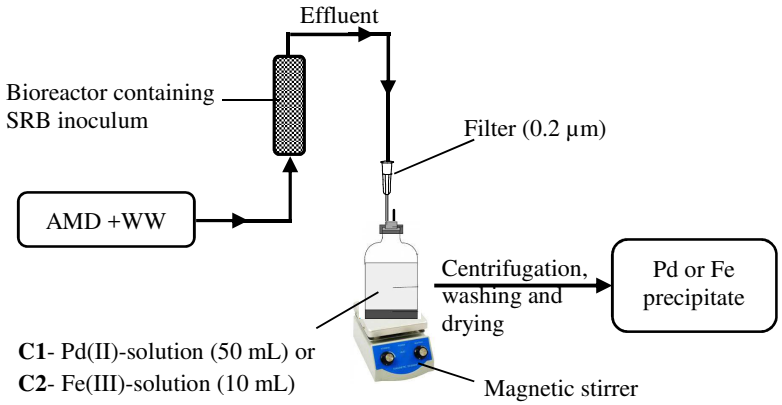


Figure 6: Schematic diagram of the continuous assays for the recovery of palladium (experiment C1) or iron (experiment C2) from aqueous solutions resulting from the stripping and scrubbing stage, respectively, of solvent extraction, using effluent from the bioremediation process for AMD treatment (AMD – Acid Mine drainage, WW – Winery Waste).

2.4 Analytical methods and precipitate characterization

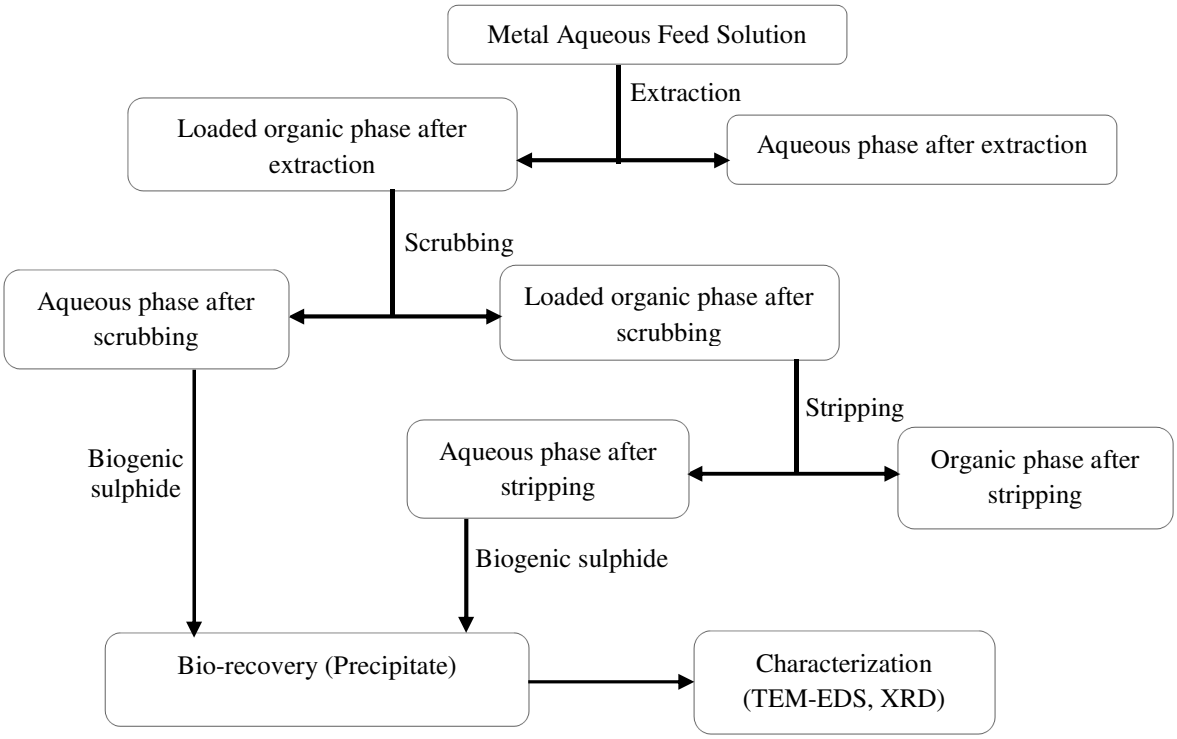
A series of standard solutions were used to calibrate the analytical instruments. Blanks were analysed to check for the presence of background absorption and spectral interferences from the blank matrices. The determination of metal ion contents in each aqueous phase, before and after extraction, and also after stripping and bio-precipitation, was carried out by FAAS, ICP-

AES or MP-AES. FAAS was only used for the analysis of single metal ion solutions, whereas ICP-AES or MP-AES were used for the analysis of solutions containing more than one metal ion.

Periodically, samples were collected from the culture medium using sterile syringe and needle via the top of the serum bottles. The sulphide concentration was measured immediately after sampling by the methyl-blue method (665 nm, Hach-Lange) using molecular UV-Vis spectrophotometry. Employing the same UV-Vis Spectroscopy, sulphate concentration was determined by the sulfaVer4 method (450 nm, Hach-Lange) and optical density at 600 nm (OD_{600}, Hach Lange) was measured. Glass pH electrode and a Pt electrode coupled with a reference saturated calomel electrode were used to measure the pH and redox potential, respectively.

The precipitate formed was separated from the supernatant solution by centrifuging at the rotation speed of 4000 rpm for 15 minutes. The pellets were washed with deionized water followed by 70% ethanol (stirred and centrifuged 4000 rpm, 15 min). Then, the precipitate was dried under vacuum (desiccator connected to a vacuum pump) to a constant weight. For the determination of the morphology, size and elemental composition of the precipitate particles XRD and TEM, coupled with EDS, were used.

General flow diagram of extraction, scrubbing, stripping and bio-recovery of metals.



2.5 Statistical analysis

Sample analysis was made in triplicate and reported at 95% confidence interval, except for those analysis results conducted to screen the extraction and stripping efficiency of reagents (single, binary and tertiary metal solutions). Statistically significant difference between the results obtained in extraction, stripping and recovery techniques were evaluated employing analysis of variance (ANOVA) and pair-wise mean comparison using student t-test (Microsoft excel 2017). Differences were considered significant at the 95% confidence level.

3. RESULTS AND DISCUSSION

In this study, solvent extraction involving DMDCHTDGA or DMDBTDGA in toluene as extractant was carried out to selectively extract the target metal ions from feed aqueous solutions. Accordingly, equal volumes of organic and aqueous phases containing the chosen metal ions were put in contact for 15 minutes. After equilibration, a clear and almost immediate separation of phases was observed. Moreover, there were no observed problems with emulsion, precipitation or suspension of solid particles for metal ion(s) extractions with both organic phases. The extracted metal ions in the loaded organic phases were subsequently subject to scrubbing and stripping by employing appropriate reagents. For the recovery of metal ions from the solutions resulting from the scrubbing and stripping stages, biogenic sulphide from Postgate B nutrient medium and an effluent from a bioremediation process for AMD treatment were engaged. In both cases sulphide generated by SRB was responsible for the recovery of metals. The overall obtained results are discussed in the upcoming sections.

3.1 Solvent extraction

3.1.1 Single metal ion solution

3.1.1.1 Extraction and stripping of Pd(II) and Pt(IV)

After extraction of Pt(IV) and Pd(II) from their respective aqueous feed (HCl solutions), the percentages of extraction were calculated to establish the performance of the organic phases (DMDCHTDGA and DMDBTDGA in toluene) towards Pt(IV) and Pd(II). The results for the percentage of Pt(IV) and Pd(II) extraction by organic phases, and their distribution ratios, are presented in Figures 7 and 8, respectively.

In this study, Pt(IV) and Pd(II) extraction efficiencies of DMDBTDGA and DMDCHTDGA were verified. Accordingly, DMDCHTDGA shows better Pt(IV) and Pd(II) extraction efficiency than DMDBTDGA at all HCl concentrations tested except for 2 M HCl, in which DMDBTDGA extracted more Pt(IV) than DMDCHTDGA. Pt(IV) extraction by DMDCHTDGA is less efficient for higher and lower HCl concentrations, with the highest percentage of extraction at 6 M HCl media (Figure 7). Previous work on Pt(IV) extraction using DMDCHTDGA in 1,2-dichloroethane showed an increasing performance of extraction with increasing HCl concentration, having the highest efficiency (100 %) above 5 M HCl (Paiva, et al., 2014b). This difference in extraction behaviour might be related with the change in the diluent for organic phase. Almost similar Pt(IV) extraction performance of DMDBTDGA were observed at all HCl concentrations studied. This trend is likely similar to the reported value for

Pt(IV) extraction using DMDBTDGA, but with the highest extraction performance observed at 7 M HCl (Paiva, et al., 2015b).

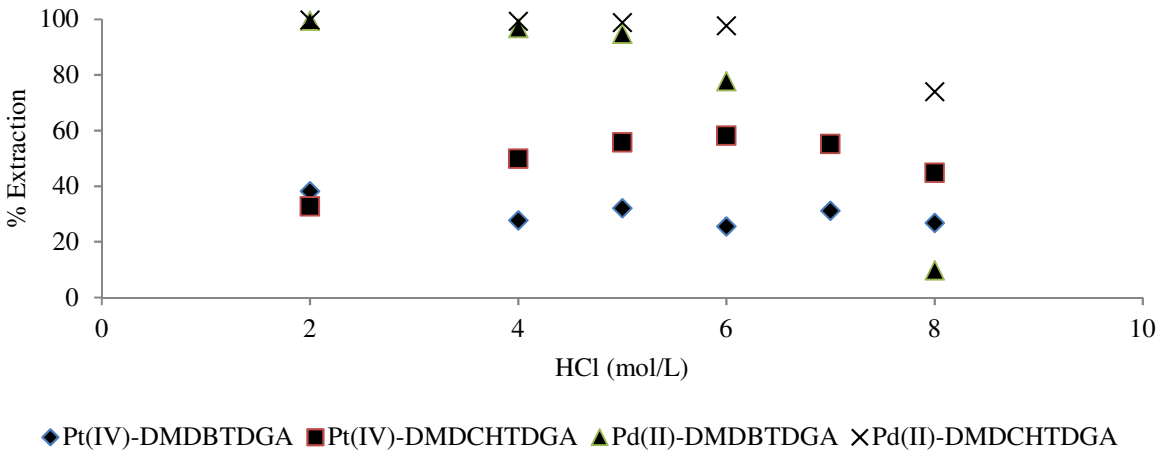


Figure 7: Pt(IV) and Pd(II) extraction performance from aqueous HCl media by 0.02M DMDBTDGA or DMDCHTDGA in toluene.

Both organic phases show a decreasing trend towards Pd(II) extraction from lower HCl concentrations to higher ones (Figure 7). Similar Pd(II) extraction behaviours from aqueous feed HCl media were reported using DMDBTDGA (Paiva, et al., 2015b). Pd(II) was extracted almost quantitatively by DMDBTDGA until 5 M HCl, and by DMDCHTDGA until 6 M HCl. A sharp decrease in Pd(II) extraction was found from more concentrated HCl aqueous media; for instance, from 8 M HCl using DMDBTDGA, only about 10% extraction of Pd(II) was observed. Previous reports on Pd(II) extraction from aqueous HCl media using DMDCHTDGA in 1,2-dichloroethane showed an efficient extraction performance regardless of HCl concentration (Paiva, et al., 2014b). However, the decreasing trend with increasing HCl concentration observed in the present study is in accordance with the data reported for Pd(II) extraction by DMDCHTDGA in toluene (Ortet & Paiva, 2015), and is perhaps due to the replacement of 1,2-dichloroethane by toluene as organic diluent. For all HCl media, higher Pd(II) extraction performances were observed for DMDCHTDGA than for DMDBTDGA. However, an almost similar result was observed for Pd(II) extraction performance using both organic phases from 2 M HCl.

Figure 8 illustrates the dependence of the distribution ratios of Pt(IV) and Pd(II) on HCl concentrations. As can be seen, the distribution ratios show that both organic phases are better extractants for Pd(II) than for Pt(IV). Generally, DMDCHTDGA showed better Pt(IV) and Pd(II) extraction performance from aqueous HCl media than DMDBTDGA. However, a

decreasing trend for higher concentrated HCl media is clearly visible for the extraction performance of both organic phases for Pd(II).

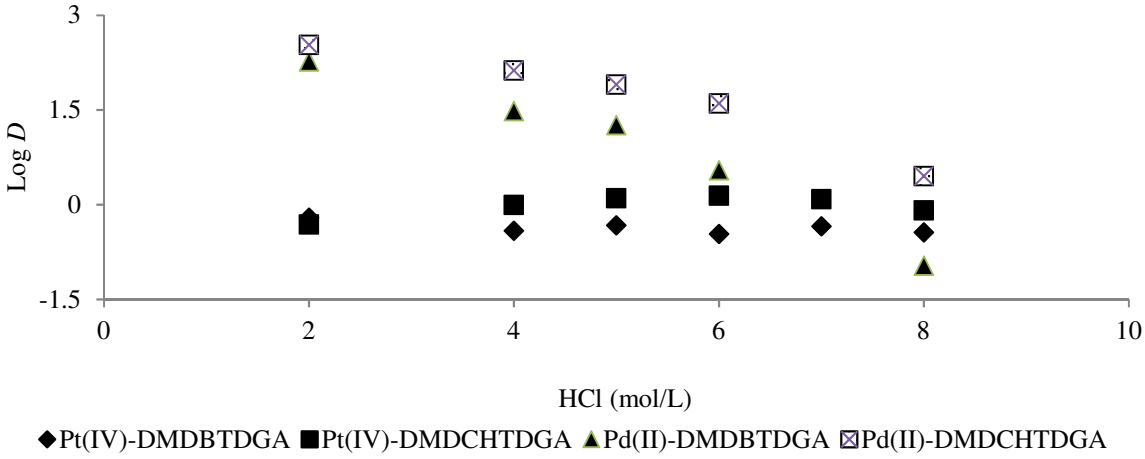


Figure 8: Variation of log *D* values for Pt(IV) and Pd(II) with HCl concentration, after extraction by DMDBTDGA and DMDCHTDGA in toluene.

Regarding the stripping of Pt(IV) and Pd(II), several researchers revealed stripping reagents such as acidic thiourea (Paiva, et al., 2014b; Ortet & Paiva, 2015), aqueous HCl (Paiva, et al., 2014b), seawater (Assunção, et al., 2016a), $HClO_4$ (Sun & Lee, 2011) and Na_2CO_3 (Sun & Lee, 2011). In these experiments, stripping of Pt(IV) and Pd(II) from loaded organic phases was carried out using seawater, which is low cost, environmentally friendly, easily and largely available. Pt(IV) and Pd(II) stripping efficiencies were established by determining the concentration of Pt(IV) or Pd(II) in aqueous phase (seawater) after stripping. The variation of Pt(IV) and Pd(II) recovery performance by the stripping agent, from both loaded organic phases, and their dependence on the initial HCl concentrations of the aqueous feed solutions, is illustrated in Figure 9.

During stripping of Pt(IV) and Pd(II) using seawater, precipitate formation was observed. As can be seen from Figure 9, seawater showed almost no stripping towards Pd(II) from both DMDCHTDGA and DMDBTDGA in toluene for all cases. In general, seawater has shown better stripping efficiency towards Pt(IV) than for Pd(II) from both organic phases. A better stripping performance of seawater towards Pt(IV) from DMDBTDGA than from DMDCHTDGA was observed. Generally, this study pointed out that seawater is not a very promising stripping agent for Pt(IV) and Pd(II). However, a previous report indicated that stripping efficiencies of 100% for Pd(II) and 86% for Pt(IV) were achieved from loaded

organic phases composed by DMDCHSA and DMDCHTDMA in 1,2-dichloroethane, respectively (Assunção, et al., 2016a).

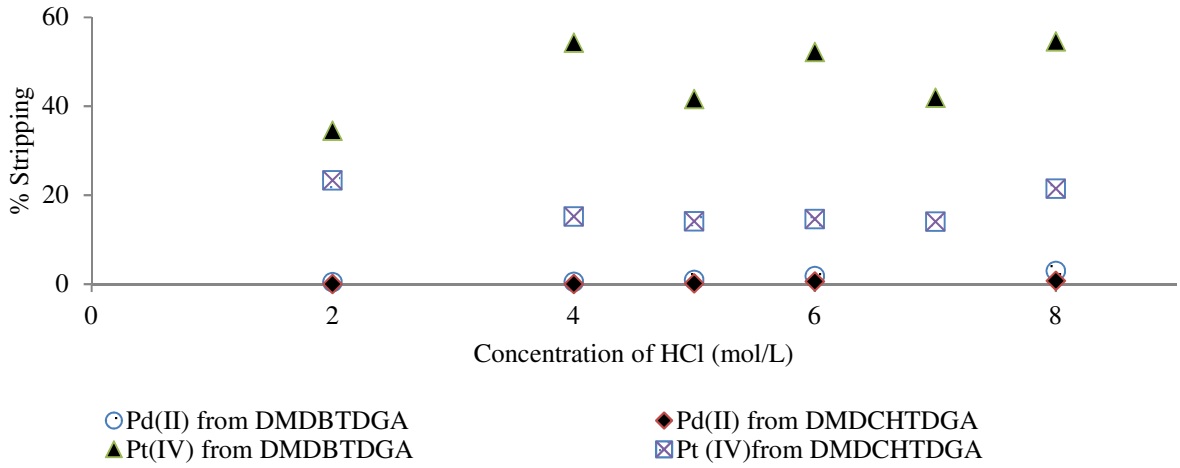


Figure 9: Pt(IV) and Pd(II) stripping efficiency from loaded organic phases by seawater, and their dependence on the initial HCl concentration of the aqueous feed solutions.

In these assays, the results revealed that both organic phases favour Pd(II) extraction over Pt(IV) from HCl media. An almost similar Pd(II) extraction performance was observed at lower HCl concentration using both organic phases. Regarding the stripping of the metal ions, nearly no stripping was observed for Pd(II) using seawater from both organic media. Concerning Pt(IV), seawater showed a better stripping performance. Moreover, seawater showed better Pt(IV) stripping performance from loaded DMDBTDGA than from DMDCHTDGA. However, the stripping performance of seawater for both metal ions is not promising. Hence, based on literature data, other stripping agents were investigated to remove Pt(IV) from DMDCHTDGA in toluene.

3.1.1.2 Extraction and stripping of Pt(IV)

For the purpose of finding a better Pt(IV) stripping reagent, six replicate extractions of Pt(IV) from 4 and 6 M HCl aqueous feed solutions, using DMDCHTDGA in toluene, were carried out. The results are illustrated in Figures 10 and 11 for 4 and 6 M aqueous HCl media, respectively. As shown in both Figures 10 and 11, in all six replicates, about half of Pt(IV) found in the initial aqueous feed solutions at both HCl media were extracted by DMDCHTDGA in toluene.

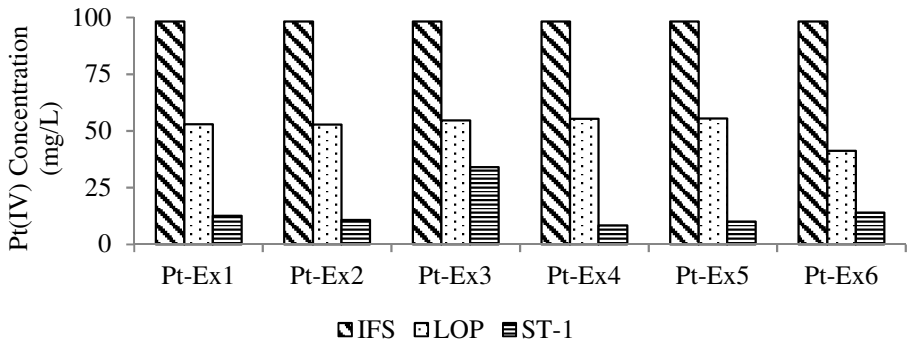


Figure 10: Pt(IV) concentration (mg/L) in the initial 4 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP) and stripped at first stage stripping (ST-1, stripping agents: Ex1 by 8.2 mM $Na_2S_2O_3$, Ex2 by 8.2 mM KSCN, Ex3 by 8.2 mM Na_2SO_3, Ex4 by 1 M HCl, Ex5 by 1 M HNO_3, and Ex6 by 0.05 M NaCl).

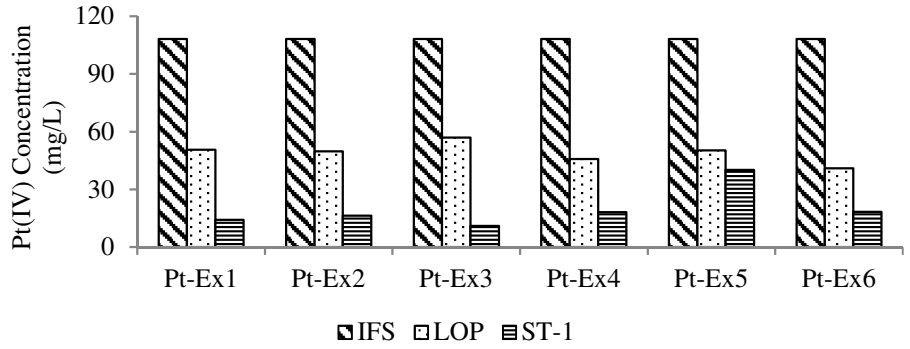


Figure 11: Pt(IV) concentration (mg/L) in the initial 6 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP) and stripped at first stage stripping (ST-1, stripping agents: Ex1 by 1 M HCl, Ex2 by 1 M HNO_3, Ex3 by water, Ex4 by 1 M H_2SO_4, Ex5 by 1 M NH_3 – 0.1 M NH_4Cl and Ex6 by 0.05 M NaCl).

Stripping of Pt(IV) from loaded DMDCHTDGA was carried out using various reagents to evaluate their stripping performance. The stripping reagents employed in this study were selected based on previously reported literature results for Pt(IV). However, the extractants employed for the extraction of Pt(IV) found in literature and used in the present study are different (Cieszynska & Wisniewski, 2010; Lee, et al., 2010; Raju, et al., 2012; Paiva, et al., 2014b; Schreier & Edtmaier, 2003; Sun & Lee, 2011). The results of the present study for a first stage stripping are illustrated in Figures 10 and 11 for 4 and 6 M aqueous HCl media, respectively. During stripping of Pt(IV) from loaded DMDCHTDGA at 4 and 6 M HCl media, a white precipitate was observed using $Na_2S_2O_3$ and with the mixture of NH_3 - NH_4Cl, respectively. For the rest of the stripping procedures, immediate separation was observed between aqueous and organic phases. Moreover, no emulsion, precipitation or solid particle formations were observed either in aqueous or organic phases.

As it is shown in Figure 10, at first stage stripping from loaded DMDCHTDGA from 4 M HCl media, none of the stripping agents employed showed promising results. However, the analysis of Pt(IV) precipitated during stripping, in addition to that stripped in the aqueous solution, showed that $Na_2S_2O_3$ has a better stripping capability (34 mg/L), about 62%. Similarly, the stripping agents employed for Pt(IV) from loaded DMDCHTDGA at 6 M HCl are not encouraging, except the mixture of NH_3 - NH_4Cl. Furthermore, the analysis of Pt(IV) precipitated during stripping, in addition to that stripped to aqueous phase, displayed about 40 mg/L, corresponding to an 80% stripping efficiency (Figure 11).

Based on the first stage stripping results obtained and indicated above, a second stripping stage was performed for those reagents showing better stripping efficiencies. Accordingly, KSCN, Na_2SO_3, $Na_2S_2O_3$, and NaCl for 4 M HCl media, and deionized water, H_2SO_4, NaCl, and NH_3-NH_4Cl mixture for 6 M HCl media were used to strip the remaining Pt(IV) left in DMDCHTDGA after the first stage stripping. A white precipitate was observed on stripping with Na_2SO_3 and mixture of NH_3-NH_4Cl for 4 and 6 M HCl media, respectively. The results of analysis are given in Figures 12 and 13 for 4 and 6 M HCl media, respectively.

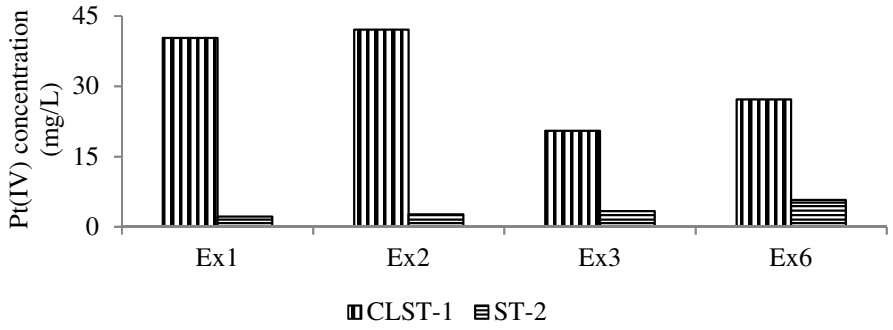


Figure 12: Pt(IV) concentration (mg/L) left in loaded DMDCHTDGA at 4 M HCl media after first stage stripping (CLST-1) and stripped at second stage stripping (ST-2, stripping agents: Ex1 by 8.2 mM $Na_2S_2O_3$, Ex2 by 8.2 mM KSCN, Ex3 by 8.2 mM Na_2SO_3, Ex6 by 0.05 M NaCl).

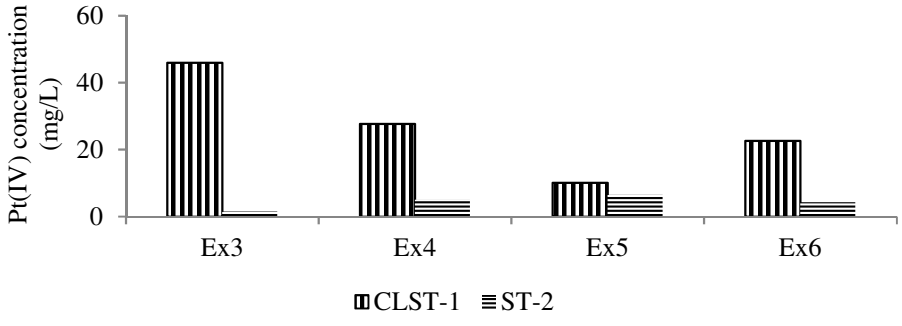


Figure 13: Pt(IV) concentration (mg/L) left in loaded DMDCHTDGA at 6 M HCl media after first stage stripping (CLST-1) and stripped at second stage stripping (ST-2, stripping agents: Ex3 by water, Ex4 by 1 M H_2SO_4, Ex5 by 1 M NH_3 - 0.1 M NH_4Cl, Ex6 by 0.05 M NaCl).

As can be seen from Figure 12, NaCl showed a better Pt(IV) stripping efficiency than the other reagents employed for the second stage stripping at 4 M HCl media. Similarly, H_2SO_4 and NH_3-NH_4Cl showed better stripping efficiencies in the second stage stripping than the other stripping agents employed at 6 M HCl media (Figure 13). However, still none of the employed reagents showed an appreciable stripping efficiency towards Pt(IV).

The overall percentages of Pt(IV) stripping by the various reagents are presented in Figures 14 and 15 for 4 and 6 M HCl media, respectively. From Figure 14, 39 mg/L (72%) and 20 mg/L (50%) of Pt(IV) were recovered from loaded DMDCHTDGA at 4 M HCl media using Na_2SO_3 and NaCl, respectively. The other stripping agents employed showed lower performances for the stripping of Pt(IV). Likewise, 47 mg/L (93%), 23 mg/L (55%) and 23 mg/L (51%) of Pt(IV) stripping from loaded DMDCHTDGA at 6 M HCl media were observed using NH_3-NH_4Cl mixture, NaCl and H_2SO_4 respectively (Figure 15). Barakat and Mahmoud reported a stripping of 97.5% of platinum from spent catalysts using a mixture of NH_3-NH_4Cl as stripping agent, which is more or less in a similar range with the results found in the present study (Barakat & Mahmoud, 2004).

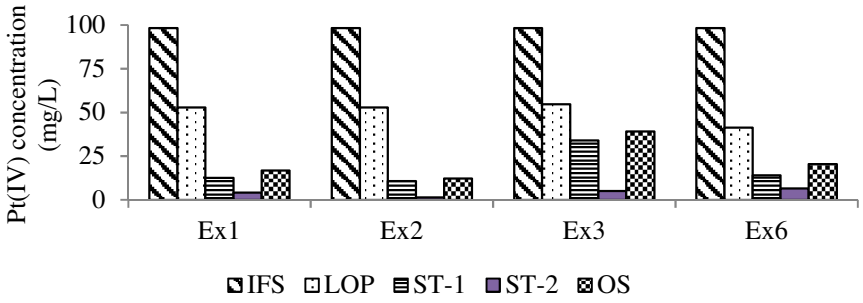


Figure 14: Pt(IV) concentration (mg/L) in the initial 4 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped at first stage stripping (ST-1), stripped at second stage stripping (ST-2) and overall stripping (OS) (stripping agents: Ex1 by 8 mM $Na_2S_2O_3$, Ex2 by 8.2 mM KSCN, Ex3 by 8.2 mM Na_2SO_3, Ex6 by 0.05 M NaCl).

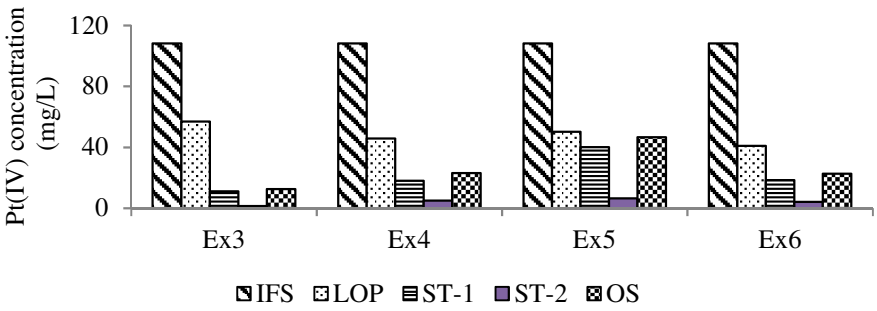


Figure 15: Pt(IV) concentration (mg/L) in the initial 6 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped at first stage stripping (ST-1), stripped at second stage stripping (ST-2) and overall stripping (OS) (stripping agents: Ex3 by water, Ex4 by 1 M H_2SO_4, Ex5 by 1 M NH_3 - 0.1 M NH_4Cl, Ex6 by 0.05 M NaCl).

The result of this trail explored that, from both 4 M and 6 M HCl aqueous feed solutions, about half of Pt(IV) was extracted by DMDCHTDGA, on average. The analysis of the aqueous phases after the first stage stripping showed that all the reagents were not encouraging for the recovery of Pt(IV) from loaded DMDCHTDGA. However, the analysis of the precipitates formed, and the Pt(IV) amounts on the aqueous phases after the first stage stripping, pointed out better Pt(IV) stripping performances by Na_2SO_3 and NH_3-NH_4Cl for 4 and 6 M HCl media, respectively. The overall stripping revealed that 39 mg/L (72%) and 47 mg/L (93%) Pt(IV) were stripped using Na_2SO_3 and a mixture of NH_3-NH_4Cl from loaded DMDCHTDGA at 4 and 6 M HCl media, respectively. However, the stripping of Pt(IV) needs a tedious work, and none of the reagents was encouraging for the stripping of Pt(IV). The extractant employed is also not efficient enough to extract Pt(IV) from HCl media. Hence, based on the results discussed above, the extractant DMDCHTDGA is used in sequence to perform the solvent extraction of Pd(II). Binary, tertiary and a mixture containing six metal ions were considered as aqueous feed solutions, to evaluate the DMDCHTDGA selectivity towards Pd(II) extraction. The stripping agents evaluated for Pd(II) recovery were deionized water, nitric acid, ammonia and acidic thiourea. The results of this investigation are presented in the proceeding sections.

3.1.2 Binary mixtures

The extraction and stripping of metal ions from binary solutions in 2, 4 and 6 M HCl media were investigated using the solvent extraction method. DMDCHTDGA in toluene was employed as extractant and aqueous ammonia, nitric acid, acidic thiourea and deionized water were evaluated for their stripping efficiency for the metal ions from loaded DMDCHTDGA. The outcomes of analysis are illustrated in Figures 16 to 21 for both Pd(II)-Cu(II) and Pd(II)-Fe(III) binary solutions in HCl media. The investigation revealed that DMDCHTDGA did not show any capability to extract Cu(II) from Pd(II)-Cu(II) binary solutions in HCl media (Figures 16-18). A similar result was observed for Cu(II) extraction from Pd(II)-Cu(II) binary solutions containing a higher concentration, about 400 mg/L, of Cu(II) (data not shown). Concerning Pd(II) extraction performance of DMDCHTDGA from Pd(II)-Cu(II) binary solutions, a quantitative extraction at all HCl media was observed. A similar Pd(II) extraction performance of DMDCHTDGA from binary solutions containing higher concentrations of Cu(II), regardless of HCl media, was observed (data not shown). Generally, DMDCHTDGA showed an excellent extraction performance towards Pd(II) with no co-extraction of Cu(II) and regardless of HCl concentration.

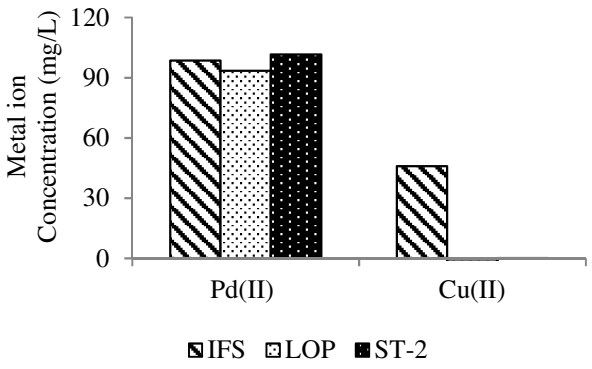


Figure 16: Metal ion concentrations (mg/L) in the initial 2 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

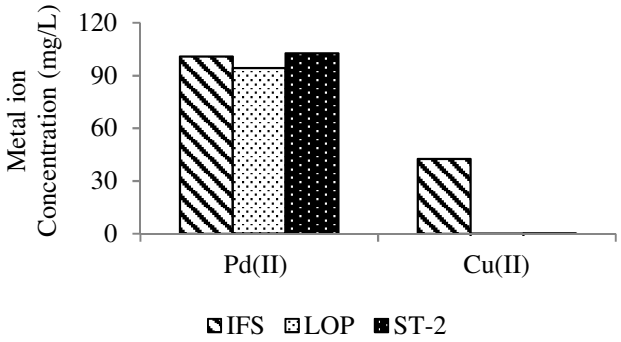


Figure 17: Metal ion concentrations (mg/L) in the initial 4 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

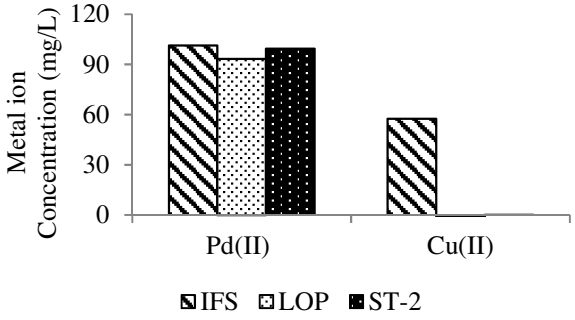


Figure 18: Metal ion concentrations (mg/L) in the initial 6 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

In all the stripping experiments carried out using acidic thiourea, the colour of the aqueous phases changed from colourless to a light-yellow colour, which might be an indication for the transfer of the metal ion(s). The DMDCHTDGA fraction loaded at 2 M HCl media was first stripped by HNO_3, and in sequence by acidic thiourea. The results revealed that HNO_3 showed almost no stripping capability towards Pd(II). On the other hand, acidic thiourea exhibited an excellent stripping efficiency towards Pd(II) regardless of HCl concentration (Figures 16-18).

A similar investigation on Pd(II) stripping performance of NH_3 revealed that about 63 % Pd(II) was stripped from Pd(II)-Cu(II) loaded DMDCHTDGA at 2 M HCl media (data not shown).

As one can clearly see from Figures 19-21, DMDCHTDGA showed an appreciable capability for extraction of Pd(II) from the feed solutions with Fe(III), regardless of HCl media. However, there was increasing co-extraction of Fe(III) at 4 and 6 M HCl media from the Pd(II)-Fe(III) binary feed solutions. A small quantity of Fe(III), extracted from the 2 M HCl feed solution was observed (only about 12 mg/L). An almost similar extraction capability of DMDCHTDGA in 1,2-dichloroethane was previously reported, but with different combinations of metal ions (Paiva, et al., 2014a). Fe(III) extraction performance by DMDCHTDGA showed an increase with increasing HCl concentration, denoting 53 mg/L (96%) extraction achieved from the binary solution at 6 M HCl.

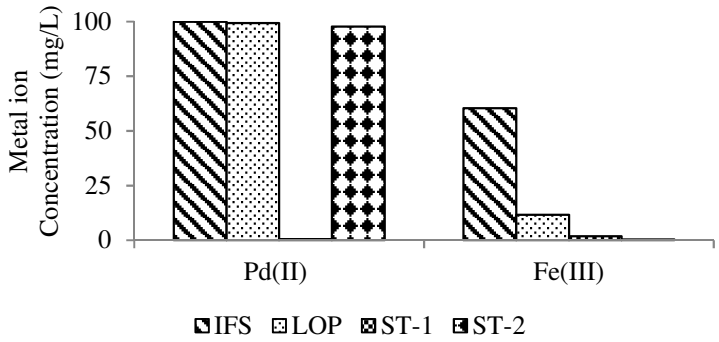


Figure 19: Metal ion concentrations (mg/L) in the initial 2 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1), stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

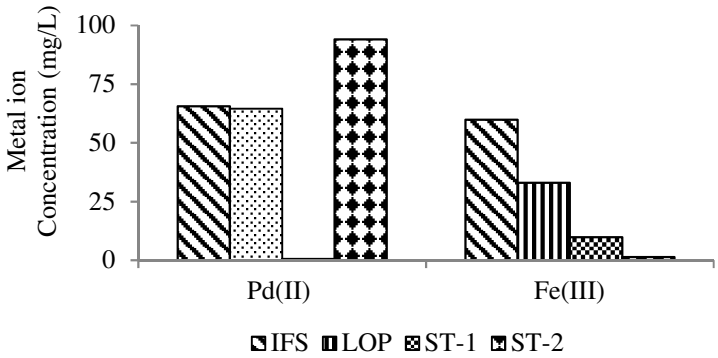


Figure 20: Metal ion concentrations (mg/L) in the initial 4 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

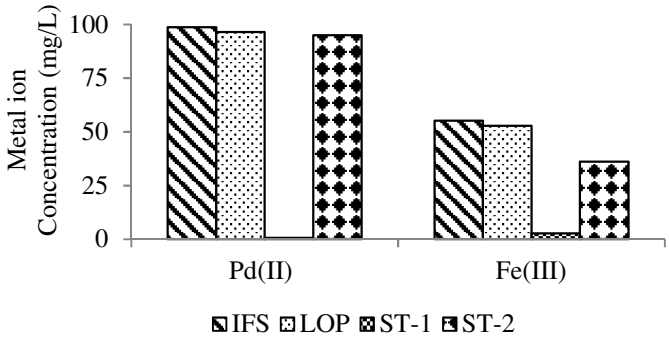


Figure 21: Metal ion concentrations (mg/L) in the initial 6 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

Metal ions extracted by DMDCHTDGA from the Pd(II)-Fe(III) binary solutions were stripped with deionized water, followed by acidic thiourea. As it is presented in Figures 19-21, almost no Pd(II) stripping using deionized water was observed, regardless of HCl concentration. Concerning the stripping of Fe(III), deionized water showed negligible stripping performance at the three HCl concentrations of the feed solutions – showing the best stripping of 10 mg/L (30%) – at 4 M HCl media. The acidic thiourea solution showed admirable stripping performances towards Pd(II) from Pd(II)-Fe(III) loaded DMDCHTDGA at all the HCl media tested. However, about 36 mg/L (72%) Fe(III) were co-stripped with Pd(II) from 6 M HCl media. From Pd(II)-Fe(III) loaded DMDCHTDGA at both 2 and 4 M HCl media, acidic thiourea exhibited a negligible or no capability towards Fe(III) recovery.

Ultimately, this experiment revealed that DMDCHTDGA showed an excellent extraction performance towards Pd(II) from both binary feed solutions, irrespective of HCl concentration. However, the co-extraction of Fe(III), which increased with increasing HCl concentration, was observed. Deionized water did not show appreciable ability to strip Pd(II) and Fe(III), though showed better capability for the stripping of Fe(III) than for Pd(II). An excellent stripping of Pd(II) using acidic thiourea, regardless of HCl concentration, was achieved. However, an almost quantitative co-stripping of Fe(III) at 6 M HCl media was observed.

3.1.3 Tertiary mixtures

The extraction and stripping of metal ions from Fe(III)-Pd(II)-Cu(II) tertiary solutions were investigated using the solvent extraction method. The results for metal ion extraction by DMDCHTDGA in toluene and stripping using deionized water, followed by acidic thiourea, are illustrated in Figures 22 to 24 at 2, 4 and 6 M HCl media, respectively. As can be seen from Figures 22, 23 and 24, DMDCHTDGA showed an excellent extraction efficiency towards

Pd(II), regardless of HCl concentration. However, both Cu(II) and Fe(III) were co-extracted. A better extraction of Cu(II) was observed from the 2 M HCl feed solution, decreasing with increasing concentrations of HCl. Concerning Fe(III), the extraction efficiency of DMDCHTDGA increases with the increase of HCl concentration, being an excellent extractant at 6 M HCl. From the 2 M HCl feed solution, no extraction of Fe(III) was observed.

Metal ions extracted by DMDCHTDGA from Fe(III)-Pd(II)-Cu(II) tertiary solutions were stripped using deionized water, followed by acidic thiourea. Using deionized water, neither Cu(II) nor Pd(II) were stripped from loaded DMDCHTDGA at 2 M HCl feed solution. As indicated in Figures 22, 23 and 24, deionized water depicted no or negligible stripping efficiency towards Pd(II) and Cu(II). A quantitative stripping of Fe(III) from loaded DMDCHTDGA was achieved using deionized water as stripping agent, and 4 and 6 M HCl feed solutions. However, negligible amounts of Cu(II) and Pd(II) were co-stripped.

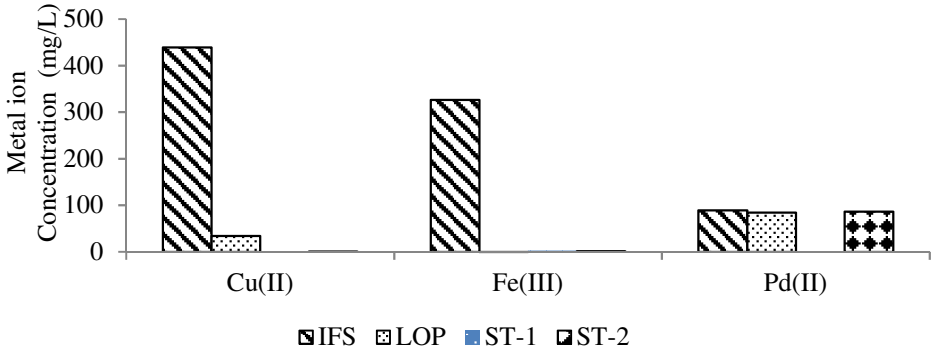


Figure 22: Metal ion concentrations (mg/L) in the initial 2 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

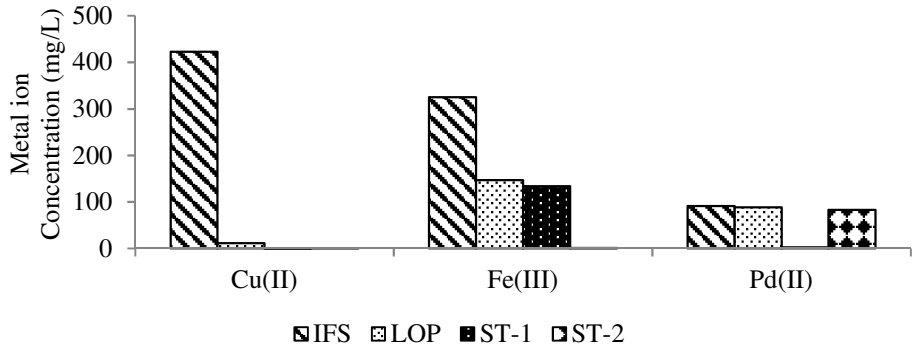


Figure 23: Metal ion concentrations (mg/L) in the initial 4 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

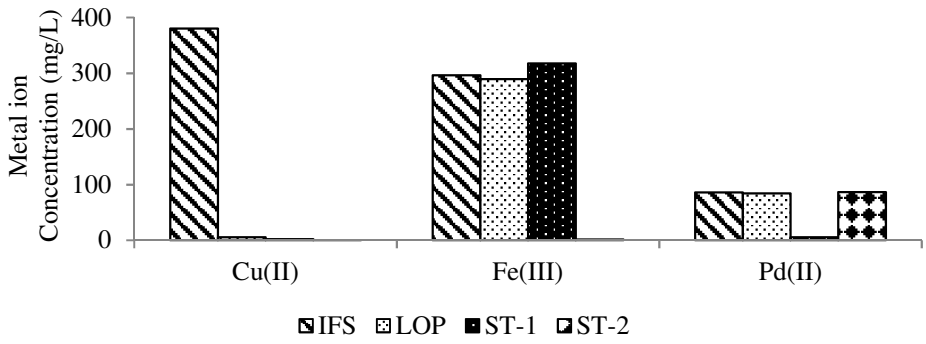


Figure 24: Metal ion concentrations (mg/L) in the initial 6 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

The stripping of Fe(III), Pd(II) and Cu(II) using acidic thiourea was carried out for the amounts left in loaded DMDCHTDGA after stripping with deionized water. Unlike for Fe(III) and Cu(II), acidic thiourea showed an excellent stripping performance for Pd(II) from loaded DMDCHTDGA at all HCl concentrations employed. However, there was negligible co-stripping of Fe(III) – only 2 mg/L at both 4 and 6 M HCl media.

In summary, DMDCHTDGA showed an incredible Pd(II) extraction performance from both binary and tertiary feed solutions. Similar Fe(III) extraction trends were observed from binary and tertiary feed aqueous media, but with no extraction from 2 M HCl tertiary feed solution. On the other hand, Cu(II) has not shown-up in the extraction from binary solutions at all acidities. A better Cu(II) extraction performance of DMDCHTDGA was observed from tertiary feed solutions – with higher concentrations of Cu(II). These variations might come from either the concentrations differences or the composition of metal ions, in binary and tertiary feed solutions.

Finally, this investigation exposed that nearly no extraction was observed for Cu(II) from feed solutions at all HCl concentrations, and Fe(III) from 2 M HCl media, whereas an excellent Pd(II) extraction ability of DMDCHTDGA from all the feed solutions was observed. The extraction performance of DMDCHTDGA towards Fe(III) increased with HCl concentration, with an excellent efficiency from the 6 M HCl feed solution. On the subject of stripping, water showed an excellent performance to strip Fe(III) over Pd(II) and Cu(II). Similarly, acidic thiourea showed an excellent stripping performance for Pd(II), rather than for Cu(II) and Fe(III), at the HCl concentrations studied.

3.1.4 Complex mixtures

Extraction and stripping of metal ions from acidic aqueous feed solutions containing Cu(II), Fe(III), Pb(II), La(III), Ni(II) and Zn(II) using liquid-liquid extraction were carried out. The extraction of metal ions was performed using DMDCHTDGA in toluene, followed by scrubbing with deionized water and subsequent stripping using acidic thiourea. The results of investigation are displayed in Figures 25 to 27 for 2, 4 and 6 M HCl media, respectively. Despite different metal ion compositions of tertiary and complex mixtures, almost the same extraction and stripping behaviours for Pd(II) and Fe(III) were observed.

It can be seen from the Figures that DMDCHTDGA showed an excellent extraction performance towards Pd(II) at all three HCl concentrations of feed solutions. However, a significant co-extraction of Fe(III) at 4 and 6 M HCl was observed. From the 2 M HCl feed solution, an excellent Pd(II) extraction performance was observed with co-extraction of Zn(II) and Fe(III) – only about 9.0 ± 2.2 mg/L and 11.5 ± 1.8 mg/L, respectively (Figure 25). A quantitative extraction of Pd(II) and about half of Fe(III) were obtained from the 4 M HCl feed solution, with almost no co-extraction of Cu(II), La(III), Ni(II) and Zn(II) (Figure 26). Similarly, an admirable extraction performance of DMDCHTDGA towards Pd(II) with a quantitative co-extraction of Fe(III) and small co-extraction of Cu(II), Ni(II), La(III) and Zn(II) – only 7.8 ± 2.3 mg/L, 9.0 ± 4.8 mg/L, 3.0 ± 1.1 mg/L and 10.0 ± 6.1 mg/L respectively, from 6 M HCl, were observed (Figure 27). The co-extraction of impurities increased with the increase of the HCl concentration of the feed solution. However, the extraction performance of DMDCHTDGA towards Pd(II) was not affected by the presence of other metal ions. A similar Pd(II) extraction performance of DMDCHTDGA from leaching solutions coming from the treatment of a spent automobile catalyst was previously reported (Paiva, et al., 2015a).

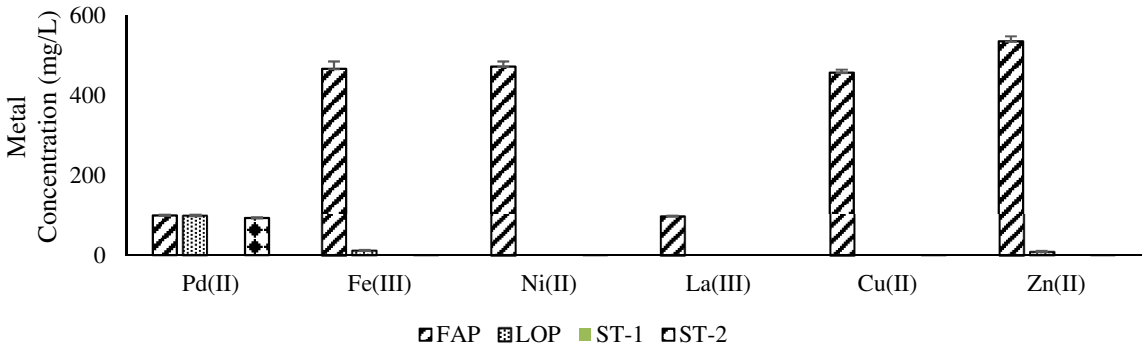


Figure 25: Metal ion concentrations (mg/L) in the initial 2 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

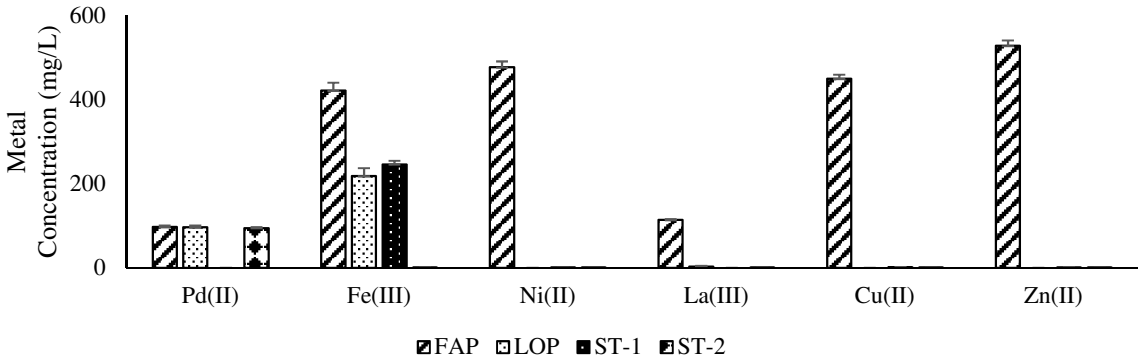


Figure 26: Metal ion concentrations (mg/L) in the initial 4 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

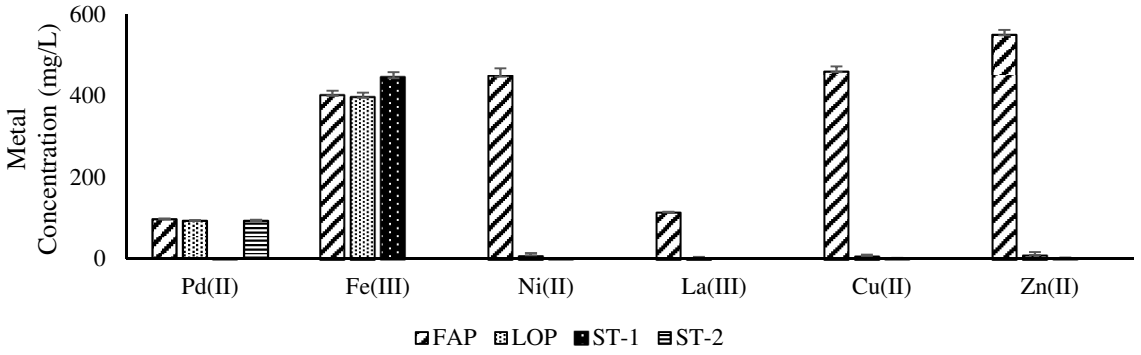


Figure 27: Metal ion concentrations (mg/L) in the initial 6 M HCl feed solution (IFS), loaded 0.02 M DMDCHTDGA in toluene (LOP), stripped by water (ST-1) and stripped by 0.1 M thiourea in 1 M HCl solution (ST-2).

Metal ions extracted by DMDCHTDGA were scrubbed with deionized water followed by stripping using acidic thiourea. Accordingly, Fe(III) was quantitatively scrubbed with deionized water, with negligible amounts of Cu(II) and Zn(II) – only about 2.2 ± 0.1 mg/L and 2.5 ± 0.1 mg/L, respectively, from 6 M HCl feed solutions. Likewise, Pd(II) was appreciably stripped using acidic thiourea, regardless of the HCl concentration of the aqueous feed solutions. However, a very negligible amount of Fe(III) - only 0.6 ± 0.1 mg/L from both 4 and 6 M HCl feed solution was co-stripped. In all the stripping experiments using acidic thiourea, co-stripping of other metal ions was not observed. This result indicates that acidic thiourea has a capability to selectively strip Pd(II).

Thus, the results of this investigation showed that Pd(II) was quantitatively extracted from 2 M HCl feed solution with co-extraction of small amounts of Ni(II) and Zn(II). However, Pd(II) was quantitatively stripped using acidic thiourea – Ni(II) and Zn(II) were not co-stripped. Furthermore, both co-extracted metals did not show-up in scrubbing with deionized water.

From 4 M HCl feed solution, a significant quantity of Fe(III) and a very negligible amount of La(III) were co-extracted with Pd(II). However, the former was completely scrubbed using deionized water, and Pd(II) was quantitatively stripped using acidic thiourea, with a negligible interference from Fe(III). Appreciable extraction efficiencies of DMDCHTDGA towards Pd(II) and Fe(III) from 6 M HCl were observed – with co-extraction of Cu(II), La(III), Ni(II) and Zn(II). However, quantitative stripping of Fe(III) and Pd(II) were achieved by scrubbing with deionized water for Fe(III), followed by stripping using acidic thiourea for Pd(II). Though, apart from Fe(III) appearing in the scrubbing solution, none of the other metals were co-stripped either using deionized water or acidic thiourea.

To explore the statistical relation for the mean extraction and stripping of Pd(II) among the three acidity values for the feed solutions employed, ANOVA and pair-wise comparison were made. Accordingly, there exists a significant difference ($p < 0.05$) between the mean results for the extraction of Pd(II) by DMDCHTDGA from 2, 4 and 6 M HCl feed solution, at 95% confidence level. Furthermore, the pair-wise comparison of mean indicated that the difference in mean were originated from the extraction results obtained from all acidity feed solutions engaged. Regarding stripping of Pd(II) by acidic thiourea, there exists a significant difference ($p < 0.05$) between the mean stripping results, at 95% confidence level. According to pair-wise comparison of mean, the significant variation in mean results were caused by the Pd(II) stripping result obtained from the loaded organic phase previously equilibrated with the 2 M HCl feed solution (Figure 28).

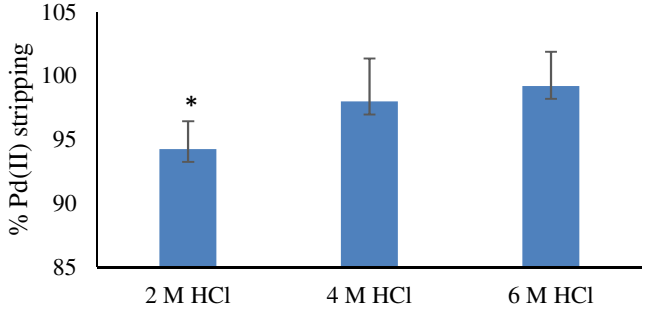


Figure 28: Statistical variation for mean Pd(II) stripping results from loaded organic phases, after equilibration with 2, 4 and 6 M HCl feed solutions (* significant difference from all).

3.2 Biological recovery of metals

The removal or recovery of metals through the action of bacteria, like SRB, from metal bearing industrial wastewaters is an environmentally friendly and cost-effective approach (Alvarez, et al., 2007). Biologically generated species, which can act as reducing or precipitating agents,

are widely used for the synthesis of metallic and metal compound nanoparticles. In this regard, biologically generated sulphides play a significant role for the recovery of metals through the synthesis of metallic and/or metal sulphide nanoparticles from aqueous solutions. The potential advantage of the recovery of metals as metal sulphide includes its ability to precipitate metal ions even at low concentrations, due to the low solubility of the precipitates, and the ability to subsequently recover the metals from sulphide sludge (Bhagat, et al., 2004). The synthesis of nanosized particles such as Au(0), Au_2S, ZnS, CuS, PdS using metabolic products from SRB communities has been previous reported (Alvarez, et al., 2007; Assunção, et al., 2016a; Costa, et al., 2012; Costa, et al., 2013; Vitor, et al., 2015; Assunção, et al., 2016b). The synthesis of nanoparticles employing biological techniques is a safe and eco-friendly method that allow to obtain functional particles with wide applications in several fields such as medicine, agriculture, waste water treatment, drug delivery, biosensors (Hulkoti & Taranath, 2014).

3.2.1 Biogenic sulphide generated in Postgate B medium

Biogenic sulphide, generated using Postage B medium, was used aiming palladium and iron recovery. For monitoring the growth of SRB in that medium, parameters such as OD_{600}, E_h, sulphate and sulphide concentration were measured weekly. Accordingly, the results of analysis are displayed in Figure 29.

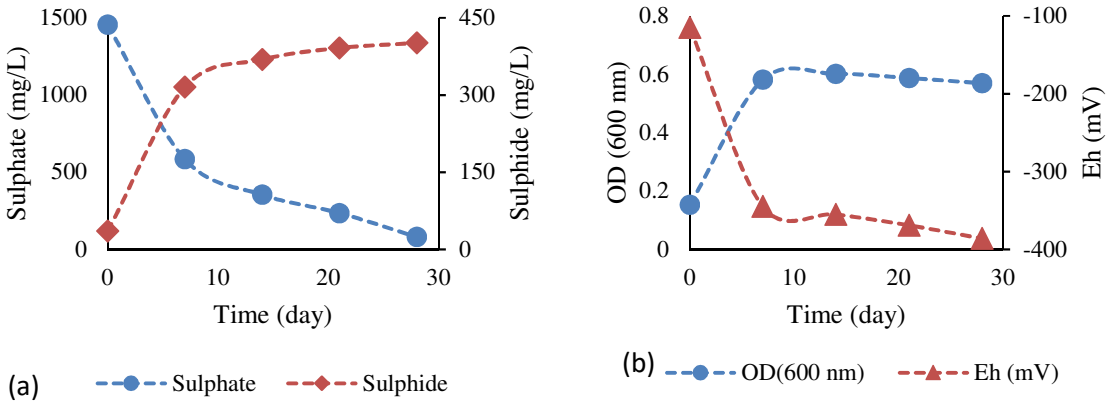


Figure 29: Evolution of growth parameters of SRB in Postage B medium measured during re-inoculation of a previously enriched inoculum; (a) sulphide and sulphate concentration and (b) optical density (OD at 600 nm) and redox potential (E_h).

After few days of operation, the colour of the medium changed to grey and the medium presented some turbidity. That was an indication of the bacterial growth with subsequent generation of sulphide, which was responsible for the formed black coloured precipitate of iron sulphide (Bhagat, et al., 2004; Lovley & Phillip, 1994). Furthermore, the values registered for OD_{600} and E_h also gave indication of SRB growth. The OD_{600} showed fast increment on the

first week of enrichment, indicating the growth of bacteria in the community, and becomes almost constant for the rest of the growth period. The E_h showed a decreasing trend until the end of the experiment, showing a rapid decline in the first week. The consumption of the initial available oxygen and the reduction of sulphate to sulphide, contribute for the decrease in the E_h during anaerobic bacterial growth. Moreover, the decrease in E_h was accompanied by a slight change (from 6.75 to 6.9) in pH at the first week of operation probably due to the adaptation of SRB to a new enrichment medium.

Regarding the sulphate and sulphide concentration, at the first week of enrichment a fast conversion of sulphate to sulphide was observed. This might be explained by the log phase of bacterial community growth. For the rest of the growth period the conversion rate was almost seeming constant. However, a gradual improvement in sulphate conversion rate was noticed after the first week of culture enrichment. These raise in sulphide and decrease in sulphate concentration signals the increasing activity of SRB (Cao, et al., 2009; Jong & Parry, 2003). The low rate of sulphate reduction can perhaps be attributed to the inhibition of the anaerobic process due to insufficient activity of SRB, which might be due to bacterial stationary phase (Cao, et al., 2009). There is a direct relation between sulphide generation and sulphate consumption (as one is formed at the expense of the other). However, the dissolved sulphide measurements considerably underestimated the amount of sulphate lost during enrichment. The slow increase in sulphide concentration after the first week might be due to the evaporation of H_2S and metal precipitation as insoluble sulphide in addition to adsorption on the wall of the growth container (Jong & Parry, 2003). SRB gain energy for growth by reduction of sulphate to hydrogen sulphide with electrons usually derived from the degradation of organic matter. After 28 days of bacterial growth period, about 94% sulphate reduction was achieved.

The pH of the enrichment culture is one of the factors that influences the reduction of sulphate. A report indicated that sulphate reduction was favoured at pH between 6 and 9. Lower pH contributes for the inhibition of the SRB (Cao, et al., 2009). However, the physical-chemical properties of the environment surrounding SRB might also be another determinant factor for the inhibition of SRB system (Jong & Parry, 2003). In this study, the pH of the batch nutrient growth was in the range of neutral medium (7.0 ± 0.3). This insures that the condition was favourable, with respect to pH, for the SRB to perform their communal activity in the enrichment culture. During the growth period, a change in pH (from 6.75 to 7.12) was noticed

and it might be attributed to the generation of alkalinity because of the metabolic activity of SRB (Sahinkaya, et al., 2009; Jong & Parry, 2003; Cao, et al., 2009).

3.2.2 Biogenic sulphides from a bioremediation process

For ensuring the efficient and economic bioremediation of AMD, the selection of suitable organic substrates play a vital importance in the treatment process. As the AMD contains low amounts of organic matter, the selection of an appropriate electron donor, or a mixture that stimulates the microbial metabolism and determines the effectiveness of the bioreactor (Abhilash, et al., 2015) is a key issue. Thus, engaging an electron donor from an easily available and cost-effective source, instead of chemically synthesised or commercialized chemicals, has merits for the sustainability of the technique. In the present study, winery waste was employed as an electron donor or nutrient source for SRB in a bioremediation process where AMD was the sulphate source. The composition of AMD and winery waste are displayed in Table 2.

Table 2: Composition of AMD (São Domingos mine, Portugal – sampling date 17-Feb-2016) and winery waste (Quinta do Barranco Longo, Lda., located in Algoz, Portugal) employed in the bioremediation process.

Acid Mine Drainage			**Winery Waste**				
Parameters	**Value**		**Parameters**	**Value**		**Parameters**	**Value**
Iron (mg/L)	172		COD (mg O_2 /L)	174650		Propionic acid (g/L)	1.85
Aluminium (mg/L)	205		Sulphate (mg/L)	34		Sucrose (g/L)	0.235
Zinc (mg/L)	43		Ethanol (g/L)	180.3		Glucose (g/L)	0.592
Copper (mg/L)	41		Lactic acid (g/L)	2.66		Arabinose (g/L)	0.489
Sulphate (mg/L)	2170		Acetic acid (g/L)	1.33		Fructose (g/L)	0.487
Eh (mV)	607		Tartaric acid (g/L)	0.20		Myo-inositol (g/L)	1.03
pH	2.49		Butyric acid (g/L)	0.16			

The bioremediation process for AMD treatment was an economic and environmentally efficient and sustainable continuous process involving winery waste as nutrient source for SRB. In that process SRB generate sulphide biologically, which is subsequently used for the removal or recovery of metals from AMD. As can be seen from Table 2, AMD contains heavy metals at lower pH which can affect the activity of SRB and the generation of sulphide (Sani, et al., 2001). Thus, an initial neutralization stage adjusts the pH to create favourable conditions for SRB growth (Figure 30). Then in the bioreactor, the remaining metals in the aqueous solution were precipitated as insoluble metal sulphides by the generated biogenic sulphide and the excess sulphides are excreted with the effluent (Figure 30). Sulphate to sulphide conversion inside the bioreactor depends on the flow rate of AMD and on the type and amount of carbon source. Hence, the flow rate of the influent was optimized previously to achieve a chemical composition of the effluent in terms of metals and sulphate concentration that allows its use for

irrigation purposes. In this way, the effluent from the bioreactor was engaged for the recovery of palladium and iron in batch and in continuous assays, as previously described in the Experimental sections (2.3.2.1 and 2.3.2.2).

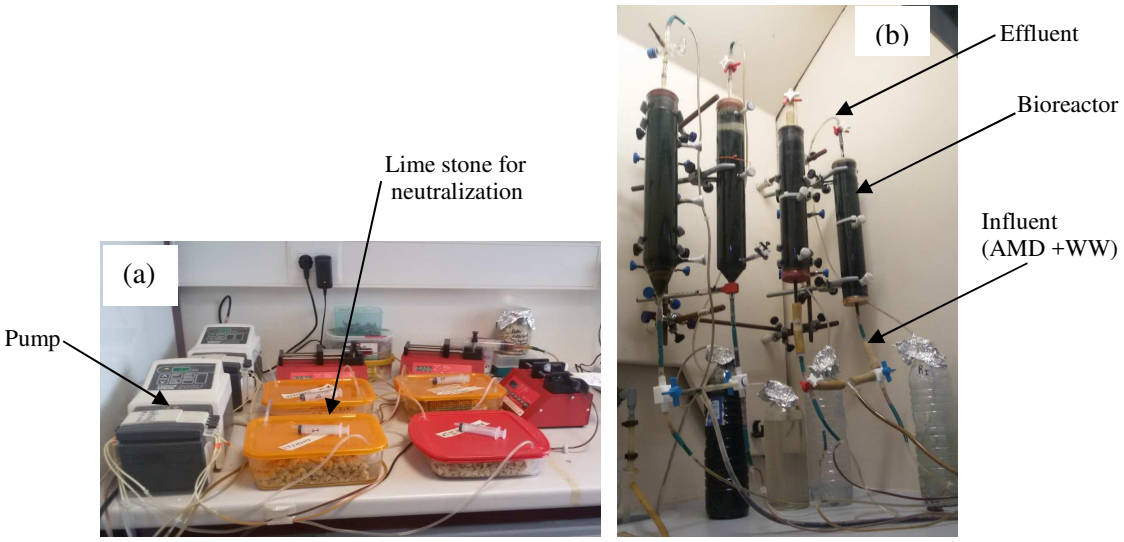


Figure 30: Bioremediation system for AMD treatment; (a) Neutralization step and the subsequent (b) biologic step.

3.2.3 Batch experiment for the recovery of metals

Recovery of palladium and iron from aqueous solution resulting from the stripping and scrubbing stage, respectively, of solvent extraction, was performed using batch assays. Consequently, Postgate B medium after 28 days of incubation (experiment A) and effluent from the bioreactor of the bioremediation process for AMD treatment (experiment B) were used. Parameters such as pH, OD_{600}, E_h, sulphate and sulphide concentration were determined in both solutions and the results are displayed in Table 3. In both experiments A and B, the solution was passed through a 0.2 µm filter before addition to the metal ion solution.

Table 3: Characterization of Postgate B nutrient medium after 28 days of incubation and effluent from the AMD bioremediation process (Expt – experiment).

Parameters	**S^{2-}(mg/L)**	**SO_4^{2-} (mg/L)**	**OD_{600}**	**Eh(mV)**	**pH**
Postgate B medium (Expt A)	395 ± 17	79.1 ± 4.4	0.57 ± 0.02	-385.7 ± 8.8	7.1 ± 0.6
Bioreactor (Expt B)	389.2 ± 8.6	177 ± 10	0.46 ± 0.05	-366.3 ± 9.5	7.0 ± 0.2

For the recovery of palladium, the solutions containing biogenic sulphide from Postgate B medium (experiment A1) and from bioremediation process (experiment B1) were engaged in batch. Consequently, in both experiment A1 and B1, immediately after the contact between the filtered solution containing biogenic sulphide and Pd(II) solution, the mixtures were turned to a brown colour which might be due to the formation of PdS precipitate. The amounts of Pd(II)

and sulphide were determined to monitor the progress of palladium recovery and sulphide consumption at 3, 24 and 48 hrs of duration of experiment A1 and B1. Accordingly, the progressive decrease of Pd(II) and sulphide amount with time and the percentage of palladium recovery are displayed in Figures 31 and 32, respectively, for both experiments. Thus, as it can be seen from Figure 31, the simultaneous decrease in the amount of Pd(II) and sulphide might be an indication of PdS formation in both experiments. Similarly, the analysis of the sample taken from both experiments after 3 hr of contact time indicated that, 96.3 ± 1.3% and 87.9 ± 0.4% palladium were recovered in experiments A1 and B1, respectively (Figure 32). However, the percentage of metal recovery slightly increases with the experiment duration. At 48 hr of duration, an almost complete palladium recovery was achieved in both experiments. Thus, the result confirmed the efficiency of biogenic sulphide, from both origins, for the recovery of palladium from aqueous solution.

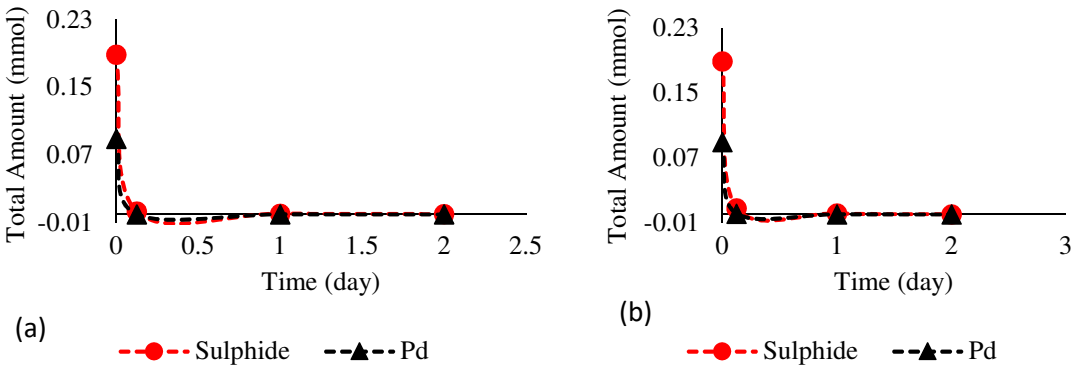


Figure 31: Time progress of Pd(II) and sulphide amount during the batch assay; (a) experiment A1 and (b) experiment B1.

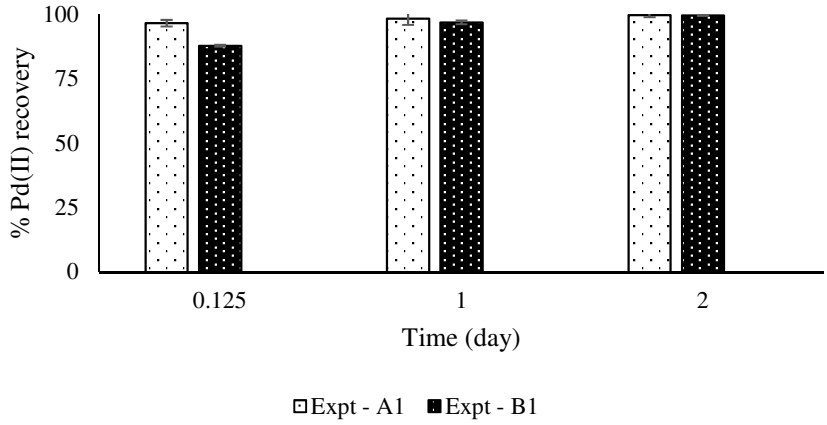


Figure 32: Percentage of palladium recovery (Pd(II) initial concentration: 94.6 ± 1.8 mg/L) in experiment A1 and experiment B1 from acidic thiourea solution resulting from the stripping stage of solvent extraction.

The concentration of sulphide, in both experiments, A1 and B1, decreased continuously during the first moments of operation (Figure 31). Even though sulphide determination was done immediately after sampling, several factors can contribute for the loss of sulphide from the sample mixture, apart from that eventually involved in metal precipitation. As the result of the volatile nature of sulphide as H_2S, all added sulphide may not be combined with the metal ion present in the test solution. The decrease in concentration of sulphide might also be attributed to escaping of sulphide as H_2S during sample withdrawal for the progress analysis. This escape might be due to high pressure inside the reaction bottle. Moreover, oxidation of sulphide by air while transferring the sample, can also eventually contribute for the loss of sulphide, resulting in lower concertation of sulphide than expected (Kuhn, et al., 1983; Jong & Parry, 2003).

A literature was publicized for the recovery of palladium from an aqueous HCl solution and from seawater using biogenic sulphide from Postgate E nutrient medium (Assunção, et al., 2016a). The authors noted that palladium was completely recovered in less than three days of assay. However, the molar ratio of sulphide to Pd(II) employed was about 3:1. The present study revealed a similar palladium recovery efficiency from acidic thiourea solution using biogenic sulphide from Postgate B medium. Furthermore, the present study employed a sulphide to Pd ratio of 2:1 and an almost complete palladium recovery was achieved within 24 hr of experiment duration. Thus, the shorter recovery time and the lower sulphide to Pd ratio can probably be due to the difference in the solution in which palladium was present (thiourea in the present study and seawater in the previous one) and/or to the composition of the biologic solution.

Theoretically, Pd(II) and sulphide reacts in one-to-one stochiometric quantity. However, the employed ratio in the assay was different from what was theoretically expected. This might be explained by the pH effect on the equilibrium of the metal sulphide and by the volatility nature of H_2S species, discussed above. Studies revealed the effect of pH on the equilibrium of metal sulphide species, the lower pH alters the equilibrium of the dissolved sulphide species towards to the H_2S formation, which can easily volatilize (Kleinjan, et al., 2005; Jiménez-Rodríguez, et al., 2009). In the present study, the Pd(II) solution under investigation was in acidic thiourea solution, which might have a significant contribution for the need of greater amounts of sulphide over Pd(II), since part of sulphide is in the form of the volatile H_2S specie. Moreover, in experiments A1 and B1 the pH decreased upon addition of biogenic sulphides to Pd(II) solution. For experiment A1, the pH was changed from values of 7.1 ± 0.6 to values of 2.5 ±

0.4, whereas for experiment B1 from values of 7.0 ± 0.2 to values of 2.7 ± 0.4. The pH of the initial Pd(II) solution used in both experiments was 1.7 ± 0.6, which contributed for the decrease in pH observed in experiment A1 and B1. Furthermore, the reaction of sulphide with Pd(II) favoured the dissociation of available H_2S, which possibly also contributed for the observed decrease in pH.

Regarding iron recovery, batch experiments were performed using solutions containing biogenic sulphides from Postgate B medium (experiment A2) and from bioremediation process (experiment B2). In both experiments, the sulphide to Fe(III) ratio employed was 2.5:1. The amount of Fe(III) in the solution decreased with addition of the solution containing biogenic sulphide. The amounts of Fe(III) and sulphide were determined as function of time to monitor the progress of iron recovery and sulphide consumption at 0.083, 1 and 5 days of the experiment and the results are displayed in Figures 33 and 34, respectively, for experiments A2 and B2, respectively. As can be seen from Figure 33a, the amount of Fe(III) progressively decreased after the addition of biogenic sulphide, which can be attributed to the consumption of iron and sulphide to form iron sulphide. Correspondingly, the analysis of the samples collected at 0.083 day (2 hrs) of experiment indicated that 97.4 ± 0.6% and 53.8 ± 1.9% of iron was recovered from the solution in experiments A2 and B2, respectively (Figure 34). In experiment A2, an almost complete recovery of iron was observed in less than 24 hr, whereas a progressive recovery of the metal occurred in experiment B2 – having 78 ± 12% recovery after 5 days of experiment (Figure 34). This difference in the recovery efficiency might be attributed to differences in the growth conditions of SRB and to the different environment in which the biogenic sulphides were generated. Iron recovery in experiment A2 is promising as an almost complete recovery was achieved in a shorter period.

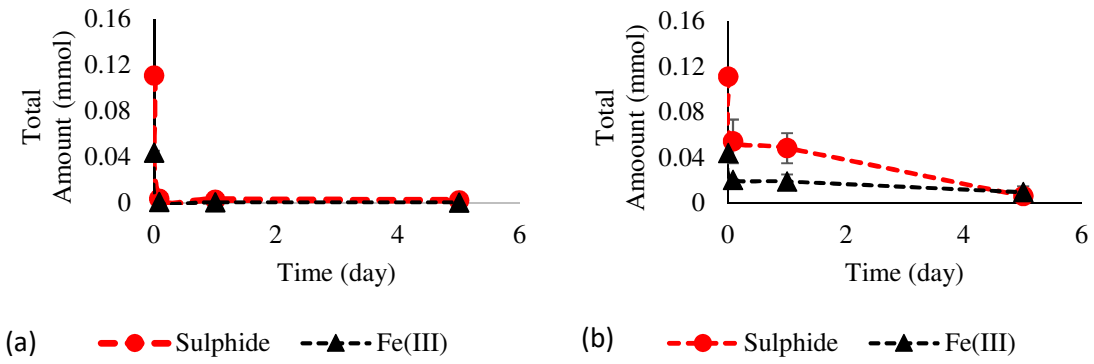


Figure 33: Progress of Fe(III) and sulphide amount in the mixed solution during the assay; (a) experiment A2 and (b) experiment B2.

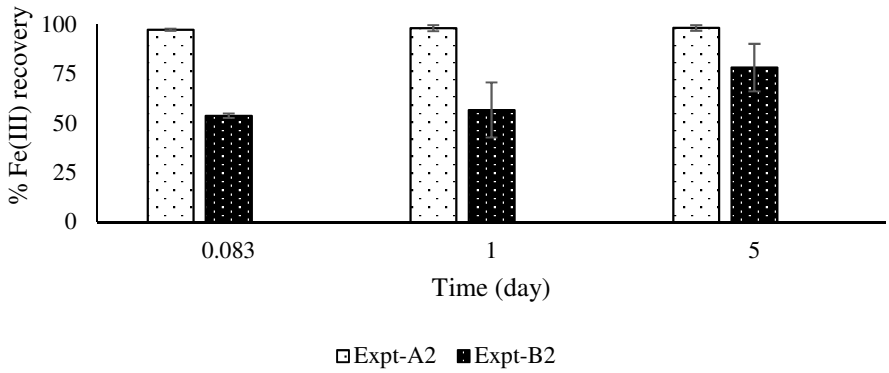


Figure 34: Percentage of iron recovery (246.1 ± 8.5 mg/L starting concentration) from the aqueous solution resulting from the scrubbing stage of solvent extraction in experiment A2 and experiment B2.

In both experiments, A2 and B2, the pH decreased upon addition of the biogenic sulphide to the Fe(III) solution. On experiment A2 and B2 the pH changed from values of 7.1 ± 0.6 to values of 4.6 ± 0.6 and from values of 7.0 ± 0.2 to values of 5.6 ± 0.6, respectively. The initial pH of the Fe(III) solution used in both experiments was 2.3 ± 0.2, which might also have contributed for the decrease in pH. Moreover, the pH decrease might also be associated with the dissociation of H_2S due to the reaction of sulphide with Fe(III), which probably caused a shift in the equilibrium of the dissolved sulphide species (Jiménez-Rodríguez, et al., 2009; Kleinjan, et al., 2005).

3.2.4 Continuous flow assay for the recovery of metals

A laboratory scale bioreactor designed for the bioremediation of AMD generates sulphide through employing SRB. As the sulphide is generated in excess (in comparison to the metals in AMD to be precipitated) and because it is an environmental hazardous, its discharge represents a huge concern. However, the use of this toxic sulphide for the recovery of metal can solve the problem related to its discharge in the environment. Accordingly, for the recovery of palladium or iron, a solution containing one of these metal ions was directly coupled to the bioremediation system aiming the continuous addition of the effluent produced (experiment C, Figure 35). The effluent from the bioreactor was characterized and the results are presented in Table 4.

Table 4: Characterization of the filtered effluent from the bioremediation process for AMD treatment.

Parameters		**E_h (mV)**	**pH**	**SO_4^{2-} (mg/L)**	**S^{2-} (mg/L)**	**Flow rate (ml/h)**
Bioreactor effluent	Expt C1	-378.0± 7.5	6.9 ± 0.4	88.5 ± 1.7	201 ± 16	1.5 ± 0.7
	Expt C2	-396.3 ± 6.3	7.0 ± 0.1	75.9 ± 2.8	196.0 ± 8.0	1.50 ± 0.37

A 0.2 µm filter was placed between the effluent exit and the bottle containing the metal solution (Figure 35). The average flow rate of the effluent after fitting the filter was 1.5 mL h^{-1}. During operation variations in the flowrate were noticed, which might be attributed to clogging of the pores of the filter by bacteria and solid particles. Furthermore, the formation of solids such as metal oxides/hydroxides and small particles from the neutralization step, can possibly, contribute for the clogging of valves and pores in the column, as well as in the filter (Vitor, et al., 2015; Jong & Parry, 2003). Thus, the filter was periodically changed to monitor the change in the flowrate and to prevent the contamination of palladium or iron precipitates.

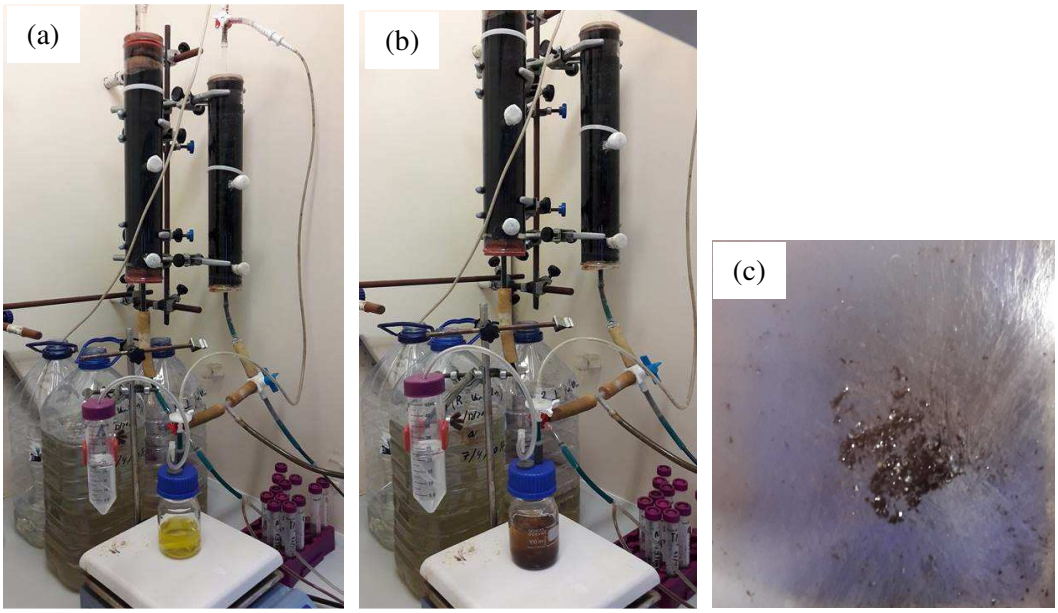


Figure 35: Picture of palladium recovery in continuous flow assay (experiment C1). Pd(II) solution coupled to the bioremediation system before (a) and during (b) effluent addition, and (c) palladium precipitate collected.

An experiment was carried out in a contentious system for the recovery of palladium using effluent from the bioremediation process (experiment C1). Consequently, when the effluent from the bioreactor started to be added to the Pd(II) solution, the colour of Pd(II) solution started changing from yellow to brown (Figure 35), which may indicate the formation of PdS. Analysis of the samples collected at 12, 18 and 24 hr showed that the concentration of Pd(II) in the solution was contentiously decreasing. As can be seen from Figure 36, at 12 hr of experiment duration 72.2 ± 0.7% of palladium was recovered. However, the recovery performance increased with the duration of the experiment through constant introduction of the effluent. The analysis of the samples taken at 18 and 24 hrs showed that 87.5 ± 3.6% and 99.8 ± 0.1% of palladium, respectively, were recovered from the solution (Figure 36). Hence, an almost complete recovery of palladium was achieved at 24 hrs of experiment duration.

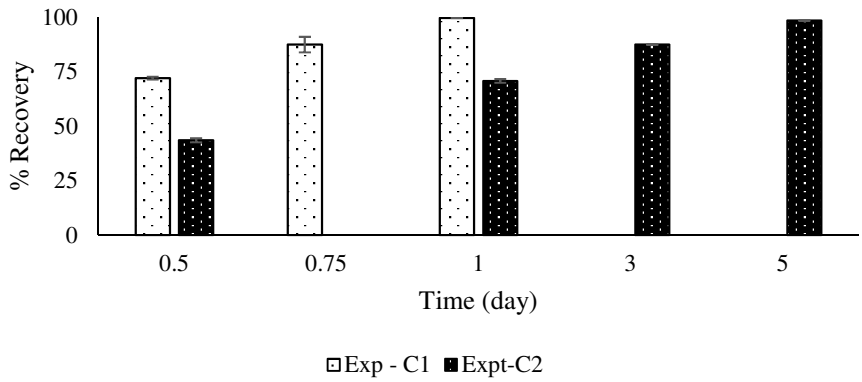


Figure 36: Percentage of palladium (experiment C1 – 94.6 ± 1.8 mg/L starting concentration) and iron (experiment C2 – 246.1 ± 8.5 mg/L starting concentration) recovered from the stripped and scrubbed solution, respectively, in the continuous flow assay.

Similar pH changes as observed in the batch assays were measured in this experiment (C1). Thus, the pH was changed from 6.9 ± 0.4 to 2.9 ± 0.7. This decrease in pH might be attributed to the reaction of Pd(II) with sulphide and to the lower pH value of the Pd(II) solution, as discussed above in the case of the batch assays. The lower pH affects the equilibrium of the sulphide species favouring the formation of H_2S species, which might be lost through volatilization.

Although some of the sulphides can be lost in operation, the amounts of sulphide reaching the metal solution were enough to precipitate Pd(II) as PdS in the sample bottle. Moreover, the increase in the concentration of sulphide in the final solution was, perhaps, an indication of the complete recovery of palladium, provided the low solubility of PdS. During the operation, the concentration of Pd(II) in the medium decreased whereas biogenic sulphides was contentiously added. As a result, the total volume of the solution was increased, which resulted in decreasing the access for the contact between available Pd(II) and incoming sulphide. Hence, more sulphide solution was needed to precipitate the remaining Pd(II). This might be the reason for the consumption of a large volume of effluent.

Regarding the recovery of iron in experiment C2, at the beginning of the operation a yellow coloured suspended solid was observed, which remained until the end of the operation (Figure 37). As previously reported by other authors (Bhagat, et al., 2004), the observed colour might be due to the reduction of sulphide to elemental sulphur. The report indicated that, Fe(III) oxidizes sulphide to elemental sulphur being itself reduced to Fe(II). Subsequently, the reduced Fe(II) combines with the incoming sulphide to form insoluble black FeS precipitate (Bhagat, et al., 2004). The solution in the sample bottle contained a mixture of black and yellow coloured

solid particles, which might be consistent with the presence of both elemental sulphur and iron sulphide. As utilised in experiment B2, a sulphide to Fe(III) ratio of 2.5:1 was enough for the complete removal of iron from the solution. However, oxidation of sulphide to elemental sulphur by Fe(III), probably contributed for the increased consumption of sulphide than stoichiometrically required for Fe-precipitation. As a result of this, more sulphide was introduced, which made the sulphide to Fe(III) ratio to be greater than expected.

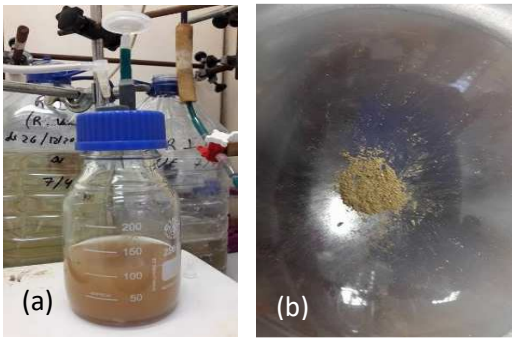


Figure 37: Picture of the recovery of Fe coupled to the bioremediation system: (a) effluent addition to Fe(III) solution and (b) iron precipitate collected.

A gradual increase in the recovery of Fe was observed in experiment C2. As can be seen from Figure 36, at 12 hr of duration, 43.6 ± 1.0 % of iron was recovered from the solution. This low recovery efficiency might be attributed to the consumption of added sulphide for the conversion of Fe(III) to Fe(II), thus, causing lower recovery efficiency due to the lower availability of sulphide species for interacting with the metal ions in the solution. However, the recovery was steadily increased having an almost complete recovery at the fifth day of operation – leaving trace amounts.

The decrease in pH was observed during the recovery of Fe in experiment C2. Thus, the pH changed from 7.0 ± 0.2 to 3.1 ± 0.6. The pH of the initial aqueous solution of Fe(III) was 1.7 ± 0.7, which contributed for the decrease in pH during the assay. As discussed above in the batch experiments, the reaction between the metal ions and sulphide might have contributed for the pH change.

Thus, experiment C revealed that, the effluent from the bioremediation process was capable to recover palladium and iron from the solutions resulting from the stripping and scrubbing stages of solvent extraction, respectively. An efficient and complete recovery of palladium and iron was achieved within 1 and 5 days of experimental duration, respectively. The technique employed for the recovery of palladium from the aqueous solution was environmentally

sustainable and promising, as it involves wastes for the generation of sulphide, which played an active role in metals recovery.

To find out the statistical relation between the mean results, one-way ANOVA and pair-wise comparison of mean were performed, for the recovery of palladium and iron using three approaches (experiment A, B and C). Accordingly, statistical evaluation of metals recovery based on the acidity of the feed solution (2, 4 and 6 M HCl feed solutions) indicated that at 95% confidence level there is no significant difference ($p > 0.05$) between the mean recovery of palladium in experiment A1 and B1. The same conclusion was obtained for iron recovery employing experiments A2 and B2. Conversely, for the statistical evaluation based on the approaches employed, there is 95% ($p < 0.05$) chance that a significant variation exists for the mean recovery of palladium employing experiment A1, B1 and C1. Furthermore, the pair-wise mean comparison indicated that at 95% confidence level, the significant variation of mean for palladium recovery results were originated from all the three approaches engaged. Similarly, significant difference ($p < 0.05$) exists between the mean iron recovery results at 95% confidence level for all three methods employed. According to the pair-wise mean test, at 95% confidence level the difference arises from experiment A2 and B2 (Figure 38).

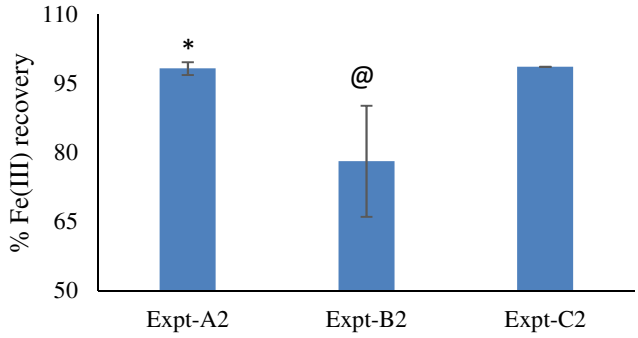


Figure 38: Statistical variation among methods employed for iron recovery; experiment A2, experiment B2 and experiment C2 (* significant difference with Expt-B2, @ significant difference with Expt-C2).

3.3 Precipitate characterization

In all the above bio-recovery experiments conducted, the formed precipitates were collected by centrifugation. Then, the precipitates were washed three times with deionized water followed by 70% ethanol. The solids were dried in a desiccator under vacuum at room temperature and grinded to powder before characterization.

3.3.1 Characterization of Pd-precipitate

To assess the mineralogical and chemical composition of the precipitates, X-ray diffraction and TEM-EDS analysis were performed. However, the diffractogram obtained from X-ray diffraction analysis were not conclusive, since they contained numerous peaks that could not be assigned to specific crystalline structures (data not shown). On the other hand, the TEM/EDS analysis revealed valuable information about the morphology and chemical composition of the collected precipitates.

The Pd precipitate obtained from experiment A1 was amorphous and composed of aggregated particles as revealed by the micrographs in Figure 39 (a and b). These results are similar to those obtained by Assunção, et al.(Assunção, et al., 2016a). The EDS spectrum (Figure 39c) shows that the precipitate was only composed by Pd and S, since the intense peaks displayed belong to those elements. Nonetheless, the analysis of the atomic percentages indicated that the ratio of Pd to S was different in various regions of the precipitate. However, the majority of the analysis resembled to atomic percentages of 33.4% and 66.6% for Pd and S, respectively, which corresponds to an approximate stochiometric ratio of 1Pd to 2S, greater than expected for PdS, this can be due to the presence of elemental sulphur. Thus, the precipitate probably contains elemental sulphur in addition to PdS. The excess S in comparison to Pd might be due to the excess sulphide used to guarantee a complete precipitation of Pd(II). The TEM image shows particles with a spherical morphology with sizes ranging between 42 to 103 nm, which is compatible with nanosized particles (Figure 39). Therefore, the evidences suggested that the precipitate contains PdS nanosized particles. An intense peak corresponding to copper was also identified in the EDS spectrum, which was due to the composition of sample grid.

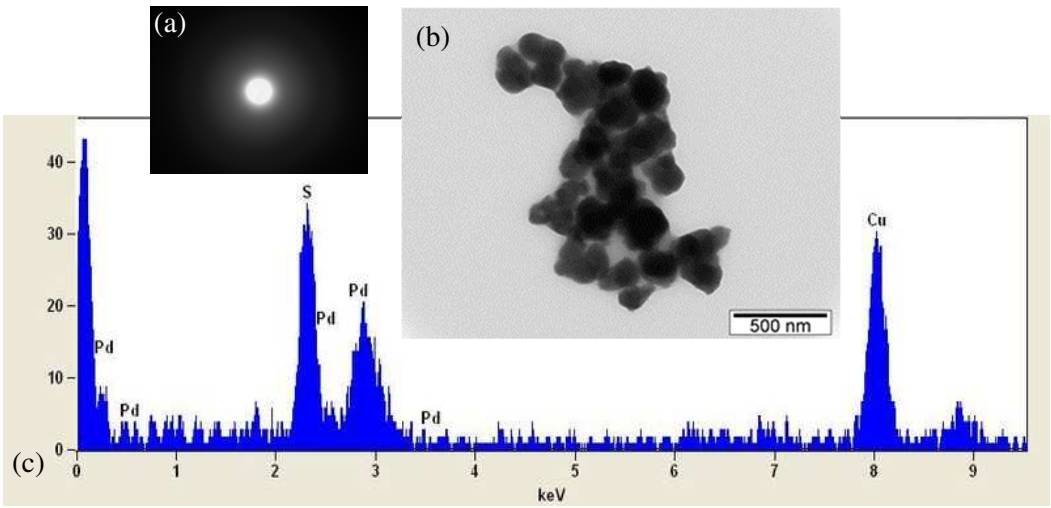


Figure 39: XRD (a), TEM image (b) and EDS spectrum (c) of the Pd-precipitate obtained from experiment A1.

The Pd-precipitate obtained from experiment B1 was crystalline as indicated by the XRD image (Figure 40a). The TEM micrograph shows aggregate particles with shape corresponding to spherical morphology having particle size ranging from 51 to 147 nm (Figures 40b). Hence, this indicates that the synthesised precipitate was composed of nanocrystalline particles with some particles above the nano-range. Similar morphological shape and sized was reported for PdS nanosized particle synthesised using biogenic sulphide from Postgate E medium (Assunção, et al., 2016a). As revealed by the EDS spectrum, S and Pd were the only elements detected in the precipitate (Figure 40c). The atomic percentage varies depending on the region of the precipitate. However, most of the regions have atomic composition of 36.0% Pd and 64.0% S, corresponding to an approximate stochiometric ratio of 1Pd to 2S. The larger atomic percentage of S, in relation to Pd, might be due to excess sulphide used to guarantee a complete precipitation of Pd(II). Hence, some elemental sulphur might be present in the precipitate and it may result from sulphide oxidation. Thus, the results suggest that the precipitate contains both nanocrystalline PdS and elemental sulphur.

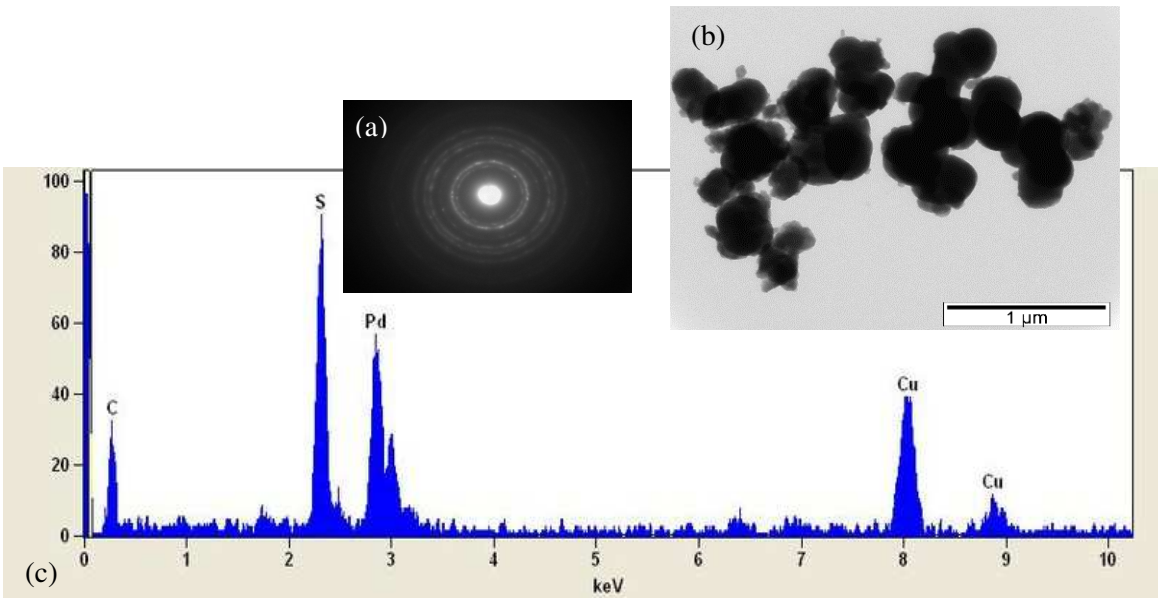


Figure 40: XRD (a), TEM image (b) and EDS spectrum (c) of the Pd-precipitate obtained from experiment B1.

The Pd-precipitate obtained from experiment C1 was crystalline as indicated by the XRD image (Figure 41a). The TEM image revealed that the particles were composed of aggregates having shapes corresponding to spherical morphology with particle size ranging from 30 to 73 nm (Figures 41b). Hence, this indicates that the synthesised precipitate was composed of nanocrystalline particles. As displayed by the EDS spectrum, S and Pd were the only elements detected in the precipitate (Figures 41c). The atomic percentage of Pd and S was 44% and 56%,

respectively, which corresponds approximately to a stochiometric ratio of 0.8Pd to 1S, close to 1:1, which is consistent with the synthesis of nanocrystalline PdS (Figure 41c). Taking into account that no other elements were detected in the EDS spectrum except Pd and S, the result suggests that the synthesised nanocrystalline PdS should be ready for future potential application without the need of further purification. Copper and carbon were also detected in the EDS spectrum, which were originated from the sample supporting grid as they are present in the background area.

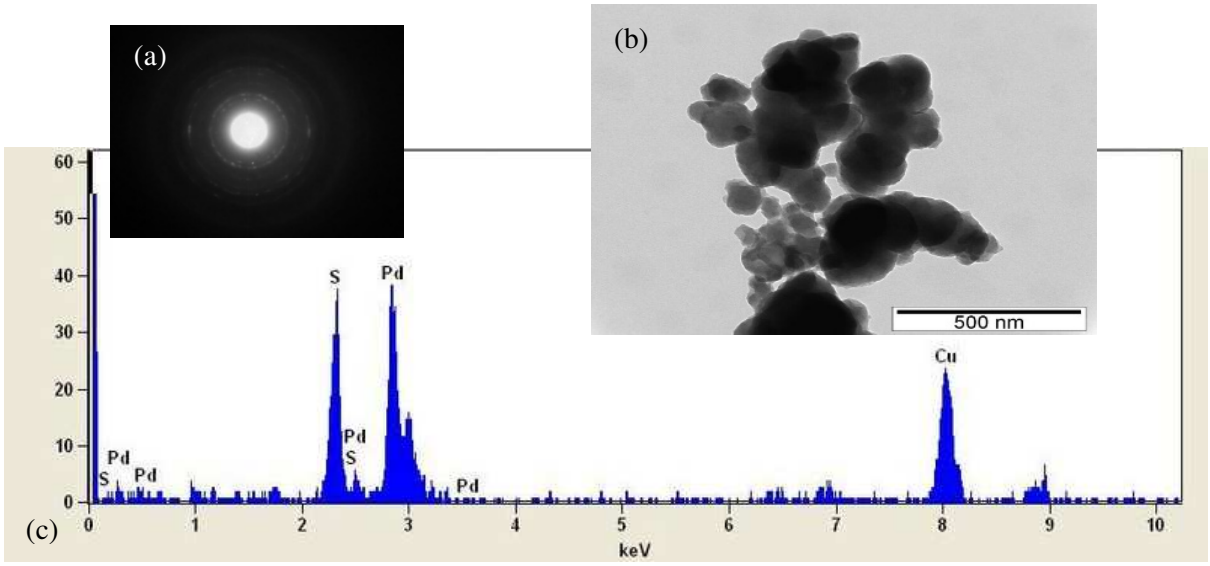


Figure 41: XRD (a), TEM image (b) and EDS spectrum (c) of the Pd-precipitate obtained from experiment C1.

A previous study reported an excellent extraction of Pd(II) using DMDCHTDMA with subsequent quantitative stripping employing HCl and seawater from the feed aqueous solution (Assunção, et al., 2016a). The researchers noticed a quantitative recovery of palladium in less than four days, employing biogenic sulphide from Postgate E medium. Furthermore, the study also indicted the synthesis of nanosized PdS particles with other elemental impurities detected. In the present study a different Pd(II) extractant, DMDCHTDGA, was used as well as a different metal stripping solution, acidic thiourea. The results revealed an excellent Pd(II) extraction and stripping efficiency of DMDCHTDGA and acidic thiourea, respectively. The investigation also showed a complete recovery of palladium from the stripping solution in less than 48 hrs by the addition of biogenic sulphide generated by SRB in Postgate B medium and in a bioremediation process for AMD treatment. Additionally, the particles were composed of Pd and S with no other elemental impurities – proving the synthesis of pure nanosized PdS. Concerning the morphological shape and size of the particles, both the present and Assunção, et al. study revealed almost similar morphology and size. Furthermore, in the present study, the precipitate obtained using the effluent from the AMD bioremediation process is composed of

PdS nanocrystalline particles. In contrast, an amorphous precipitate was obtained by employing biogenic sulphide generated in Postgate B medium, which is a similar result to that one reported by Assunção, et al. nevertheless with sulphide generated in Postgte E medium (Assunção, et al., 2016a).

3.3.2 Characterization of Fe precipitate

For determination of the morphology and elemental composition of the iron-containing precipitates collected from experiments A2, B2 and C2, TEM-EDS and XRD analysis were performed. The results of the analysis are displayed in Figures 42 to 44. As revealed by the XRD all the precipitates are amorphous. Moreover, they are composed of aggregate particles with various morphologies and sizes.

A sheet like shape was displayed by TEM micrograph of the precipitate collected from experiment A2 (Figure 42a). A TEM image with a similar shape was reported for nanocrystalline FeS (Ohfuji & Rickard, 2006; Dai, et al., 2009). The wrap and crackle (such as marked arrows in Figures 42) makes the determination of the exact size of the particles to be difficult. Nevertheless, they appeared in the range between 30 to 70 nm. The EDS spectrum revealed that Fe, S, O, Ca and P were detected in the precipitate (Figure 42c). Variable atomic percentages were observed in different region of the precipitate, which makes the determination of the possible combination of the detected elements to be ambiguous. However, the EDS spectrum displays an atomic ratio of Fe to S of about 0.91:1, suggesting the synthesis of FeS nanoparticle. There might also be the possibility for the precipitate to contain oxides and hydroxides of Fe.

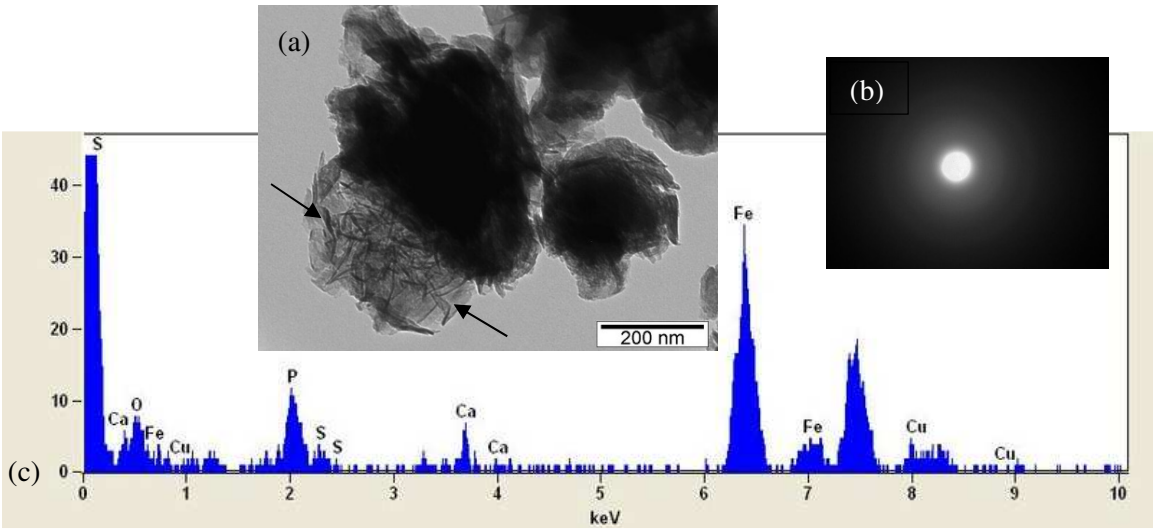


Figure 42: TEM image (a), XRD (b) and EDS spectrum (c) for the Fe-precipitate obtained from experiment A2.

Ca, O and P might be originated from the chemical composition of Postgate B medium used to generate biogenic sulphide. The peak corresponding to Cu on the EDS spectrum was introduced from the sample grid.

Regarding the Fe-precipitate collected from experiment B2, XRD revealed its amorphous nature (Figure 43b). The TEM displayed a similar morphology to that one presented by the precipitate collected from experiment A2, having particle size ranging from 50 to 147 nm (Figure 43a). This suggests that the precipitate is composed of a mixture of nanosized particles and particles above the nanorange. The EDS spectrum displayed peaks assigned to Fe, S, O, and Cu (Figure 43c). The most intense peak of the spectrum corresponds to Fe. Cu and O might be originated from the AMD and winery waste used in the bioremediation system. Variable atomic percentages were observed depending on the different region on the precipitate. However, an atomic ratio of Fe to S of about 1:3.5, suggests the synthesis of either FeS or FeS_2 nanoparticles, and elemental sulphur, which is also supported by the dark yellowish colour of the precipitate, as discussed above. Nevertheless, oxides and hydroxides of Fe might also exist. As the precipitate was analysed using a Ni sample grid, the spectral peak corresponding to Ni also appears. The percentage of atomic O is greater than all other elements detected. It is not clear if O is associated either with Fe, or with other elements in the precipitate.

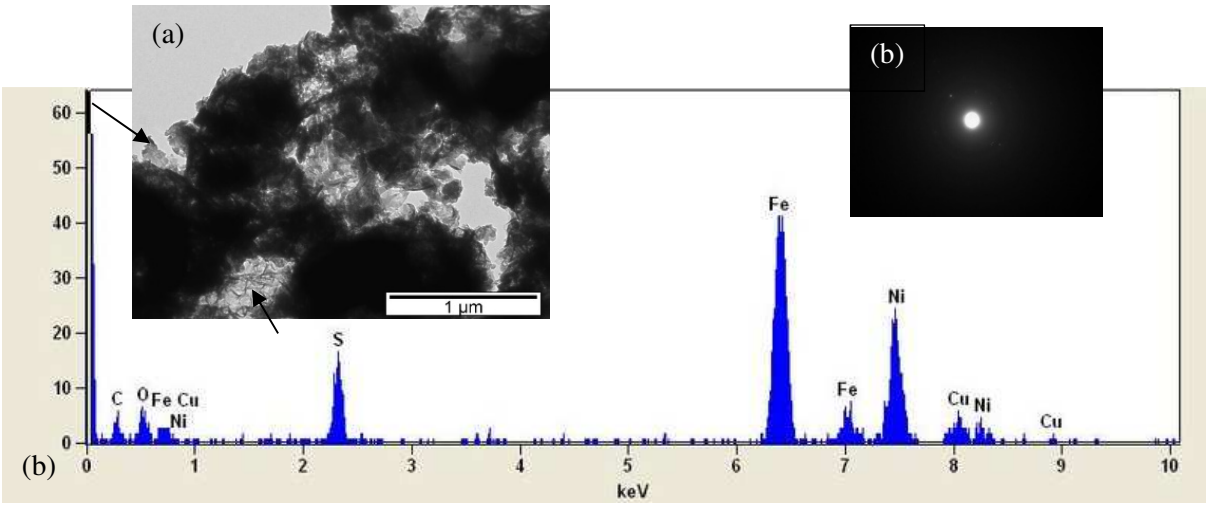


Figure 43: TEM image (a), XRD (b) and EDS spectrum (c) for the Fe-precipitate collected from experiment B2.

Concerning the iron precipitate collected from experiment C2, TEM micrograph revealed that it is composed of clusters (aggregates) of particles. Moreover, the precipitate was composed of particles with various morphologies and sizes, of which spherical and nanorod are the major contributors (Figure 44a). The determination of the exact size of the particles is difficult due to

overlapping, although size ranges from 57 to 187 nm were determined. Similar TEM images containing nanorods were assigned to nanocrystalline FeS_2 synthesized in ethylenediamine (Xuefeng, et al., 2001). The spot profile of EDS spectrum showed that the precipitate was composed of Fe, O, Cu and S (Figure 44c). O and Cu might be originated from AMD and winery waste used in the bioremediation process. O has the highest atomic percentage among the detected elements. Although the association of O with other elements is difficult to determine, the precipitate might contain oxide and hydroxides of Fe. Peaks assigned to Ni appear in the spectrum and are originated from the sample grid. The correlation between the elements detected in the spectrum is ambiguous, as the atomic percentages vary in different regions of the precipitate.

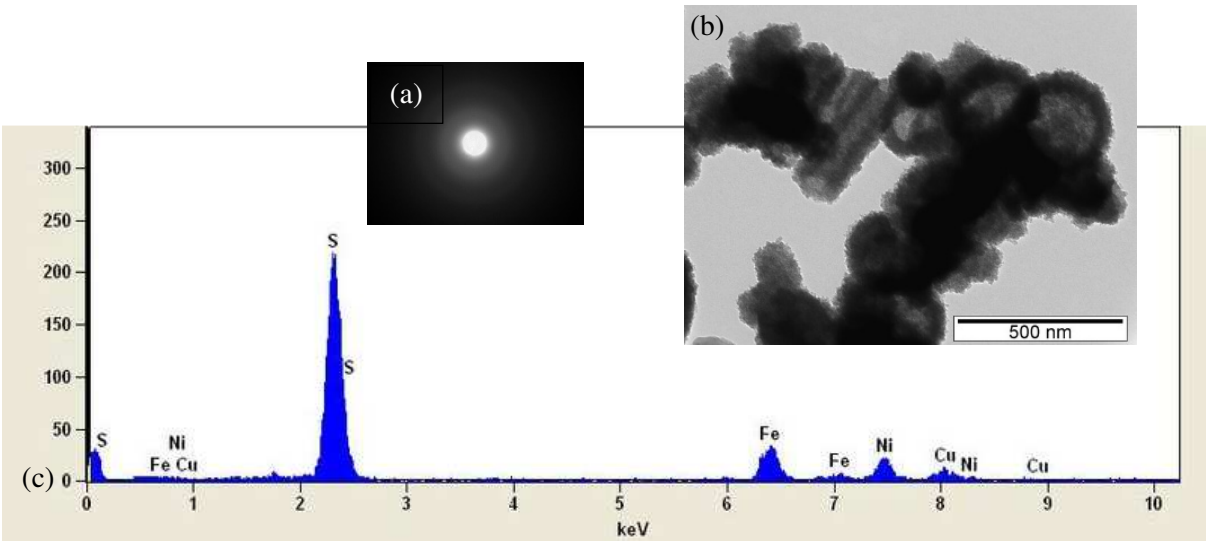


Figure 44: XRD (a), TEM image (b) and EDS spectrum (c) for the Fe-precipitate collected from experiment C2.

Ultimately, this study demonstrated the efficiency and effectiveness of the recovery of palladium and iron by combining liquid-liquid extraction with biological techniques based on the use of SRB communities. Liquid-liquid extraction technique, involving a suitable organic extractant (DMDCHTDGA), was employed to selectively pick-out the target metal ion (Pd(II)) from complex matrices containing elements such as La(III), Fe(III), Cu(II), Ni(II) and Zn(II). However, co-extraction of Fe(III) was observed, which was removed by scrubbing the loaded organic phase with deionized water. Then, the target metal ion, Pd(II), was transferred from the metal loaded organic phase to a new purified aqueous phase through stripping with acidic thiourea solution. Biological techniques based on the use of SRB communities were then employed for the recovery of palladium and iron from the stripped and scrubbed solution, respectively. Accordingly, a SRB community enriched from sludge from a WWTP was used for the generation of sulphide, which played a significant role in the metals recovery process.

The biogenic sulphide from both origin, Postgate B medium and contained in the effluent of a bioremediation process for AMD treatment, showed good affinity for the recovery of palladium and iron from aqueous solutions. A complete recovery of palladium and 78 – 99% recovery of iron from aqueous media were achieved. Henceforth, successful synthesis of PdS nanoparticles was achieved. Thus, biogenic sulphide is an excellent alternative to conventional techniques for the recovery of those metals. The "raw materials" employed for the generation of sulphide by SRB in the bioremediation process were AMD and winery waste as sulphate and carbon sources, respectively. Therefore, the technique utilized wastes for the recovery of valuable materials from metal-bearing effluents (waste-to-waste approach), which is an environmentally safe, sustainable, cheap and easy approach. The employed technique also reduces the cost needed for the cultivation of bacteria by avoiding the use of commercial chemicals. Therefore, the combination of solvent extraction with biological techniques, based on the use of SRB, helps to achieve efficient recovery of metals from aqueous solutions.

3.4 Possible applications of metal-sulphide

Due to their recognised catalytic activity, low cost, abundance, easy manufacture and adequate transport properties (mainly low electrical resistivity) metal sulphides are used in various areas (Barawi, et al., 2014). According to the literature (Ermakova, et al., 2002), PdS showed increasing selectivity toward the catalysis of tetrahydrothiophene. Chen et al., 2013 reported the synthesis of nanostructured PdS by *in-situ* coprecipitation and demonstrated its highest photocatalytic activity for hydrogen evolution under visible light irradiation from an aqueous solution containing sulphide and sulphate. The same report indicates that PdS synthesized through a hydrothermal technique demonstrated high stability for hydrogen evolution (Chen, et al., 2013). Due to its adequate optical and transport properties, nanostructure PdS was employed as a photoanode in photoelectrochemical cells for hydrogen evolution (Barawi, et al., 2014)**.** PdS was also employed as co-catalyst to enhance the photocatalytic activity of CdS for hydrogen production from water. Accordingly, an efficient activity for the generation of hydrogen was reported. Ermakova et al., 2002, reported the selective catalysis activity of PdS for the synthesis of tetrahydrothiophene through liquid-phase and gas-phase hydrogenation of thiophene (Ermakova, et al., 2002; Zirka & Mashkina, 2000).

Regarding FeS, it has got wide application in biosensors, biotechnology and environmental chemistry due to its special properties, such as good stability and ease of production (Dai, et al., 2009). A study by Dai, et al revealed that FeS has got a novel application in areas such as

biosensing and biocatalysis due to its specific electron transfer ability, good absorption and lower band gaps than FeO. The researchers also noticed the intrinsic performance of peroxidase-like activity of the sheet-like FeS nanostructure, which was confirmed by the ability to catalyse the oxidation of organic substrates. The sheet-like nanostructured FeS can also be used as an “artificial peroxidase” for the potential development of amperometry transducers and biocatalysts (Dai, et al., 2009).

4 CONCLUSION

A liquid-liquid extraction technique involving DMDBTDGA and DMDCHTDGA in toluene as organic phases, was tested to check the efficiency and selectivity of both extractants towards the recovery of Pt(IV) and Pd(II) from HCl media. The results revealed that both organic phases showed a better preference for the extraction of Pd(II) over Pt(IV). However, decreasing extraction performances for Pd(II) were observed with increasing HCl concentrations of feed solutions. For the extraction of Pt(IV), DMDCHTDGA showed a better efficiency than DMDBTDGA. Regarding stripping, seawater showed poor efficiencies to strip both metal ions. Attempts to find a suitable stripping agent to separate Pt(IV) prior to Pd(II) from loaded DMDCHTDGA were unsuccessful.

DMDCHTDGA showed an excellent performance for the extraction of Pd(II) from binary, tertiary and more complex metallic HCl feed solutions. However, the co-extraction of Fe(III) and other metal ions increased with increase in acidity of the feed solutions. The former metal ion was quantitatively cleaned-up by scrubbing with deionized water. A significant recovery of Pd(II) was achieved in the subsequent stripping using acidic thiourea solution. None of the other impurities appeared either in the scrubbing stage with deionized water or in the stripping using acidic thiourea. The developed liquid-liquid extraction schemes involving complex aqueous media containing six metal ions at 2, 4 and 6 M HCl were successfully reported to produce acidic thiourea solutions containing about ~100 mg/L Pd(II).

For the final recovery of metals, chemical strategies need commercial reagents which are not cost effective and eco-friendly. To overcome this limitation, biological techniques employing naturally existing bacterial communities were explored as possible alternatives. Hence, biologically generated sulphide from Postgate B medium and from a bioremediation process for AMD treatment were used for the recovery of metal ions. The experiments were conducted in batch and in continuous. Successful palladium and iron recovery was achieved. TEM analysis revealed that the precipitate is composed by nanosized particles. Furthermore, EDS and atomic percentage analysis suggest that the collected precipitates from both assays were composed of Pd and S only, which is consistent with the formation of nanosized PdS particle, although Pd and S ratio lower than 1, also suggests the presence of elemental sulphur. Regarding iron, the precipitate obtained from both assays are amorphous and aggregated particles. The elemental analysis of iron precipitate showed the presence of other metals besides Fe and S.

The fact that waste materials and naturally occurring organisms were employed for the recovery of metals, make this technique to be cost effective and eco-friendly with ease of operation. Thus, this study explored the efficiency and "greenness" of the technique employed for the recovery of Pd as PdS nanosized particles. Likewise, Fe was also effectively recovered using biogenic sulphide from the scrubbing solution produced during the solvent extraction scheme.

Future Perspective: In relation with the wide application and demand of PGMs in the current high-techs, biologically mediated recovery from waste can be an environmentally and economically appreciable approach. This is more meaningful, if the recovery process ends up in the synthesis of nanoparticles. The complex composition of the secondary sources makes the recovery of PGMs more difficult. In this regard, the present study was focussed on the selective extraction and isolation of the metals by solvent extraction followed by metal recovery through a biologic approach. Based on the results achieved, employing the techniques explored in this study to real metal bearing effluents would result in the successful recovery of palladium. Furthermore, taking into account the effectiveness of the effluent from the bioremediation process for the recovery of Pd, it is strongly recommended to investigate the possibility of using a similar approach for the recovery of other PGMs. During the characterization of the obtained precipitates aggregates of particles affected the morphological and size determination of the nanoparticles. Hence, the possibility of using disaggregation techniques during or subsequently to the synthesis procedure should be explored. The potential applications of the nanoparticles obtained in this study should be tested further. Taking into account that there might be differences in the properties and in the potential applications of the nanoparticles depending on the way they were synthesised, via chemical and biological techniques. Thus, exploring potential applications of the biologically synthesised nanoparticle, not only for the applications already explored, but also for new applications, such as photocatalytic activity is required. Regarding the precipitates obtained through the recovery of Fe, the results obtained in this study were ambiguous. Thus, the application of further characterization techniques such as X-ray photoelectron spectroscopy, scanning electron microscopy (SEM) in addition to those already applied should be done.

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APPENDIX

1. Calibration curves

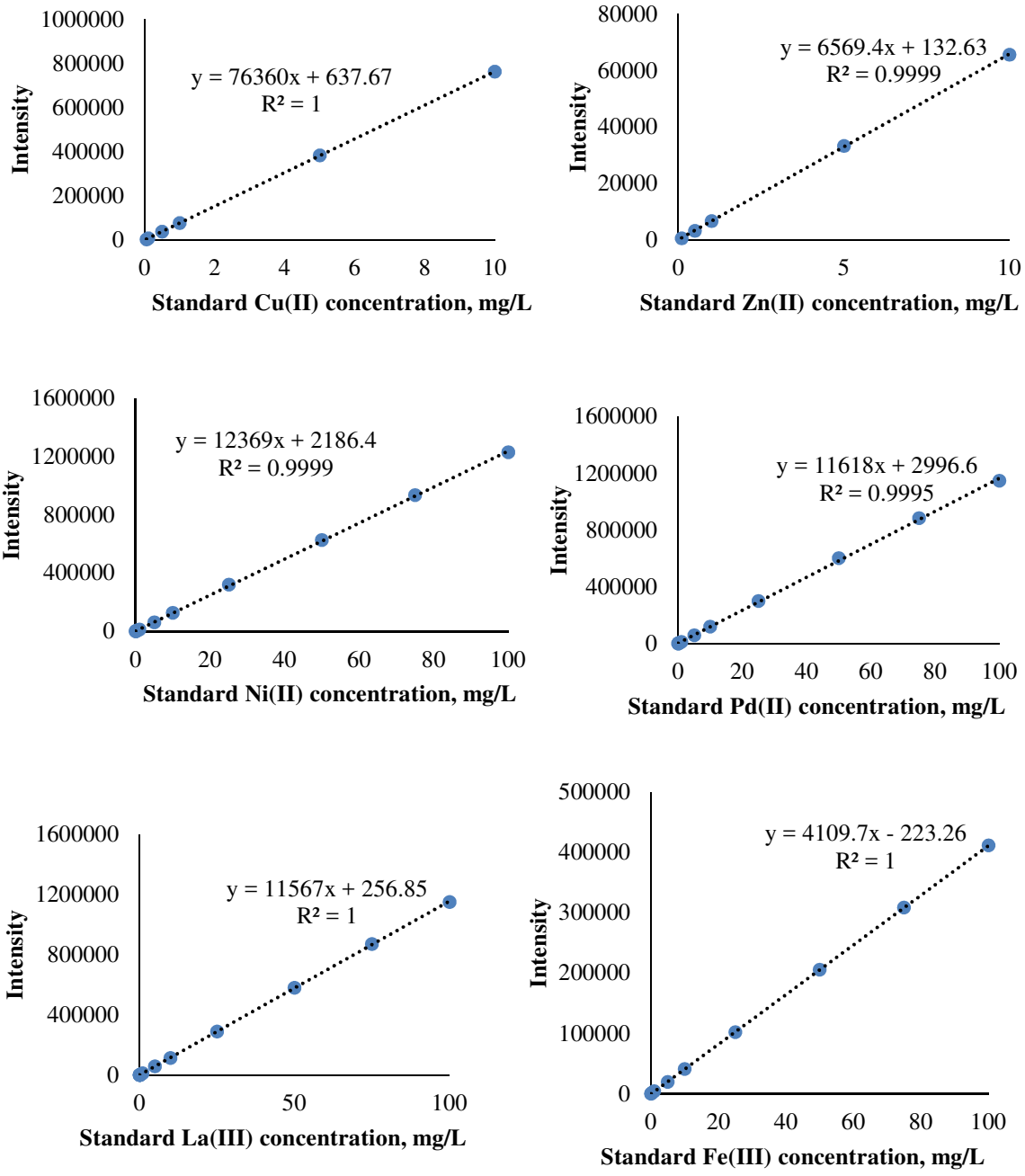

Figure A1: Example of calibration curve for (a) Cu(II), (b) Zn(II), (C) Ni(II), (d) Pd(II), (e) La(III) and (f) Fe(III) obtained by MP-AES for determination of metal contents.

2. X-ray powder diffractograms of Pd and Fe precipitates obtained during bio-recovery from aqueous solution resulting from stripping and scrubbing stage in solvent extraction, respectively, through employing biogenic sulphide.

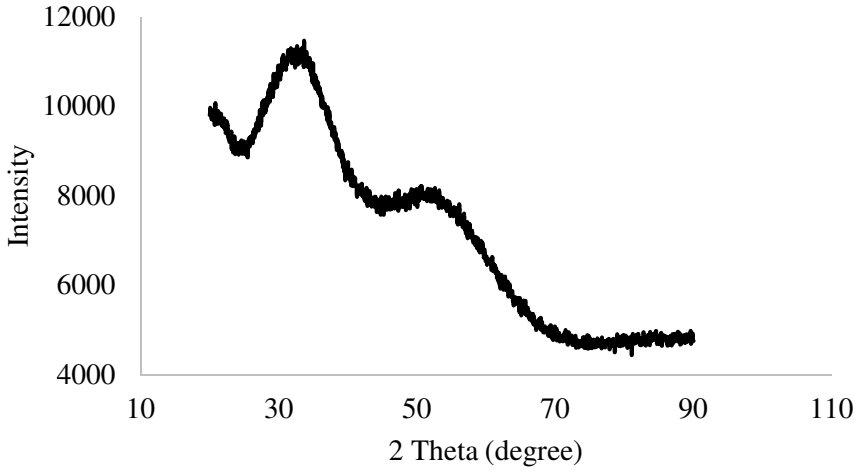

Figure A2: X-ray powder diffractogram of Pd precipitate obtained from batch experiment using Pd(II) solution resulted from stripping stage in solvent extraction and Postgate B nutrient medium containing biogenic sulphide after 28 days of inoculation.

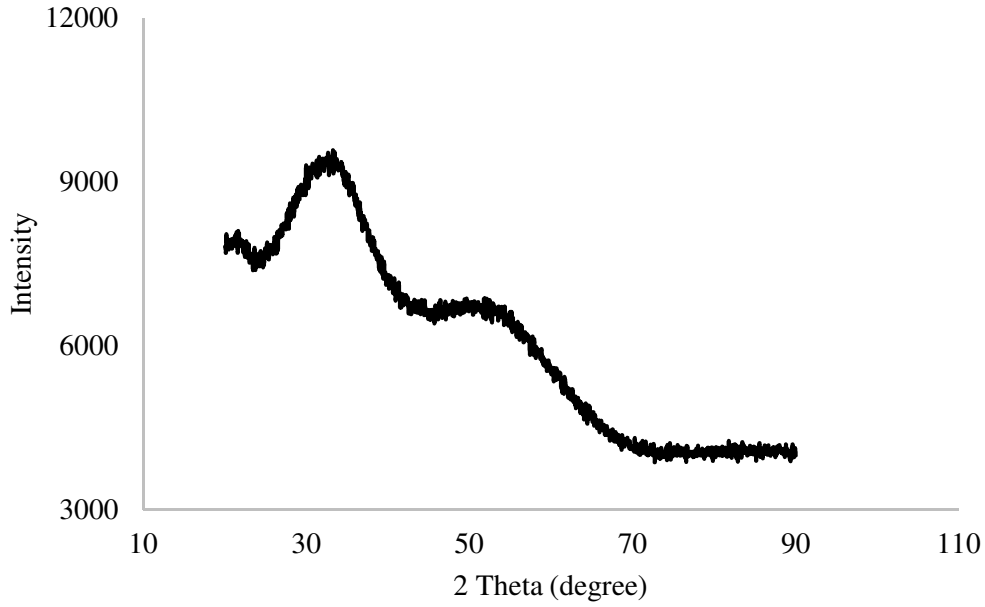

Figure A3: X-ray powder diffractogram of Pd precipitate obtained from batch experiment using Pd(II) solution resulted from stripping stage in solvent extraction and effluent containing biogenic sulphide from bioreactor of the bioremediation process for AMD treatment.

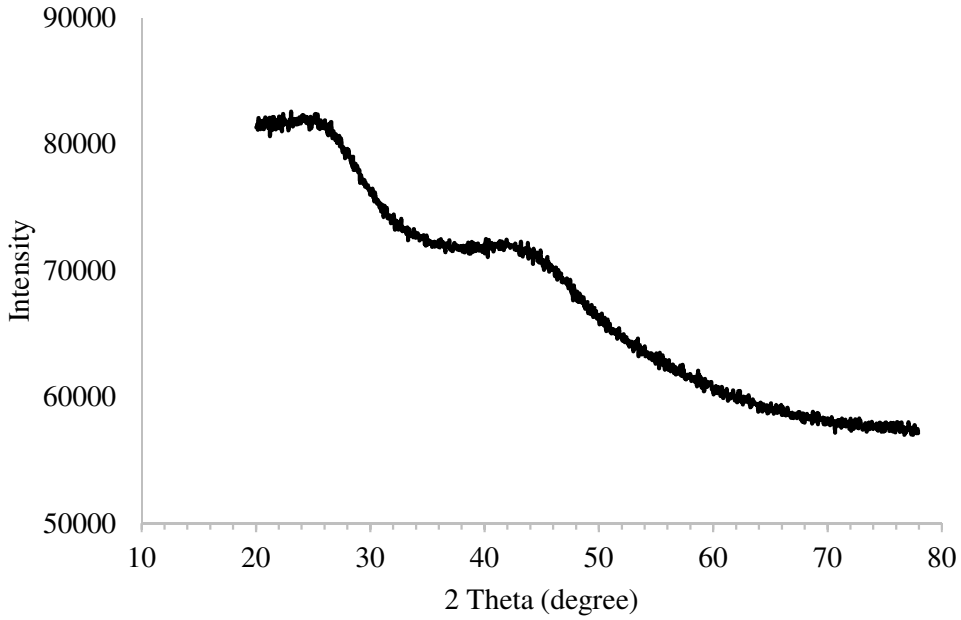

Figure A4: X-ray powder diffractogram of Fe precipitate obtained from batch experiment using Fe(III) solution resulted from scrubbing stage in solvent extraction and Postgate B nutrient medium containing biogenic sulphide after 28 days of inoculation.