
Master Thesis : Application of Bacterial Bioleaching for Gold Recovery from Refractory Pyritic Flotation Tailings (including an introduction to research methodology)

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Research on Refractory Gold Recovery from BERALB Sh.A. Flotation Tailings by Bio-oxidation and Thiourea Leaching

Thesis presented to obtain the degree of :

**Master of Science in Advanced Materials and
Innovative Recycling**

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Abstract

The recovery of gold from sulphide-bearing flotation tailings is challenging because gold may remain finely disseminated or associated with sulphide minerals, limiting its accessibility to conventional leaching. This study investigated a combined bio-oxidation and thiourea-leaching route to improve gold extraction from flotation tailings.

The flotation tailings were characterised by particle-size analysis, X-ray diffraction (XRD), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDS), and Au deportment analysis. Bio-oxidation was investigated using shake-flask cultures and five mechanically stirred reactor experiments. Changes in pH, oxidation-reduction potential (Eh), and dissolved Fe, S, and Zn were monitored to evaluate the treatment. The results were consistent with oxidation and dissolution of sulphide-bearing phases, although no simple relationship was observed between bulk Fe or S dissolution and subsequent Au extraction.

Following bio-oxidation, the residues were subjected to 6 h thiourea leaching under acidic conditions. Direct leaching of untreated flotation tailings was used as a reference, and the influence of ferric-ion addition was examined in paired tests. Au was not detected in the pregnant leach solution samples; therefore, extraction was evaluated from initial and final solid-phase Au grades and is reported as apparent Au extraction.

The untreated flotation tailings, containing 0.81 ppm Au, produced an apparent Au extraction of 18.5%. Bio-oxidised residues from Reactor Experiments 1, 2, and 3 yielded 24.7%, 22.2%, and 27.2%, respectively, with Experiment 3 providing the highest extraction. Ferric-ion addition increased apparent extraction from 13.6% to 19.8% in the paired Experiment 4 tests, while the pyrite-rich concentrate yielded 22.9%.

Overall, bio-oxidation produced a modest improvement in apparent Au extraction under selected conditions, but the maximum extraction remained below 30%. The absence of quantifiable Au in the liquid phase prevented establishment of a complete Au mass balance, indicating that further optimisation of the bio-oxidation and thiourea-leaching conditions and improved analytical sensitivity are required.

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1. CHAPTER 1

INTRODUCTION

1.1 Background and Context

Gold is an important metallic resource whose value extends beyond jewellery and investment. Its chemical stability, high electrical conductivity and resistance to corrosion support applications in electronics, advanced materials and other technologies. At the same time, depletion of easily processed high-grade deposits has increased the importance of lower-grade and mineralogically complex resources [1,2,51].

Mineral-processing operations generate large quantities of tailings after the recovery of the principal economic minerals. These materials may retain residual valuable metals that were not recovered during the original beneficiation process. Improvements in characterisation, mineral processing and hydrometallurgy have therefore renewed interest in tailings as potential secondary resources rather than only as wastes [1,4].

Recent studies have demonstrated the technical feasibility of recovering both base metals and gold from old copper-sulphide flotation tailings through combined leaching and bioleaching approaches [45].

Gold recovery from flotation tailings can be particularly difficult when gold is associated with sulphide minerals. Pyrite and arsenopyrite can physically encapsulate fine gold particles, while gold may also occur as submicroscopic or structurally bound “invisible” gold. In these cases, direct leaching is limited because the lixiviant cannot adequately contact the gold-bearing domains [1,2,5,6].

An oxidative pretreatment may therefore be required to modify the sulphide matrix before gold leaching. Bio-oxidation is attractive because acidophilic microorganisms can promote the oxidation of ferrous iron and reduced sulphur species under acidic conditions, thereby contributing to the breakdown of sulphide minerals at comparatively moderate temperature and pressure [14,15,20,41].

This thesis investigates bio-oxidation as a pretreatment for sulphide-bearing flotation tailings prior to acidic thiourea leaching. The overall route consists of biological oxidation of the sulphide-bearing material followed by chemical leaching of the resulting solids with thiourea.

1.2 Gold Association with Sulphide Minerals

The response of a gold-bearing material to hydrometallurgical treatment depends strongly on gold deportment. Gold may occur as liberated particles, at mineral boundaries, as inclusions within other minerals, or as invisible gold within sulphide phases. Particle size,

mineral association and degree of liberation therefore influence the selection of comminution, concentration, pretreatment and leaching operations [1,2,5,6].

Pyrite is especially relevant in refractory and partially refractory gold resources. Gold associated with pyrite may occur as microscopic inclusions, nanoparticles or structurally incorporated species. Grinding can increase exposed surface area, but it may not fully liberate gold that occurs at scales below practical grinding limits [2,5,6].

The mineralogical characteristics of the feed must therefore be established before selecting a treatment route. Oxidative pretreatment can alter pyrite and other sulphide minerals and may expose gold-bearing regions that were previously inaccessible. Available pretreatment technologies include roasting, pressure oxidation, ultrafine grinding and biological oxidation, with process selection depending on mineralogy, sulphide content, particle size, scale and economic constraints [1,2,4].

For low-grade secondary materials such as flotation tailings, bio-oxidation is of particular interest because it operates under relatively mild conditions. Its performance nevertheless depends on microbial activity, oxygen transfer, nutrient availability, solution chemistry and effective contact between the microorganisms and mineral surfaces [14,15,20,41].

1.3 Bio-oxidation of Sulphide Minerals

Bio-oxidation uses acidophilic iron- and sulphur-oxidising microorganisms to generate and maintain oxidising conditions in sulphide-bearing systems. These microorganisms can regenerate ferric iron and promote formation of sulphuric acid, both of which contribute to mineral breakdown and metal release [14,15,20].

In pyrite-bearing systems, ferrous iron is biologically oxidised to ferric iron. Ferric iron then participates in chemical oxidation of the sulphide mineral, while microbial activity continuously regenerates the oxidant. Current understanding describes these reactions through indirect mechanisms involving thiosulphate or polysulphide intermediates rather than direct enzymatic electron transfer from the mineral to the cells [21].

Bio-oxidation can therefore produce measurable changes in dissolved Fe and S concentrations, pH and oxidation-reduction potential. These parameters are useful for monitoring process development, although their interpretation must also consider solution replacement, pH correction and the initial composition of the process medium [14,20,21].

Process performance is influenced by temperature, pH, particle size, pulp density, aeration, agitation and residence time. Efficient solid suspension is particularly important for dense sulphide particles because settling can reduce mineral exposure and create non-uniform

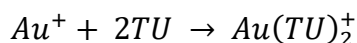
reactor conditions. Stirred-tank and heap-based biomining applications consequently require careful control of hydrodynamics and oxygen supply [15,20,41].

In the present study, biological treatment was first examined in shake flasks and then in mechanically stirred reactors. This sequence allowed preliminary culture behaviour to be assessed before production of sufficient bio-oxidised solids for subsequent thiourea leaching.

1.4 Gold Leaching after Bio-oxidation

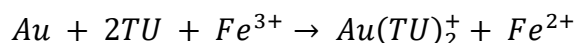
Following oxidative pretreatment, an appropriate lixiviant is required to dissolve the newly accessible gold. Cyanidation remains the dominant industrial process because of its maturity and effectiveness for many ores. However, environmental and process concerns associated with cyanide have encouraged continued investigation of alternative lixivants [1,2,38].

Thiourea is one of the most extensively studied alternatives. It forms a stable cationic bis(thiourea) gold(I) complex in acidic media and can dissolve gold rapidly when an appropriate oxidant is present [38]. The simplified complexation step may be represented as:



where TU represents thiourea. Because metallic gold must first be oxidised to Au(I), ferric ions are commonly used as the oxidant.

The coupled overall reaction can be represented in simplified form as:



The performance of acidic thiourea leaching depends on thiourea concentration, ferric-ion concentration, pH, redox potential, temperature, pulp density and leaching time. Ferric ions promote gold oxidation, but excessive oxidising conditions can also accelerate thiourea degradation and increase reagent consumption [38,39].

Recent studies have shown that control of ferric-ion speciation and the use of suitable additives can reduce excessive thiourea oxidation, while mechanical activation may improve reagent access to mineral surfaces [39,40]. These developments demonstrate that thiourea performance is governed by both solution chemistry and the physical condition of the feed.

For sulphide-bearing materials, thiourea leaching also depends on the effectiveness of the preceding pretreatment. If gold remains encapsulated within unoxidised sulphides, changes in leaching chemistry alone may not produce high extraction. Bio-oxidation and thiourea leaching must therefore be evaluated as an integrated sequence [15,38,41].

1.5 Problem Statement

The flotation tailings investigated in this study contain low-grade residual gold together with sulphide-bearing mineral phases. Recovery of this gold presents two linked challenges. First, part of the gold may be inaccessible because of its association with or encapsulation within sulphide minerals. Second, after pretreatment, the exposed gold must be dissolved under chemical conditions that maintain an effective gold complex while limiting reagent degradation.

The central research question is whether bio-oxidation of sulphide-bearing flotation tailings can improve gold accessibility sufficiently to enhance subsequent extraction by acidic thiourea leaching. The study also examines how bio-oxidation conditions, feed preparation, reactor operation and ferric-ion addition influence the performance of the combined treatment route.

1.6 Aim and Objectives

The main aim of this study was to investigate a combined bio-oxidation and thiourea-leaching process for gold recovery from sulphide-bearing flotation tailings.

1. Characterise the flotation tailings with respect to particle-size distribution, mineralogical composition and relevant mineral associations.
2. Investigate biological oxidation of the flotation tailings using preliminary shake-flask tests and mechanically stirred reactor experiments.
3. Monitor bio-oxidation through pH, Eh and temperature measurements and through dissolved Fe, S and Zn concentrations.
4. Compare bio-oxidation behaviour under different feed preparation, reactor scale, solid loading, agitation and aeration conditions.
5. Investigate a pyrite-rich concentrate produced from the flotation tailings by gravity separation as an alternative bio-oxidation feed.
6. Apply acidic thiourea leaching to the bio-oxidised residues and evaluate the subsequent apparent Au extraction based on changes in the solid-phase Au grade.
7. Compare bio-oxidised materials with untreated flotation tailings subjected directly to thiourea leaching.
8. Assess the effect of ferric-ion addition through paired thiourea tests performed with and without ferric sulphate.
9. Identify relationships and limitations connecting sulphide oxidation with subsequent apparent gold extraction.

1.7 Hypothesis

The central hypothesis of this study is that the bio-oxidation of sulphide minerals in the flotation tailings will partially decompose the pyritic matrix, thereby increasing the exposure and accessibility of the associated gold. This increased accessibility is expected to result in a measurably higher gold extraction during subsequent acidic thiourea leaching compared to direct thiourea leaching of the untreated tailings. Furthermore, it is hypothesised that the addition of ferric ions as an oxidant during thiourea leaching will enhance gold dissolution by providing a more favourable electrochemical environment.

1.8 Scope of the Study

The experimental work focused on the flotation tailings material selected for this thesis. Characterisation included particle-size analysis, X-ray diffraction, SEM-EDS observations, diagnostic leaching and Au deportment analysis. The biological programme consisted of shake-flask experiments followed by five mechanically stirred reactor experiments. Reactor Experiments 1–4 used flotation tailings, while Experiment 5 used a pyrite-rich concentrate produced from the flotation tailings by gravity separation.

During bio-oxidation, pH, Eh and temperature were monitored, and Fe, S and Zn were used as the principal dissolved elements for evaluating sulphide-related changes. The second stage consisted of thiourea leaching of bio-oxidised residues, direct leaching of untreated flotation tailings, and paired testing of the Experiment 4 residue with and without ferric-ion addition.

The study was intended as a laboratory-scale evaluation of the feasibility and limitations of the combined treatment route and did not aim to establish a fully optimised industrial flowsheet.

1.9 Thesis Structure

Chapter 1 introduces the research problem, scientific context, objectives and scope.

Chapter 2 reviews gold occurrence in sulphide-bearing materials, flotation tailings as secondary resources, bio-oxidation of sulphide minerals and acidic thiourea leaching.

Chapter 3 describes the materials, analytical methods and experimental procedures.

Chapter 4 presents and discusses the characterisation, bio-oxidation and thiourea-leaching results.

Chapter 5 summarises the conclusions and provides recommendations for future work.

2. CHAPTER 2

LITERATURE REVIEW

2.1 Gold Ores and Refractory Gold Ores

Gold has been one of the most valuable metals throughout human history because of its rarity, exceptional chemical stability, high corrosion resistance, excellent electrical conductivity, and wide range of industrial applications. In addition to its traditional use in jewellery and financial investment, gold plays an increasingly important role in modern technologies including electronics, aerospace engineering, medical devices, and renewable energy systems. As global demand continues to increase while high-grade deposits become progressively depleted, the mining industry is increasingly forced to exploit lower-grade and more mineralogically complex ores, making efficient extraction technologies more important than ever before [1,2].

Gold mineralisation occurs in numerous geological environments and exhibits considerable mineralogical variability. Depending on the mode of occurrence of gold and its association with gangue or sulphide minerals, different processing routes are required to achieve economically viable recoveries. Consequently, a comprehensive understanding of gold ore classification and mineralogical characteristics is essential for selecting appropriate beneficiation and hydrometallurgical processes [1,3].

From a metallurgical perspective, gold ores are generally classified as free-milling, complex, or refractory according to their response to conventional cyanidation. Free-milling ores permit direct gold recovery by cyanide leaching after comminution, whereas refractory ores require an additional pretreatment stage because a significant proportion of the gold remains inaccessible during direct leaching. This classification is widely adopted in both academic research and industrial practice because it directly determines process flowsheet selection and plant economics [1].

The increasing exploitation of refractory resources represents one of the greatest challenges facing the modern gold industry. Refractory ores commonly contain gold encapsulated within sulphide minerals such as pyrite and arsenopyrite, incorporated as invisible gold within crystal lattices, or associated with naturally carbonaceous materials that adsorb dissolved gold complexes during cyanidation. Without appropriate pretreatment, gold recoveries from such ores are often significantly lower than those obtained from free-milling deposits, making oxidation technologies an essential component of modern refractory gold processing [1,2].

For sulphide-rich refractory ores, pretreatment technologies such as roasting, pressure oxidation, ultrafine grinding, and bio-oxidation have been developed to destroy the sulphide matrix before chemical leaching. Among these alternatives, bio-oxidation has attracted increasing attention because it operates under relatively mild conditions, requires lower

energy input than thermal processes, and offers a more environmentally sustainable approach for exposing encapsulated gold prior to cyanidation or alternative lixiviants such as thiourea [1,2].

2.1.1 Classification of Gold Ores

Gold ores can be classified according to different criteria, including their geological origin, mineralogical composition, oxidation state, and metallurgical behaviour. While geological classifications are primarily used for mineral exploration and resource evaluation, metallurgical classifications are more relevant to mineral processing because they determine the most appropriate extraction route and the expected gold recovery. Consequently, modern gold processing plants generally classify ores according to their response to conventional cyanidation rather than solely by deposit type [1,2].

Although thiourea was employed as the lixiviant in the present study, conventional cyanidation is used here as the reference criterion because the metallurgical classification of gold ores as free-milling or refractory is traditionally based on their response to cyanide leaching.

From a geological perspective, gold deposits are commonly grouped into several major categories, including orogenic, epithermal, Carlin-type, porphyry, placer, and volcanogenic massive sulphide (VMS) deposits. Each deposit type exhibits distinct mineralogical characteristics, ore-forming processes, and gangue mineral assemblages, all of which influence downstream metallurgical performance. For example, placer deposits generally contain coarse liberated native gold and are readily treated by gravity separation, whereas Carlin-type deposits are characterised by extremely fine-grained and chemically bound gold associated with arsenian pyrite, making them among the most refractory gold resources currently exploited [2].

Although geological classification is essential for understanding ore genesis, it provides only limited information regarding processing behaviour. Two ores formed in different geological environments may respond similarly during leaching, while ores from the same deposit type may require completely different processing routes because of differences in gold deportment, sulphide content, particle size distribution, or the presence of carbonaceous matter. Therefore, metallurgical classification has become the preferred approach in process design [1].

From a metallurgical standpoint, gold ores are generally divided into free-milling, complex, and refractory ores. Free-milling ores typically achieve gold recoveries greater than 90–95% by conventional cyanidation after crushing and grinding. Complex ores contain constituents such as copper minerals, cyanicides, oxygen-consuming sulphides, or other components that increase reagent consumption but still permit economically viable gold recovery. In contrast,

refractory ores cannot achieve acceptable recoveries through direct cyanidation and therefore require an additional pretreatment stage before leaching [1,2].

The degree of refractoriness is often further classified according to the gold recovery obtained by direct cyanidation. A widely adopted classification considers ores with recoveries above approximately 95% as free-milling, 80–90% as mildly refractory, 50–80% as moderately refractory, and below 50% as highly refractory. Although these recovery thresholds may vary slightly among authors and ore types, they provide a practical framework for selecting suitable pretreatment technologies and comparing metallurgical performance between different deposits [1,4].

The principal causes of refractoriness include physical encapsulation of gold within sulphide minerals, chemical incorporation of gold into pyrite or arsenopyrite crystal lattices (invisible gold), adsorption of dissolved gold complexes by naturally carbonaceous materials (preg-robbing), and the presence of minerals that consume cyanide or dissolved oxygen during leaching. Since these mechanisms differ significantly in their mineralogical origin, proper ore classification represents the first and one of the most critical steps in designing an efficient gold extraction flowsheet [1,2].

2.1.2 Mineralogical Occurrence of Gold

The mineralogical occurrence of gold is one of the most important factors controlling its recovery during mineral processing. Gold may occur as native metallic particles, as natural alloys, within telluride minerals, or in association with sulphide minerals. The size, morphology, distribution, and mineral association of gold particles directly influence the selection of beneficiation and extraction processes, as well as the overall metallurgical recovery [1,2].

Native gold (Au) is the most common naturally occurring gold mineral and typically contains minor amounts of silver, copper, mercury, or other trace elements. When the silver content exceeds approximately 20 wt.%, the

mineral is generally referred to as electrum. Native gold and electrum commonly occur in quartz veins, placer deposits, and hydrothermal ore bodies, where relatively coarse particles can often be recovered efficiently by gravity concentration before chemical leaching [2,5].

In addition to native gold, several gold-bearing telluride minerals occur in hydrothermal deposits, including calaverite (AuTe_2), krennerite ($(\text{Au,Ag})\text{Te}_2$), sylvanite ($(\text{Au,Ag})\text{Te}_4$), and petzite (Ag_3AuTe_2). Although these minerals represent a relatively small proportion of global gold resources, they are economically important in several deposits because their metallurgical behaviour differs considerably from that of native gold. Gold tellurides often require oxidative pretreatment or specialised hydrometallurgical processes to achieve satisfactory recovery [2,4].

A significant proportion of the world's remaining gold resources is hosted by sulphide minerals, particularly pyrite (FeS_2) and arsenopyrite (FeAsS). In these ores, gold may occur either as microscopic inclusions trapped during crystal growth or as invisible gold, where individual gold atoms or nanometre-sized particles are incorporated within the crystal structure of the host mineral. Because cyanide solution cannot readily access these forms of gold, sulphide-associated ores frequently exhibit refractory behaviour and require oxidative pretreatment before leaching [1,2].

The distribution of gold within an ore body is commonly described by the term gold deportment, which refers to the mineralogical location and physical form of gold. Gold deportment studies evaluate whether gold occurs as liberated particles, locked grains, grain-boundary inclusions, lattice-bound invisible gold, or particles associated with gangue minerals. This information is fundamental for selecting appropriate comminution, flotation, oxidation, and leaching strategies, and it is routinely determined during metallurgical characterisation of refractory ores [2,6].

Modern process mineralogy combines automated mineralogical techniques, such as Mineral Liberation Analyzer (MLA) and QEMSCAN, with scanning electron microscopy (SEM), electron probe microanalysis (EPMA), and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). These complementary techniques provide quantitative information on mineral abundance, liberation characteristics, particle size distribution, and trace-element distribution within individual mineral grains. Such techniques can provide valuable information for the design of processing flowsheets and for understanding the causes of poor gold recovery in refractory deposits [6].

The growing application of advanced mineralogical characterisation has significantly improved the understanding of refractory gold ores over the past two decades. In particular, detailed investigations of pyrite and arsenopyrite have demonstrated that gold occurrence is controlled not only by mineral abundance but also by crystal chemistry, trace-element substitution, and the geological history of ore formation. These findings have provided the scientific basis for the development of modern pretreatment technologies such as bio-oxidation and pressure oxidation, which specifically target the destruction of sulphide minerals before gold leaching [2,6].

2.1.3 Free-Milling and Refractory Gold Ores

From a metallurgical perspective, the most widely accepted classification of gold ores is based on their response to conventional cyanide leaching. Accordingly, gold ores are generally categorised as free-milling, complex, or refractory. This classification is more useful than a purely geological classification because it directly determines the processing route, expected gold recovery, reagent consumption, and overall process economics [1,2].

Free-milling ores contain gold that is either fully liberated or only weakly associated with gangue minerals. After conventional crushing and grinding, cyanide solution can readily access the gold surface, and recoveries exceeding 90–95% are commonly achieved without any oxidative pretreatment. Native gold occurring in quartz veins and many placer deposits typically belongs to this category, making gravity concentration followed by cyanidation the preferred processing route [1,2].

Complex gold ores differ from free-milling ores because they contain minerals that interfere with cyanidation even though the gold itself may not be encapsulated. Sulphide minerals, copper-bearing phases, organic matter, or cyanide-consuming minerals may increase reagent consumption, reduce dissolved oxygen availability, or complicate downstream recovery. Although acceptable gold recoveries can still be obtained, additional process optimisation is generally required [2,4].

Refractory gold ores represent the greatest challenge in modern gold metallurgy. In these ores, conventional cyanidation cannot achieve economically acceptable recoveries because a significant proportion of the gold remains inaccessible. Depending on the deposit, direct cyanidation may recover less than 80% of the contained gold, while highly refractory ores may yield recoveries below 50% without pretreatment [1,4].

The refractory nature of a gold ore may arise from several different mechanisms. The most common is physical refractoriness, in which gold particles are completely encapsulated within sulphide minerals such as pyrite or arsenopyrite. Because the cyanide solution cannot penetrate the intact sulphide matrix, gold remains isolated from the leaching solution. Grinding alone is often insufficient because many inclusions are significantly smaller than the practical grinding limit [1,2].

A second mechanism is chemical refractoriness, commonly referred to as invisible gold. In this case, gold may occur as structurally bound Au species within arsenian pyrite or arsenopyrite, or as extremely fine nanoparticles that cannot be readily identified by conventional microscopic techniques. Extensive mineralogical investigations have demonstrated a strong relationship between arsenic concentration and the amount of structurally bound gold in pyrite. Reich et al. (2005) showed that arsenian pyrites with low Au/As ratios contain gold predominantly in solid solution, whereas higher Au/As ratios are associated with the formation of native gold nanoparticles, providing a quantitative framework for understanding invisible gold occurrence [5].

Another important source of refractoriness is the presence of naturally carbonaceous materials capable of adsorbing dissolved gold complexes during cyanidation. This phenomenon, known as preg-robbing, reduces dissolved gold concentration before recovery on activated carbon or by zinc precipitation can occur. Preg-robbing is particularly significant

in several sediment-hosted and Carlin-type deposits and often requires alternative process strategies or specific pretreatment methods [1,2].

Because refractory behaviour originates from different mineralogical mechanisms, proper ore characterisation is essential before process selection. Modern metallurgical test work typically combines chemical assays, diagnostic leaching, automated mineralogy, and microscopic observations to determine whether poor recovery results from sulphide encapsulation, invisible gold, preg-robbing, or a combination of these mechanisms. This information forms the basis for selecting appropriate pretreatment technologies such as roasting, pressure oxidation, ultrafine grinding, or bio-oxidation [2,4].

The increasing depletion of easily processed free-milling deposits has made refractory ores an increasingly important source of global gold production. Consequently, the development of efficient and

environmentally sustainable pretreatment technologies has become one of the major research priorities in extractive metallurgy. Among these technologies, bio-oxidation has gained particular attention because it selectively oxidises sulphide minerals under relatively mild operating conditions while reducing energy consumption and atmospheric emissions compared with thermal oxidation processes [2,4].

2.1.4 Invisible Gold and Gold Association with Sulphide Minerals

Invisible gold is an important cause of refractoriness in sulphide-bearing gold ores. It may occur as structurally bound gold within sulphide minerals or as submicroscopic to nanoscale particles that cannot be readily identified by conventional microscopic techniques. Pyrite and arsenopyrite are among the most important host minerals for these forms of gold [2,5,6].

The occurrence and distribution of invisible gold depend on several factors, including mineral chemistry, arsenic content, crystal growth history and the physicochemical conditions of ore formation. These characteristics are metallurgically important because gold that remains enclosed within or structurally associated with an intact sulphide matrix may be poorly accessible to direct leaching. Oxidative pretreatment can therefore be required to alter the sulphide host and improve gold accessibility [5–7].

The principal modes of gold occurrence in pyrite and arsenopyrite, together with the solid-solution and nanoparticle models used to describe invisible gold, are discussed in the following sections.

2.1.4.1 Gold Hosted by Pyrite and Arsenopyrite

Pyrite (FeS_2) and arsenopyrite (FeAsS) are the two most important host minerals for invisible gold and account for a substantial proportion of the world's refractory gold resources. Although these sulphides are chemically similar, their crystal structures, trace-element

chemistry, and gold incorporation mechanisms differ considerably, resulting in different metallurgical responses during oxidative pretreatment and leaching [2,5,7].

Among the two minerals, arsenian pyrite is generally regarded as the most significant host for invisible gold. The substitution of arsenic for sulphur within the pyrite lattice produces structural defects and changes in the electronic environment that favour the incorporation of gold. Consequently, arsenian pyrite commonly contains much higher concentrations of invisible gold than arsenic-poor pyrite, making it one of the principal causes of ore refractoriness [5,7].

Detailed mineralogical investigations have shown that gold hosted by pyrite may occur in several different forms:

- Structurally bound Au(I) within the pyrite lattice;
- Native gold nanoparticles measuring only a few nanometres;
- Submicroscopic inclusions trapped during crystal growth;
- Enrichment along crystal defects, fractures, and grain boundaries.

The relative abundance of these forms depends on the physicochemical conditions prevailing during mineral formation, including temperature, sulphur activity, arsenic concentration, fluid composition, and cooling history [5,7].

A major breakthrough in understanding invisible gold was achieved by Palenik et al. (2004). Using high-resolution transmission electron microscopy (HRTEM) together with high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), the authors directly observed native gold nanoparticles approximately 5–10 nm in diameter within As-rich overgrowths on pyrite from a Carlin-type deposit. Before this study, metallic nanoparticles had been hypothesised but had never been directly imaged. Their observations demonstrated that invisible gold is not exclusively present as structurally bound Au(I), but may also occur as discrete nanoparticles when the gold concentration exceeds its solubility limit in arsenian pyrite [8].

This discovery complemented the model proposed by Reich et al. (2005). While Reich and co-workers established the relationship between arsenic concentration and the solubility of gold in pyrite, Palenik et al. provided direct microstructural evidence explaining why some pyrite grains contain lattice-bound gold whereas others contain metallic nanoparticles. Together, these studies form the foundation of the modern understanding of invisible gold mineralisation [5,8].

Arsenopyrite exhibits similar behaviour but differs in several important respects. Owing to its crystal chemistry and higher arsenic content, arsenopyrite can host significant quantities of invisible gold through both structural incorporation and nanoparticle formation. However,

oxidation of arsenopyrite is generally more complex than that of pyrite because the dissolution process releases both iron and arsenic species, which may

subsequently precipitate as secondary iron arsenates or other surface products. These secondary phases can partially passivate the mineral surface if oxidation conditions are not properly controlled, thereby reducing the efficiency of subsequent gold leaching [2,4].

From a metallurgical perspective, the occurrence of invisible gold within pyrite and arsenopyrite explains why simple particle size reduction is often insufficient to achieve satisfactory gold recovery. Even ultrafine grinding cannot liberate lattice-bound gold because the gold atoms remain chemically incorporated within the sulphide structure. Consequently, oxidative pretreatment technologies such as roasting, pressure oxidation, and bio-oxidation are designed to destroy the sulphide lattice itself, creating porosity and exposing the encapsulated gold to the leaching solution [1,2,5].

2.1.4.2 Solid-Solution and Nanoparticle Models of Invisible Gold

Although the existence of invisible gold has been recognised for several decades, its structural form within sulphide minerals has been the subject of extensive scientific debate. Two principal models have been proposed to explain the occurrence of invisible gold in pyrite and arsenopyrite: the solid-solution model and the nanoparticle model. Rather than being mutually exclusive, current evidence indicates that both mechanisms may operate simultaneously within the same mineral grain, depending on the physicochemical conditions prevailing during ore formation [5,7].

Solid-Solution Model

The solid-solution model proposes that gold is incorporated directly into the crystal structure of pyrite as chemically bound Au(I). In this mechanism, gold does not occur as metallic particles but is bonded to sulphur atoms within the sulphide lattice. Gold incorporation is facilitated by lattice defects, arsenic substitution, and local charge-compensation mechanisms. Because the gold atoms become part of the crystal structure itself, they cannot be liberated by conventional grinding, regardless of particle size reduction [5].

Experimental investigations have demonstrated that arsenic plays a crucial role in stabilising structurally bound gold. As arsenic concentration increases, the crystal structure of pyrite becomes progressively more favourable for Au(I) incorporation, leading to higher concentrations of invisible gold. Reich et al. quantified this relationship and proposed an

empirical Au–As solubility model that remains one of the most widely accepted frameworks for interpreting refractory gold mineralisation [5].

Subsequent spectroscopic studies have shown that chemically bound gold is predominantly coordinated with reduced sulphur species rather than existing as Au(III). These findings

support the interpretation that invisible gold in arsenian pyrite mainly occurs in the monovalent oxidation state, Au(I), bonded to sulphur ligands in a manner similar to gold–sulphur complexes present in hydrothermal fluids [9].

Nanoparticle Model

The nanoparticle model proposes that invisible gold consists of metallic Au(0) particles measuring only a few nanometres in diameter. Because these particles are significantly smaller than the resolution of conventional optical microscopy and many routine SEM analyses, they appear "invisible" during standard mineralogical investigations. Their existence was conclusively demonstrated by high-resolution electron microscopy, providing direct evidence that not all invisible gold is structurally bound within the sulphide lattice [8].

Formation of gold nanoparticles generally occurs when the concentration of gold exceeds the solubility limit of arsenian pyrite. Under these conditions, excess gold precipitates as discrete metallic particles while remaining enclosed within the host mineral. These nanoparticles are commonly distributed along crystal defects, growth zones, dislocations, and grain boundaries, indicating that crystal growth history strongly influences their formation [5,8].

Current Scientific Understanding

Recent studies indicate that the solid-solution and nanoparticle models should not be considered competing hypotheses. Instead, they represent complementary mechanisms operating under different geological conditions. Modern analytical investigations combining atom probe tomography (APT), synchrotron X-ray spectroscopy, transmission electron microscopy, and LA-ICP-MS have shown that a single pyrite grain may simultaneously contain structurally bound Au(I), nanometre-scale Au(0) particles, and gold enriched along crystal defects [7,9].

Furthermore, recent thermodynamic and experimental work has demonstrated that the partitioning of gold between hydrothermal fluids and pyrite is controlled not only by arsenic concentration but also by temperature, sulphur speciation, fluid composition, and redox conditions.

These studies explain why pyrites formed in different geological environments may exhibit markedly different gold concentrations despite having similar bulk arsenic contents [9].

Metallurgical Significance

The distinction between structurally bound gold and gold nanoparticles has important metallurgical implications. Structurally bound Au(I) can only be recovered after oxidation destroys the sulphide lattice, whereas nanoparticulate gold may become accessible once oxidation creates sufficient porosity and microfractures within the mineral. In both cases, however, oxidative alteration of the pyrite or arsenopyrite host can substantially improve gold accessibility and subsequent extraction. This explains why oxidative pretreatment

technologies including roasting, pressure oxidation, and particularly bio-oxidation remain indispensable for processing refractory sulphide ores [1,2,5].

2.1.4.3 Analytical Techniques for Characterising Invisible Gold

The characterisation of invisible gold has evolved considerably over the past three decades owing to advances in mineralogical and microanalytical techniques. Since invisible gold cannot generally be detected by conventional optical microscopy, modern analytical methods are required to determine its concentration, oxidation state, spatial distribution, and mode of occurrence within sulphide minerals. These techniques have significantly improved the understanding of refractory gold ores and have become indispensable tools for both academic research and industrial process development [2,5,7].

Among the most widely used techniques, Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDS) provides high-resolution imaging of mineral morphology and identifies major mineral phases. SEM is particularly useful for locating visible gold particles, evaluating mineral liberation, and identifying textural relationships between sulphides and gangue minerals. However, its spatial resolution and detection limits are generally insufficient for identifying structurally bound gold or nanoparticles smaller than a few tens of nanometres [10].

To overcome these limitations, Electron Probe Microanalysis (EPMA) has been extensively applied for quantitative elemental mapping of pyrite and arsenopyrite. EPMA enables accurate determination of iron, sulphur, arsenic, and other trace elements at the micrometre scale. Although its

detection limit for gold is relatively high, EPMA has proven particularly valuable for identifying arsenic zoning within pyrite, which is closely associated with the occurrence of invisible gold [7,10].

One of the most important advances in process mineralogy has been the application of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). This technique enables direct in situ measurement of trace gold concentrations within individual mineral grains while simultaneously determining the distribution of associated elements such as As, Co, Ni, Cu, and Ag. LA-ICP-MS has become one of the principal techniques for investigating the relationship between arsenic enrichment and invisible gold because of its excellent sensitivity and spatial resolution [7,9].

More recently, Atom Probe Tomography (APT) has revolutionised the study of invisible gold. Unlike conventional analytical methods, APT reconstructs the three-dimensional atomic arrangement of a mineral and identifies individual atoms with near-atomic spatial resolution. This technique has provided direct evidence that gold may occur simultaneously as structurally bound Au(I), metallic Au(0) nanoparticles, and clusters associated with crystal

defects within a single pyrite grain. Consequently, APT has become one of the most powerful methods for investigating gold deportment at the atomic scale [7,9].

Automated mineralogical systems, including the Mineral Liberation Analyzer (MLA) and QEMSCAN, have also become indispensable tools in modern gold processing. These systems combine automated scanning electron microscopy with quantitative image analysis to determine mineral abundance, grain size distribution, mineral associations, and liberation characteristics. Although MLA and QEMSCAN do not directly detect invisible gold, they provide essential information for evaluating the distribution of pyrite, arsenopyrite, gangue minerals, and other phases that control the metallurgical behaviour of refractory ores [11].

The integration of these complementary analytical techniques has transformed the understanding of refractory gold mineralisation. Rather than relying on a single method, modern studies routinely combine SEM, EPMA, LA-ICP-MS, APT, X-ray spectroscopy, and automated mineralogy to establish a comprehensive gold deportment model. Such integrated characterisation can provide valuable information for selecting appropriate pretreatment technologies, particularly for ores intended for bio-oxidation or pressure oxidation [2,7,9].

2.1.4.4 Metallurgical Implications and Transition to Bio-oxidation

The recognition that invisible gold is predominantly associated with pyrite and arsenopyrite has fundamentally changed the approach to refractory gold processing. Traditional cyanidation assumes that gold is exposed on the particle surface and therefore accessible to the leaching solution. However, when gold is structurally bound within sulphide minerals or enclosed as nanometre-scale particles, cyanide cannot reach the gold unless the host mineral is first destroyed or sufficiently altered [1,2].

Consequently, oxidative pretreatment has become an essential stage in the processing of sulphide-rich refractory ores. The objective of pretreatment is not to dissolve gold directly but rather to oxidise the sulphide matrix, thereby generating pores, fractures, and new reactive surfaces that expose previously inaccessible gold. Roasting, pressure oxidation, and bio-oxidation all operate according to this general principle, although they differ considerably in operating conditions, environmental impact, and economic performance [2,4].

Among these technologies, bio-oxidation has emerged as one of the most environmentally sustainable alternatives because it utilises naturally occurring acidophilic microorganisms to catalyse the oxidation of pyrite and arsenopyrite under relatively mild conditions. Instead of relying on high temperatures or elevated pressures, microorganisms accelerate the oxidation of ferrous iron and reduced sulphur compounds, gradually decomposing the sulphide lattice and exposing encapsulated gold for subsequent leaching. This biological process has become the basis of several commercial operations and has attracted increasing attention as the mining industry seeks lower-energy and lower-emission processing technologies [2,4].

Therefore, a thorough understanding of invisible gold mineralogy is not only of academic importance but also forms the scientific foundation for selecting appropriate pretreatment technologies. Since bio-oxidation specifically targets the sulphide minerals responsible for hosting invisible gold, the following section reviews the microbiological principles, oxidation mechanisms, and industrial applications of bio-oxidation in refractory gold processing.

2.2 Bio-oxidation of Refractory Gold Ores

Bacterial leaching, bioleaching and bio-oxidation are related but distinct concepts. In the context of refractory gold processing, microorganisms primarily act as agents of sulphide oxidation rather than as direct gold lixiviants. Their activity promotes the regeneration of ferric iron and the oxidation of reduced sulphur species, contributing to the breakdown of the sulphide matrix and improving gold accessibility. In the process investigated in this study, gold dissolution is subsequently carried out by thiourea leaching. Therefore, the biological stage is referred to primarily as bio-oxidation pretreatment throughout this thesis [18,20–22].

2.2.1 Historical Development of Bio-oxidation

The application of microorganisms in mineral processing has transformed the treatment of refractory sulphide ores by providing an environmentally sustainable alternative to conventional oxidative pretreatment methods. Bio-oxidation is based on the metabolic activity of acidophilic chemolithotrophic microorganisms that derive energy from the oxidation of ferrous iron and reduced sulphur compounds. Rather than attacking gold directly, these microorganisms oxidise pyrite and arsenopyrite, thereby destroying the sulphide matrix that encapsulates gold and making the precious metal accessible to subsequent leaching. Today, bio-oxidation is recognised as one of the most mature and commercially successful applications of biotechnology in the mining industry [2,4,12].

The scientific foundations of bioleaching and bio-oxidation can be traced back to the late 1940s when acid mine drainage was first investigated. In 1947, Colmer and Hinkle reported that acid-producing bacteria were responsible for the oxidation of pyrite in coal mines, providing the first evidence that microorganisms could catalyse sulphide mineral oxidation. Their pioneering work marked the beginning of modern biomining research and demonstrated that biological oxidation could proceed at rates far exceeding purely abiotic reactions [12].

During the 1950s and 1960s, research focused primarily on the recovery of copper from low-grade sulphide ores through bacterial leaching. The discovery of *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) established the microbiological basis of these processes. This acidophilic bacterium was shown to oxidise both ferrous iron and reduced sulphur compounds, generating ferric iron and sulphuric acid that further promoted

sulphide mineral dissolution. These findings formed the conceptual framework later applied to refractory gold ores [13,14].

Interest in the biological pretreatment of gold concentrates increased substantially during the 1980s as the proportion of refractory gold resources expanded worldwide and environmental concerns limited the use of roasting. Extensive laboratory and pilot-scale investigations demonstrated that microbial oxidation could effectively decompose pyrite and arsenopyrite, significantly improving subsequent cyanide extraction. These encouraging results ultimately led to the commercialisation of bio-oxidation technology for gold processing [4,15].

A major milestone was achieved in 1986 with the development of the BIOX® process in South Africa. The BIOX® technology employs continuously stirred tank reactors in which mixed cultures of acidophilic microorganisms oxidise refractory sulphide concentrates under carefully controlled conditions. Compared with roasting, BIOX® operates at atmospheric pressure and relatively low temperatures, producing substantially lower atmospheric emissions while enabling high gold recoveries during subsequent leaching. Since its first commercial implementation, BIOX® has been successfully adopted in numerous gold operations worldwide and has become the benchmark against which other biological pretreatment technologies are evaluated [15,16].

Over the past three decades, bio-oxidation has continued to evolve through improvements in microbial ecology, reactor engineering, process control, and molecular biology. Modern operations no longer rely on a single bacterial species but instead employ complex microbial consortia capable of adapting to variations in temperature, mineral composition, dissolved oxygen, and pulp density. Advances in genomic sequencing and microbial community analysis have also provided deeper insight into the interactions among acidophilic microorganisms, enabling the development of more robust and efficient bio-oxidation systems [17,18].

The growing importance of sustainable mining has further strengthened the role of bio-oxidation within the gold industry. Compared with roasting and pressure oxidation, biological pretreatment generally requires lower energy consumption, produces fewer greenhouse gas emissions, and avoids the generation of sulphur dioxide associated with thermal oxidation. Consequently, bio-oxidation is increasingly regarded as a key enabling technology for the sustainable exploitation of low-grade and refractory gold resources [2,15,18].

The following sections describe the microbiological principles governing bio-oxidation, the metabolic pathways involved in sulphide oxidation, and the physicochemical factors that determine process efficiency.

2.2.2 Microorganisms Involved in Bio-oxidation

The efficiency of bio-oxidation depends primarily on the metabolic activity of specialised acidophilic microorganisms capable of oxidising ferrous iron and reduced sulphur compounds. Unlike heterotrophic microorganisms, these species obtain energy from inorganic substrates while fixing carbon dioxide as their principal carbon source. Through these metabolic processes, they generate ferric iron and sulphuric acid, both of which play central roles in the oxidative dissolution of pyrite and arsenopyrite. Consequently, the composition and activity of the microbial community directly determine the rate and extent of sulphide oxidation [15,17,18].

Commercial bio-oxidation processes rarely rely on a single microorganism. Instead, they employ mixed microbial consortia, in which different species perform complementary metabolic functions. Iron-oxidising microorganisms regenerate ferric iron, whereas sulphur-oxidising microorganisms remove elemental sulphur and other intermediate sulphur compounds that could otherwise accumulate on mineral surfaces and inhibit oxidation. This synergistic interaction contributes to greater process stability and higher oxidation efficiencies than those achieved by individual species alone [15,17].

Acidithiobacillus ferrooxidans

Among all bioleaching microorganisms, *Acidithiobacillus ferrooxidans* is the best-known and most extensively studied species. It is a Gram-negative, acidophilic, mesophilic chemolithoautotrophic bacterium that grows optimally at pH values between approximately 1.5 and 2.5 and temperatures of 25–35 °C. The organism derives energy by oxidising both ferrous iron (Fe^{2+}) and reduced inorganic sulphur compounds, making it particularly effective during the initial stages of sulphide mineral oxidation [13,15].

The oxidation of ferrous iron by *A. ferrooxidans* regenerates ferric iron (Fe^{3+}), which subsequently acts as a powerful oxidising agent for pyrite and arsenopyrite. Because ferric iron is continuously regenerated by microbial metabolism, it functions as a recyclable oxidant throughout the bio-oxidation process, substantially accelerating sulphide dissolution compared with purely chemical oxidation [15,18].

Acidithiobacillus thiooxidans

While *A. ferrooxidans* oxidises both iron and sulphur, *Acidithiobacillus thiooxidans* derives its energy exclusively from reduced sulphur compounds. This microorganism oxidises elemental sulphur, thiosulphate, tetrathionate, and other sulphur intermediates to sulphate, thereby producing sulphuric acid and preventing sulphur passivation on mineral surfaces. Its activity is therefore particularly important during prolonged bio-oxidation, where sulphur accumulation could otherwise reduce reaction rates [17,18].

The complementary metabolic functions of *A. ferrooxidans* and *A. thiooxidans* explain why both species are frequently detected together in industrial bio-oxidation reactors.

Leptospirillum Species

Species belonging to the genus *Leptospirillum*, particularly *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum*, are now recognised as dominant microorganisms in many commercial bio-oxidation operations. Unlike *Acidithiobacillus*, these bacteria oxidise ferrous iron exclusively and exhibit exceptionally high iron oxidation rates under strongly acidic conditions. Furthermore, *Leptospirillum ferriphilum* tolerates higher ferric iron concentrations than most other acidophilic bacteria, making it particularly valuable in industrial reactors operating at elevated redox potentials [17,19].

Numerous molecular ecological studies have shown that *Leptospirillum* species frequently become dominant as bio-oxidation progresses, especially in reactors treating refractory gold concentrates. Their ability to maintain high ferric iron concentrations contributes significantly to sustained sulphide oxidation and stable reactor performance [17,19].

Moderately Thermophilic Microorganisms

Industrial bio-oxidation is not limited to mesophilic microorganisms. At operating temperatures between approximately 40 and 55 °C, moderately thermophilic species such as *Sulfobacillus thermosulfidooxidans* and *Alicyclobacillus* spp. become increasingly important. These microorganisms exhibit greater tolerance to high pulp densities and elevated metal concentrations while maintaining rapid oxidation kinetics. Consequently, moderately thermophilic consortia are widely employed in high-capacity industrial operations where increased reaction rates are required [15,18].

Thermophilic Archaea

At temperatures exceeding approximately 60 °C, bacterial populations are gradually replaced by thermophilic archaea, including species of *Acidianus*, *Sulfolobus*, and *Metallosphaera*. These microorganisms possess highly efficient sulphur and iron oxidation pathways under extreme environmental conditions and are particularly attractive for treating highly refractory concentrates containing arsenopyrite or complex sulphide assemblages. Their ability to remain metabolically active at elevated

temperatures enables faster sulphide oxidation while reducing the risk of microbial inhibition associated with mesophilic systems [20].

Microbial Consortia

Mixed microbial communities are widely used in industrial bio-oxidation because different microorganisms can perform complementary metabolic functions and adapt to variations in operating conditions. Within these consortia, different microorganisms occupy distinct ecological niches according to their temperature tolerance, substrate preference, oxygen

demand, and metabolic specialisation. This functional diversity increases process robustness, enhances resistance to fluctuations in mineral composition, and minimises the accumulation of inhibitory reaction intermediates [15,17].

Advances in molecular biology, particularly 16S rRNA sequencing and metagenomic analysis, have demonstrated that microbial communities within commercial BIOX® reactors are highly dynamic and continuously adapt to changing operating conditions. These findings have shifted the focus of bio-oxidation research from individual microorganisms toward understanding the interactions and cooperative behaviour of complex microbial ecosystems [17,20].

2.2.3 Mechanisms of Bio-oxidation

The bio-oxidation of refractory sulphide minerals involves interactions between acidophilic microorganisms, dissolved iron species, reduced sulphur compounds, oxygen, and mineral surfaces. The microorganisms do not directly dissolve gold. Instead, their metabolic activity promotes the oxidation of sulphide minerals, thereby altering the mineral matrix and improving the accessibility of associated or encapsulated gold during subsequent leaching [2,15,18].

A central role of iron-oxidising microorganisms is the regeneration of ferric iron (Fe^{3+}) from ferrous iron (Fe^{2+}). Ferric iron acts as an important chemical oxidant of sulphide minerals, while the ferrous iron produced during mineral oxidation can subsequently be re-oxidised by microorganisms. This continuous regeneration of ferric iron contributes to sustained sulphide oxidation under acidic conditions [15,18].

Microorganisms may occur either attached to mineral surfaces or suspended in the surrounding solution. Attachment is facilitated by extracellular polymeric substances (EPS), which can promote the formation of biofilms and create favourable conditions for reactions at the mineral-solution interface. Consequently, sulphide oxidation can involve both surface-associated microbial activity and reactions mediated by oxidising species present in the bulk solution [20–22].

2.2.3.1 Contact and Non-contact Bio-oxidation

Bio-oxidation may be described in terms of contact and non-contact mechanisms. In the contact mechanism, microorganisms attach to the sulphide mineral surface through EPS and form a biofilm. Ferric iron and other reactive species generated within this interfacial environment can promote oxidation close to the mineral surface [20,22].

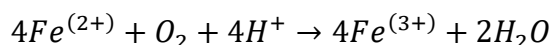
In the non-contact mechanism, microorganisms do not need to remain attached to the mineral surface. Iron-oxidising microorganisms regenerate ferric iron in the bulk solution, and the ferric iron subsequently reacts chemically with sulphide minerals. Ferrous iron generated during these reactions is returned to the microbial oxidation cycle [15,18].

These mechanisms are not mutually exclusive and may operate simultaneously. Their relative importance depends on factors such as mineralogy, microbial community, solution chemistry, ferric and ferrous iron concentrations, oxygen availability, and operating conditions.

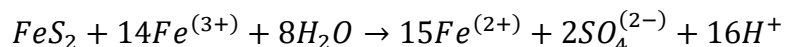
2.2.3.2 Ferric Iron-mediated Oxidation

Ferric iron plays a central role in the oxidative dissolution of sulphide minerals during bio-oxidation. Iron-oxidising microorganisms regenerate Fe^{3+} from Fe^{2+} according to the biological oxidation reaction:

The biological regeneration of ferric iron can be represented by the following reaction:



The regenerated ferric iron can subsequently oxidise pyrite, producing ferrous iron and oxidised sulphur species:

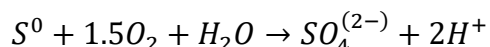


The ferrous iron generated during mineral oxidation can then be biologically re-oxidised to ferric iron, establishing a cyclic process in which the oxidising capacity of the solution is continuously regenerated. This cycle is one of the principal mechanisms responsible for sustained sulphide oxidation in bio-oxidation systems [15,18].

2.2.3.3 Sulphur Oxidation Pathway

During pyrite and arsenopyrite oxidation, reduced sulphur species are released as elemental sulphur, polysulphides, thiosulphate, tetrathionate, and other intermediate sulphur compounds. If these intermediates accumulate on mineral surfaces, they may form passivating layers that inhibit further oxidation.

Sulphur-oxidising microorganisms, particularly *Acidithiobacillus thiooxidans*, oxidise these intermediate sulphur species to sulphate:



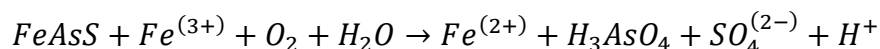
This reaction performs two important functions. First, it removes sulphur deposits that would otherwise hinder mineral dissolution. Second, it generates sulphuric acid, thereby maintaining the strongly acidic conditions required for efficient microbial activity and ferric iron stability [17,18].

The simultaneous oxidation of iron and sulphur therefore represents one of the fundamental characteristics of successful bio-oxidation systems.

2.2.3.4 Oxidation of Arsenopyrite

Arsenopyrite is generally more reactive than pyrite owing to its lower structural stability. During bio-oxidation, ferric iron attacks arsenopyrite, releasing iron, sulphate, and dissolved arsenic species.

A simplified reaction may be expressed as:



The released arsenic is subsequently oxidised from As(III) to As(V), which may precipitate with ferric iron as ferric arsenate minerals under suitable operating conditions. The oxidation of As(III) to As(V) can facilitate subsequent arsenic immobilisation through the formation of ferric arsenate phases under suitable conditions [2,15].

2.2.3.5 Overall Bio-oxidation Cycle

The bio-oxidation process may therefore be summarised as a cyclic sequence:

1. Microorganisms oxidise Fe^{2+} to Fe^{3+} .
2. Ferric iron chemically oxidises pyrite and arsenopyrite.
3. Sulphide minerals release ferrous iron and sulphur compounds.
4. Sulphur-oxidising microorganisms convert sulphur intermediates into sulphate.
5. Newly produced Fe^{2+} is biologically re-oxidised to Fe^{3+} .
6. The oxidation cycle continues until the sulphide matrix is largely destroyed.

As oxidation progresses, the sulphide particles become increasingly porous, fractured, and permeable. These structural changes can improve the accessibility of previously encapsulated gold to the lixiviant during the subsequent leaching stage. Consequently, the effectiveness of bio-oxidation is governed not by direct gold dissolution but by the progressive destruction of the sulphide host minerals that contain invisible gold [2,4,15].

2.2.4 Industrial Bio-oxidation Technologies

The successful commercialisation of bio-oxidation has demonstrated that microbial pretreatment is not merely a laboratory concept but a mature industrial technology capable of processing millions of tonnes of refractory gold concentrates. Since the late 1980s, several commercial bio-oxidation processes have been developed to improve gold recovery while reducing the environmental impact associated with conventional oxidative pretreatment technologies. Although these processes differ in reactor configuration, operating conditions, and microbial populations, they all rely on the biological oxidation of pyrite and arsenopyrite prior to gold leaching [2,4,15].

BIOX® Process

The BIOX® process is the world's most widely implemented bio-oxidation technology for refractory gold concentrates. Developed in South Africa during the 1980s, BIOX® employs a series of continuously stirred tank reactors (CSTRs) operated under controlled conditions of temperature, pH, dissolved oxygen, and pulp density. The process utilises naturally occurring mixed cultures of acidophilic microorganisms to oxidise sulphide minerals, thereby exposing encapsulated gold before cyanidation or alternative leaching processes [16,23].

Typically, the process operates at temperatures between 40 and 45 °C, with pH maintained between 1.2 and 1.8. Adequate aeration is essential because oxygen serves as the terminal electron acceptor during microbial metabolism. Nutrient supplementation, agitation, and strict control of redox potential are also required to maintain high microbial activity and stable oxidation performance [16,23].

One of the principal advantages of BIOX® is its relatively low energy requirement compared with roasting or pressure oxidation. Because oxidation occurs at atmospheric pressure and moderate temperatures, capital investment and operating costs are generally lower than those associated with high-temperature technologies. Furthermore, the process produces negligible sulphur dioxide emissions, making it significantly more environmentally sustainable [2,15].

Commercial BIOX® plants have been successfully operated in South Africa, Ghana, Brazil, China, Australia, and Kazakhstan, collectively processing several million tonnes of refractory concentrate annually. Following bio-oxidation and subsequent gold leaching, gold recoveries can exceed 90% for suitable refractory concentrates, depending on ore mineralogy and process conditions [16,23].

BacTech Process

Another important commercial development is the BacTech bio-oxidation technology. Although based on the same microbiological principles as BIOX®, BacTech has focused primarily on modular reactor systems and the treatment of refractory concentrates and mine wastes. The technology has also attracted considerable attention for its potential application in the remediation of historical tailings containing sulphide minerals and residual precious metals [24].

BacTech emphasises flexible process design, allowing adaptation to ores with varying mineralogical compositions. In addition to gold recovery, the process has been investigated for the stabilisation of arsenic-bearing residues, thereby contributing to improved environmental management of mining wastes [24].

Other Emerging Bio-oxidation Technologies

Several additional biological pretreatment technologies have been investigated over the past two decades. The GEOCOAT® process combines bio-oxidation with heap leaching by coating coarse support particles with finely ground sulphide concentrates. This approach improves oxygen transfer, enhances microbial colonisation, and facilitates the treatment of concentrates that are unsuitable for conventional heap leaching [25].

More recently, biocyanidation has emerged as a complementary biotechnological approach to conventional bio-oxidation pretreatment, addressing some of the operational and economic challenges associated with refractory gold ore processing [44].

Other research has explored bio-oxidation in heap reactors, packed-bed systems, fluidized-bed reactors, and airlift bioreactors. Although these technologies have shown promising laboratory and pilot-scale performance, most have not yet achieved the same degree of commercial success as BIOX® owing to challenges associated with oxygen transfer, heat management, and long-term microbial stability [15,18].

Advantages and Limitations of Industrial Bio-oxidation

The increasing adoption of bio-oxidation reflects several important advantages over conventional pretreatment technologies. Biological oxidation operates under relatively mild conditions, resulting in lower energy consumption and reduced greenhouse gas emissions. The absence of high-temperature roasting also minimises sulphur dioxide production and decreases the need for expensive gas-cleaning systems. Furthermore, the relatively mild operating conditions of bio-oxidation may offer advantages for the treatment of refractory sulphide-bearing materials for which energy-intensive pretreatment routes such as roasting or pressure oxidation may be less attractive [2,15,18].

Nevertheless, bio-oxidation also presents several limitations. The oxidation process is slower than thermal pretreatment, requiring residence times ranging from several days to more than one week. Microbial activity is highly sensitive to operating conditions, including pH, temperature, oxygen availability, nutrient supply, and pulp density. In addition, excessive concentrations of dissolved metals or arsenic may inhibit microbial growth if process control is inadequate [15,20].

Despite these challenges, continuous improvements in reactor design, microbial community management, and process monitoring have

significantly enhanced the reliability and economic viability of industrial bio-oxidation. Consequently, biological pretreatment is increasingly regarded as a key technology for the sustainable processing of refractory gold ores, particularly in the context of declining ore grades and increasing environmental regulations [17,18].

Transition to Process Parameters

Although the commercial success of bio-oxidation demonstrates its technical feasibility, process performance is strongly influenced by operating conditions. Parameters such as temperature, pH, dissolved oxygen concentration, pulp density, particle size, nutrient availability, and redox potential directly affect microbial activity and sulphide oxidation rates. Therefore, understanding these operational variables is essential for optimising bio-oxidation efficiency. The following section examines the principal factors controlling the performance of bio-oxidation systems.

2.3 Factors Affecting Bio-oxidation

2.3.1 Temperature

Temperature is one of the most critical operating parameters influencing the efficiency of bio-oxidation because it directly affects microbial metabolism, enzyme activity, oxidation kinetics, oxygen transfer, and mineral dissolution. Each group of acidophilic microorganisms possesses a relatively narrow optimum temperature range, and deviations from this range can significantly reduce sulphide oxidation rates. Consequently, maintaining an appropriate operating temperature is essential for achieving stable microbial activity and effective sulphide oxidation [15,17,18].

Acidophilic microorganisms involved in bio-oxidation are generally classified into three temperature groups:

- Mesophiles (20–40 °C)
- Moderately thermophiles (40–55 °C)
- Extreme thermophiles (60–80 °C)

Mesophilic microorganisms, including *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, dominate conventional bioleaching systems operating at approximately 30–35 °C. However, industrial BIOX® reactors commonly operate at slightly higher temperatures (approximately 40–45 °C) because moderately thermophilic microbial consortia exhibit faster oxidation kinetics and greater tolerance to elevated metal concentrations [16,18].

Temperature affects bio-oxidation through several mechanisms. An increase in temperature generally accelerates enzymatic reaction rates, enhances microbial growth, and increases the chemical oxidation rate of sulphide minerals. Higher temperatures also improve diffusion rates within porous mineral particles, facilitating ferric iron transport into newly formed pores. Nevertheless, excessive temperatures may denature essential enzymes, disrupt membrane integrity, and inhibit microbial metabolism, ultimately decreasing oxidation efficiency [17,20].

In addition to its effects on microbial physiology, temperature strongly influences oxygen solubility. As temperature increases, the solubility of oxygen in aqueous solutions decreases, potentially limiting microbial respiration if aeration is insufficient. Industrial reactors therefore require careful optimisation of both temperature and oxygen supply to maintain adequate dissolved oxygen concentrations throughout the oxidation process [15,18].

Temperature also influences the mineralogical behaviour of sulphide ores. Elevated temperatures generally increase the oxidation rate of pyrite and arsenopyrite, promoting faster destruction of the sulphide matrix. However, excessively high temperatures may favour the precipitation of secondary iron compounds, such as jarosite, which can partially coat mineral surfaces and reduce ferric iron accessibility. Consequently, reactor temperature must be optimised not only for microbial growth but also for minimising passivation phenomena [2,20].

Industrial experience has shown that maintaining relatively constant temperatures is considerably more important than simply maximising temperature. Sudden temperature fluctuations may alter microbial community composition, reduce biological activity, and destabilise reactor performance. Modern commercial plants therefore employ automated temperature-control systems to ensure stable operating conditions throughout the bio-oxidation process [16,23].

Overall, temperature influences nearly every aspect of bio-oxidation, including microbial metabolism, ferric iron regeneration, sulphur oxidation, oxygen availability, mineral dissolution, and microbial community structure. It is therefore regarded as one of the primary parameters controlling the efficiency and stability of industrial bio-oxidation systems.

2.3.2 Effect of pH

Among all operating variables, pH is one of the most influential factors governing the efficiency of bio-oxidation because it affects microbial growth, enzyme activity, ferric iron stability, sulphur oxidation, and the precipitation of secondary mineral phases. Acidophilic microorganisms used in industrial bio-oxidation are highly adapted to acidic environments and generally exhibit optimum activity within a narrow pH range. Consequently, even relatively small deviations from the optimum pH can significantly reduce sulphide oxidation rates and impair overall process performance [15,17,18].

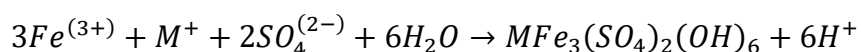
Most commercial bio-oxidation systems operate at a pH between 1.2 and 2.0, although the optimum value depends on the microbial consortium, mineral composition, operating temperature, and reactor configuration. Within this acidic range, ferric iron remains sufficiently stable in solution and conditions are generally favourable for the activity of acidophilic microorganisms and the oxidation of sulphide minerals [16,20].

The importance of pH is closely related to the iron cycle that drives indirect bio-oxidation. Ferric iron (Fe^{3+}), which is continuously regenerated by iron-oxidising microorganisms, serves as the primary oxidising agent for sulphide minerals. At excessively high pH values, ferric iron readily hydrolyses and precipitates as ferric hydroxides or jarosite-group minerals, reducing the concentration of dissolved Fe^{3+} available for mineral oxidation. Consequently, the oxidation rate decreases despite the continued presence of microorganisms [2,15].

Conversely, extremely low pH values may also adversely affect bio-oxidation. Although acidophilic microorganisms tolerate highly acidic environments, excessive proton concentrations can inhibit enzyme systems, disrupt membrane transport, and increase cellular maintenance energy requirements. As a result, microbial growth rates decline, reducing ferric iron regeneration and slowing sulphide oxidation [17,26].

The influence of pH extends beyond microbial physiology to the dissolution behaviour of sulphide minerals. Pyrite oxidation generally proceeds efficiently over a relatively broad acidic pH range, whereas arsenopyrite oxidation is more sensitive to changes in acidity because of the simultaneous release of arsenic species. Appropriate acidic and oxidising conditions can favour the conversion of As(III) to As(V) and may facilitate subsequent arsenic immobilisation through the formation of ferric arsenate phases [2,15].

One of the most significant operational consequences of inadequate pH control is the formation of jarosite, a secondary iron sulphate mineral that may precipitate according to the following simplified reaction:



where M^+ represents monovalent cations such as K^+ , Na^+ , or NH_4^+ . Jarosite precipitation is particularly undesirable because it forms a coating on sulphide particles, limiting ferric iron diffusion and restricting microbial access to the mineral surface. Excessive jarosite formation therefore decreases oxidation efficiency and increases reactor residence time [20,22].

In industrial practice, pH is continuously monitored and adjusted through the controlled addition of sulphuric acid or, when necessary, alkaline reagents. Automated pH-control systems are commonly integrated into commercial bio-oxidation reactors to maintain stable operating conditions and prevent abrupt fluctuations that could disturb microbial community structure. Stable pH control not only enhances oxidation efficiency but also improves process reproducibility and operational reliability [16,23].

Recent studies have further demonstrated that pH influences the composition of microbial communities. Iron-oxidising bacteria, sulphur-oxidising bacteria, and thermophilic archaea exhibit different pH tolerances, and prolonged shifts in reactor acidity may alter species dominance within the consortium. These ecological changes subsequently affect ferric iron regeneration, sulphur oxidation, and overall bio-oxidation kinetics [17,26].

Overall, pH affects nearly every aspect of bio-oxidation, including microbial metabolism, ferric iron stability, sulphur oxidation, arsenic behaviour, secondary mineral precipitation, and microbial community dynamics. For this reason, precise pH control is regarded as one of the most critical requirements for achieving efficient and stable industrial bio-oxidation.

2.3.3 Effect of Pulp Density

Pulp density, defined as the mass percentage of solid particles suspended in the liquid phase, is one of the most influential operational parameters affecting the performance of bio-oxidation. It governs the physical, chemical, and biological environment within the reactor by influencing oxygen transfer, microbial activity, nutrient availability, slurry rheology, heat transfer, and mineral–microorganism interactions. Consequently, optimisation of pulp density is essential for achieving high sulphide oxidation rates while maintaining stable microbial growth [15,18,20].

In industrial bio-oxidation systems, pulp density is generally maintained between 10 and 20 wt.% solids, although the optimum value depends on ore mineralogy, particle size distribution, reactor configuration, and microbial consortium. Lower pulp densities provide favourable conditions for microbial activity due to improved oxygen transfer and reduced slurry viscosity; however, they decrease reactor productivity because less mineral is treated per unit volume. Conversely, excessively high pulp densities increase throughput but often reduce bio-oxidation efficiency due to biological and hydrodynamic limitations [16,18].

One of the primary effects of increasing pulp density is the reduction of oxygen transfer efficiency. Acidophilic microorganisms require dissolved oxygen as the terminal electron acceptor during iron and sulphur oxidation. As the concentration of suspended solids increases, slurry viscosity rises and bubble dispersion becomes less effective, resulting in lower oxygen transfer coefficients (kLa). Oxygen limitation directly decreases microbial respiration and slows the regeneration of ferric iron, thereby reducing sulphide oxidation rates [18,20].

Higher pulp densities also influence microbial activity through increased concentrations of dissolved metal ions. During the oxidation of sulphide-bearing materials, substantial quantities of Fe, S and, depending on the mineral assemblage, other elements such as As, Cu and Zn may be released into solution. At elevated solid loadings, these dissolved species may accumulate to concentrations that inhibit microbial metabolism or alter the composition of the microbial consortium. The accumulation of dissolved metals at elevated solid loadings may inhibit microbial activity and can alter the composition and activity of the microbial consortium, depending on the species present and their tolerance to the prevailing solution chemistry [17,20].

Another important consequence of increasing pulp density is the change in slurry rheology. High solid concentrations increase viscosity and reduce mixing efficiency, which can create localised

regions with limited oxygen availability and uneven distribution of nutrients. Poor mixing also promotes particle settling, decreases the frequency of microorganism– mineral contact, and reduces the overall homogeneity of the reactor. Industrial bio-oxidation systems therefore require adequate agitation to maintain particles in suspension while minimising excessive shear stress that could damage microbial biofilms [15,23].

Pulp density also affects the concentration of ferric iron available for indirect oxidation. Although larger quantities of sulphide minerals provide more substrate for microbial oxidation, they simultaneously consume ferric iron at higher rates. If microbial regeneration of ferric iron cannot match its consumption, the oxidation process becomes limited by oxidant availability rather than microbial growth itself. Maintaining an appropriate balance between mineral loading and ferric iron regeneration is therefore essential for sustaining efficient sulphide dissolution [15,21].

From a mineralogical perspective, high pulp densities may also enhance the formation of secondary precipitates such as jarosite and ferric hydroxides due to increased concentrations of dissolved iron and sulphate. These precipitates may partially coat sulphide particles, restricting ferric iron diffusion and decreasing the accessibility of mineral surfaces to both microorganisms and oxidising agents. Consequently, excessive pulp density can indirectly promote mineral passivation and prolong reactor residence time [2,22].

Industrial studies have shown that the optimum pulp density represents a compromise between biological efficiency and economic productivity. Lower solid concentrations generally maximise sulphide oxidation rates but require larger reactor volumes to achieve a given production capacity. In contrast, higher pulp densities increase throughput while potentially reducing oxidation efficiency. Modern commercial bio-oxidation plants therefore optimise pulp density together with aeration rate, agitation intensity, residence time, and nutrient supply to maximise overall process performance [16,23].

Recent investigations have demonstrated that improvements in reactor hydrodynamics, oxygen distribution, and microbial adaptation enable successful operation at higher pulp densities than previously considered feasible. Nevertheless, excessive solid loading remains one of the principal factors limiting industrial bio-oxidation performance, particularly when processing concentrates containing high sulphide contents or elevated arsenic concentrations [18,20].

Overall, pulp density exerts a profound influence on microbial metabolism, oxygen availability, ferric iron regeneration, slurry hydrodynamics, mineral passivation, and reactor

productivity. Careful optimisation of this parameter is therefore essential for balancing biological performance with economic efficiency in commercial bio-oxidation operations.

2.3.4 Effect of Particle Size

Particle size is a critical parameter influencing the efficiency of bio-oxidation because it determines the available mineral surface area, the degree of sulphide liberation, mass transfer characteristics, and the accessibility of microorganisms and oxidising agents to reactive mineral surfaces. Appropriate particle size distribution is therefore essential for maximising sulphide oxidation while minimising energy consumption associated with comminution [2,15,18].

The oxidation of refractory sulphide minerals occurs primarily at the mineral surface. Consequently, reducing particle size increases the specific surface area available for microbial attachment and ferric iron attack, thereby accelerating oxidation kinetics. Finer particles also improve the liberation of pyrite and arsenopyrite from gangue minerals, exposing previously inaccessible sulphide surfaces to biological and chemical oxidation [2,4].

However, the relationship between particle size and bio-oxidation efficiency is not linear. Although finer grinding generally enhances sulphide oxidation, excessive size reduction may produce large quantities of ultrafine particles ($<10\ \mu\text{m}$). These ultrafines tend to agglomerate because of their high surface energy, reducing the effective surface area available for oxidation. In addition, very fine particles may coat microbial cells or mineral surfaces, limiting microbial attachment and decreasing oxidation efficiency [15,18].

Particle size also strongly influences slurry hydrodynamics. Fine particles remain suspended more easily, promoting uniform mixing and improving contact between microorganisms and mineral surfaces. Conversely, coarse particles settle more rapidly, creating concentration gradients within the reactor and reducing oxidation efficiency. Effective agitation is therefore required to maintain homogeneous suspension throughout the bio-oxidation process [23].

From a microbiological perspective, particle size affects biofilm formation. Acidophilic microorganisms preferentially colonise fresh mineral surfaces where iron and sulphur substrates are readily available. Increasing mineral surface area generally enhances microbial attachment and biofilm development. Nevertheless, excessively smooth or compact surfaces may support weaker biofilm formation than fractured surfaces produced during controlled grinding, indicating that mineral texture is also an important factor influencing microbial colonisation [20,22].

Particle size further affects the diffusion of ferric iron and oxygen into sulphide particles. Particle size also affects the accessibility of reactive mineral surfaces to ferric iron and other oxidising species. Smaller particles provide shorter characteristic diffusion distances and

greater specific surface area, while oxidation-induced pores and fractures can progressively increase access to previously enclosed regions of the sulphide matrix. These effects can accelerate sulphide oxidation and improve the exposure of associated or encapsulated gold [15,21].

The degree of mineral liberation is particularly important in refractory gold concentrates. In many ores, pyrite and arsenopyrite occur as composite particles intergrown with quartz, silicates, or other gangue minerals. If grinding is insufficient, significant portions of sulphide minerals remain locked within gangue, preventing microbial access and reducing oxidation efficiency. Conversely, excessive grinding increases energy consumption while potentially providing only limited additional improvement in sulphide liberation and subsequent bio-oxidation performance. Therefore, the optimum particle size should be determined based on mineralogical characterisation rather than simply minimising particle diameter [2,10].

Industrial bio-oxidation plants typically process flotation concentrates with particle sizes ranging from approximately $P_{80} = 35\text{--}75\text{ }\mu\text{m}$, depending on ore characteristics and process design. This size range generally provides an appropriate balance between sulphide liberation, oxidation kinetics, slurry handling, and grinding costs. Modern process mineralogy techniques, including MLA and QEMSCAN, are increasingly used to determine the optimum grind size by quantifying mineral liberation and sulphide associations before bio-oxidation [10,23].

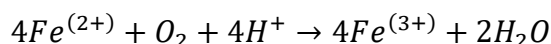
Recent studies have also highlighted that particle size interacts with several other operating parameters, including pulp density, dissolved oxygen concentration, and agitation intensity. Consequently, optimisation of particle size should not be considered independently but rather as part of an integrated process design strategy in which mineral liberation, microbial activity, oxygen transfer, and reactor hydrodynamics are simultaneously optimised [18,20].

Overall, particle size exerts a significant influence on sulphide liberation, microbial colonisation, ferric iron diffusion, oxidation kinetics, and reactor performance. Careful optimisation of grinding conditions is therefore essential for maximising bio-oxidation efficiency while maintaining acceptable energy consumption and operating costs.

2.3.5 Effect of Dissolved Oxygen and Aeration

Dissolved oxygen (DO) is one of the most important factors controlling the efficiency of bio-oxidation because oxygen serves as the terminal electron acceptor in the respiratory metabolism of acidophilic microorganisms. The oxidation of ferrous iron and reduced sulphur compounds is therefore directly dependent on an adequate oxygen supply. Insufficient dissolved oxygen limits microbial growth, decreases ferric iron regeneration, and ultimately reduces sulphide oxidation rates. Consequently, effective aeration is regarded as one of the principal requirements for successful industrial bio-oxidation [15,17,18].

Iron-oxidising microorganisms such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum* utilise oxygen during the biological oxidation of ferrous iron according to the overall reaction:



The ferric iron produced subsequently oxidises pyrite and arsenopyrite, creating a cyclic regeneration process that sustains sulphide dissolution. Because oxygen is consumed continuously during microbial respiration, industrial reactors must maintain sufficient dissolved oxygen concentrations throughout the entire slurry volume [15,20].

The concentration of dissolved oxygen depends on several interacting variables, including aeration rate, agitation intensity, reactor geometry, pulp density, particle size distribution, and operating temperature. Among these factors, the oxygen transfer coefficient (kLa) is widely recognised as one of the most important engineering parameters describing oxygen transport from gas bubbles into the liquid phase. Higher kLa values generally improve oxygen availability, resulting in enhanced microbial activity and increased sulphide oxidation rates [18,23].

In continuously stirred tank reactors (CSTRs), oxygen is supplied by compressed air introduced through spargers located near the base of the reactor. Agitation disperses the air into fine bubbles, increasing the gas– liquid interfacial area available for oxygen transfer. Efficient mixing also prevents particle settling and ensures uniform oxygen distribution throughout the reactor. However, excessive agitation may increase energy consumption and generate shear forces that disrupt microbial aggregates and biofilms. Consequently, both aeration and agitation must be carefully optimised to maximise oxygen transfer while preserving microbial activity [16,23].

Oxygen limitation becomes particularly significant at high pulp densities. As the concentration of suspended solids increases, slurry viscosity rises and bubble movement is restricted, reducing gas dispersion and decreasing oxygen transfer efficiency. Under these conditions, oxygen consumption by microorganisms may exceed oxygen supply, creating localised oxygen-deficient zones that inhibit microbial metabolism and slow ferric iron regeneration [18,20].

Temperature also influences dissolved oxygen availability. Although elevated temperatures accelerate microbial metabolism and sulphide oxidation, they simultaneously decrease the solubility of oxygen in aqueous solutions. This creates a trade-off between faster biochemical reaction rates and reduced oxygen availability. Industrial bio-oxidation plants therefore compensate for lower oxygen solubility at higher temperatures by increasing aeration rates or improving reactor hydrodynamics [17,18].

In addition to influencing microbial respiration, dissolved oxygen affects the composition of microbial communities. Iron-oxidising bacteria generally exhibit higher oxygen demand than sulphur-oxidising microorganisms, and prolonged oxygen limitation may alter the relative abundance of different species within the consortium. Such ecological shifts can reduce ferric iron regeneration efficiency and negatively affect overall reactor performance [17,26].

Recent advances in reactor engineering have improved oxygen transfer efficiency through optimised sparger designs, enhanced impeller configurations, computational fluid dynamics (CFD)-based reactor design, and real-time monitoring of dissolved oxygen. These developments have enabled commercial bio-oxidation plants to operate at higher pulp densities while maintaining sufficient oxygen availability for stable microbial activity [23,27].

Overall, dissolved oxygen influences microbial respiration, ferric iron regeneration, sulphur oxidation, microbial community structure, and reactor productivity. Maintaining adequate oxygen transfer is therefore essential for sustaining microbial activity and effective sulphide oxidation throughout the bio-oxidation process.

2.3.6 Effect of Redox Potential (Eh)

Oxidation–reduction potential (Eh) is one of the most informative operational parameters in bio-oxidation because it reflects the oxidative capacity of the leaching system and provides an indirect measure of microbial activity, ferric iron availability, and sulphide oxidation efficiency. Unlike pH, which describes proton activity, Eh represents the tendency of the solution to accept or donate electrons. In industrial bio-oxidation reactors, continuous monitoring of Eh is widely used to evaluate process stability and optimise operating conditions [15,18,20].

The importance of Eh arises primarily from the central role of the $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox couple. Ferric iron (Fe^{3+}) is the principal oxidising agent responsible for the chemical dissolution of pyrite and arsenopyrite, whereas ferrous iron (Fe^{2+}) is continuously regenerated during sulphide oxidation. Iron-oxidising microorganisms subsequently oxidise Fe^{2+} back to Fe^{3+} , thereby maintaining the oxidation cycle. Consequently, reactor Eh is closely related to the ratio of ferric to ferrous iron present in solution [15,21].

According to the Nernst equation, the redox potential of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple increases as the concentration of ferric iron rises relative to ferrous iron. A higher Eh generally reflects a more oxidising solution environment and, in iron-containing systems, is commonly associated with a higher $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio. However, Eh should not be interpreted as a direct measure of microbial activity or the extent of sulphide oxidation. Conversely, decreasing Eh values often indicate insufficient microbial activity, oxygen limitation, or excessive consumption of ferric iron during mineral oxidation [20,22].

Industrial BIOX® reactors typically operate within an Eh range of approximately 650–750 mV (Ag/AgCl reference electrode), although the optimum value varies depending on ore mineralogy, microbial consortium, and reactor configuration. Maintaining Eh within this range generally ensures efficient iron oxidation while minimising the formation of undesirable secondary precipitates [16,23].

Redox potential also influences microbial physiology. Iron-oxidising microorganisms exhibit maximum metabolic activity under oxidising conditions where sufficient ferrous iron is available for biological oxidation but ferric iron concentrations remain high enough to sustain indirect mineral dissolution. Excessively low Eh values may indicate oxygen deficiency or inadequate microbial growth, whereas excessively high Eh values may increase oxidative stress and alter microbial community composition [17,26].

In addition to its biological significance, Eh affects the oxidation behaviour of sulphide minerals. Elevated redox potentials generally accelerate the oxidation of pyrite and arsenopyrite by increasing the concentration of ferric iron available for chemical attack. However, excessively high Eh values may also promote the precipitation of secondary iron compounds

such as jarosite, particularly when accompanied by elevated ferric iron concentrations and appropriate pH conditions. These precipitates may partially passivate mineral surfaces and reduce oxidation efficiency [2,22].

Continuous measurement of Eh has therefore become an essential component of industrial process control. Modern bio-oxidation plants employ online redox probes integrated with automated control systems to monitor reactor performance in real time. Sudden decreases in Eh may indicate oxygen limitation, nutrient deficiency, reduced microbial activity, or excessive sulphide loading, enabling operators to implement corrective measures before significant deterioration in reactor performance occurs [16,23].

Recent studies have also demonstrated that Eh should not be interpreted independently. Instead, it should be evaluated together with dissolved oxygen concentration, pH, ferric-to-ferrous iron ratio, temperature, and microbial population dynamics. The integration of these parameters provides a more comprehensive understanding of reactor performance than any single measurement alone [17,20].

Overall, redox potential represents one of the most valuable operational indicators in industrial bio-oxidation because it integrates information regarding microbial metabolism, ferric iron regeneration, sulphide oxidation kinetics, and process stability. Maintaining an appropriate Eh is therefore essential for achieving efficient sulphide oxidation and maximising subsequent gold recovery.

2.3.7 Effect of Nutrient Availability

Although acidophilic microorganisms derive their metabolic energy from the oxidation of ferrous iron and reduced sulphur compounds, they still require adequate supplies of essential nutrients for cellular growth, maintenance, and reproduction. Macronutrients such as nitrogen, phosphorus, potassium, magnesium, calcium, and sulphur, together with trace elements including cobalt, copper, zinc, and molybdenum, are indispensable for enzyme synthesis, nucleic acid formation, membrane stability, and numerous metabolic pathways. Consequently, nutrient availability directly influences microbial growth kinetics and the overall efficiency of bio-oxidation [15,17,20].

Unlike heterotrophic microorganisms, the bacteria and archaea involved in bio-oxidation obtain carbon primarily through the fixation of carbon dioxide via the Calvin–Benson–Bassham cycle or alternative autotrophic

pathways. Therefore, dissolved carbon dioxide or bicarbonate in the reactor environment is essential for biomass production. Insufficient inorganic carbon availability may limit microbial proliferation even when adequate energy substrates such as ferrous iron and sulphur are present [17,26].

Nitrogen is one of the most important macronutrients because it is required for the synthesis of amino acids, proteins, nucleic acids, and numerous cellular cofactors. In industrial bio-oxidation systems, nitrogen is commonly supplied as ammonium sulphate or ammonium salts, which provide a readily assimilable nitrogen source for acidophilic microorganisms. Nitrogen deficiency results in reduced cell growth, slower microbial adaptation, and decreased regeneration of ferric iron, ultimately lowering sulphide oxidation rates [20,28].

Phosphorus plays an equally important role because it is an essential component of ATP, nucleic acids, phospholipids, and intracellular energy-transfer systems. Most commercial bio-oxidation operations supplement phosphorus in the form of phosphate salts to maintain continuous microbial growth. Limitation of phosphate availability reduces ATP synthesis, slows cell division, and decreases the metabolic activity of iron- and sulphur-oxidising microorganisms [17,20].

Magnesium serves as an essential cofactor for numerous enzymes involved in DNA replication, RNA synthesis, ATP utilisation, and cellular metabolism. It also contributes to ribosome stability and membrane integrity. Similarly, potassium regulates intracellular osmotic balance and enzyme activation, while calcium contributes to cell envelope stability and may influence microbial attachment to mineral surfaces. Deficiencies in these nutrients generally result in reduced microbial viability and slower sulphide oxidation [20,26].

In addition to macronutrients, several trace elements are required in very small concentrations for optimal microbial metabolism. Iron-oxidising microorganisms possess numerous

metalloenzymes containing copper, molybdenum, cobalt, nickel, or zinc as catalytic cofactors. Although these elements are naturally released during sulphide oxidation, their concentrations may occasionally become limiting, particularly during laboratory-scale experiments using purified mineral substrates. Consequently, defined nutrient media used in bio-oxidation research often include carefully balanced trace-element solutions [15,29].

The concentration of nutrients must also be carefully controlled because excessive supplementation may have adverse effects. High concentrations of ammonium salts or phosphate can alter solution chemistry, increase ionic strength, or promote unwanted precipitation reactions that interfere with microbial activity. Furthermore, excessive nutrient addition increases operating costs without necessarily improving sulphide oxidation rates. Industrial bio-oxidation plants therefore optimise nutrient dosing according to microbial demand and reactor performance rather than applying fixed concentrations [15,18].

Nutrient availability also influences the composition of microbial consortia. Different acidophilic microorganisms exhibit varying nutritional requirements and competitive abilities under nutrient-limited conditions. For example, sulphur-oxidising bacteria may remain metabolically active at nutrient concentrations that are insufficient for rapid growth of iron-oxidising species. Consequently, prolonged nutrient limitation may alter microbial community structure, indirectly affecting ferric iron regeneration and sulphide oxidation kinetics [17,26].

Recent developments in microbial ecology have demonstrated that nutrient utilisation within bio-oxidation reactors is considerably more complex than previously recognised. Modern metagenomic and transcriptomic studies indicate that microbial interactions, cross-feeding mechanisms, and nutrient recycling contribute significantly to the stability of industrial microbial consortia. These findings suggest that optimisation of nutrient supply should consider the metabolic requirements of the microbial community as a whole rather than individual species alone [17,20].

Overall, nutrient availability influences microbial growth, enzyme activity, biomass production, ferric iron regeneration, sulphur oxidation, and microbial community dynamics. Appropriate nutrient management is therefore essential for maintaining stable microbial activity and maximising the efficiency of industrial bio-oxidation processes.

2.3.8 Effect of Mineralogical Composition and Toxic Elements

The mineralogical composition of refractory gold concentrates plays a decisive role in determining the efficiency of bio-oxidation. Although microbial metabolism provides the driving force for sulphide oxidation, the response of an ore to biological pretreatment is ultimately governed by the abundance, texture, and chemical composition of the constituent minerals. Variations in sulphide mineralogy, gangue composition, trace-element distribution,

and the presence of toxic impurities can significantly influence microbial activity, sulphide oxidation kinetics, and the overall effectiveness of bio-oxidation [2,4,10].

Among sulphide minerals, pyrite (FeS_2) and arsenopyrite (FeAsS) constitute the principal hosts of invisible gold and are therefore the primary targets of bio-oxidation. However, their oxidation behaviour differs considerably. Pyrite and arsenopyrite can exhibit different oxidation behaviours during bio-oxidation, depending on their mineralogical characteristics and the prevailing operating conditions. Consequently, the relative proportions of pyrite and arsenopyrite strongly influence the design and operating conditions of industrial bio-oxidation systems [2,15,18].

Ore texture is equally important. Finely disseminated sulphide minerals with intimate associations between pyrite, arsenopyrite, and gangue require more extensive oxidation to expose encapsulated gold than coarse-grained ores with well-liberated sulphides. Modern process mineralogy techniques such as MLA and QEMSCAN are therefore routinely employed to evaluate mineral liberation, grain size distribution, and mineral associations before selecting an appropriate pretreatment strategy [10,23].

The presence of gangue minerals also affects bio-oxidation performance. Carbonate minerals, including calcite and dolomite, consume sulphuric acid during neutralisation reactions and increase the acid requirement of the process. Excessive carbonate contents may elevate slurry pH beyond the optimum range for acidophilic microorganisms, thereby reducing microbial activity and ferric iron stability. Similarly, clay minerals may increase slurry viscosity, impair oxygen transfer, and reduce mixing efficiency, particularly at high pulp densities [2,18].

Among trace elements, arsenic exerts the greatest influence on bio-oxidation. Although arsenic is released naturally during the oxidation of arsenopyrite, elevated dissolved arsenic concentrations may inhibit microbial growth by interfering with enzyme function and cellular metabolism. Many acidophilic microorganisms have developed resistance mechanisms, including arsenite oxidation, arsenate reduction, and active efflux systems, enabling them to tolerate concentrations that would inhibit non-adapted species. Nevertheless, excessive arsenic accumulation remains a major operational challenge in industrial bio-oxidation plants [17,20,30].

Other potentially toxic elements include antimony, mercury, lead, copper, and zinc, which may also be released during sulphide oxidation. At moderate concentrations, some of these metals serve as essential micronutrients; however, elevated concentrations may inhibit enzyme systems, damage cell membranes, and alter microbial community composition. The degree of toxicity depends on metal speciation, solution chemistry, pH, and the adaptive capacity of the microbial consortium [17,18].

Chloride ions represent another important inhibitory factor. Elevated chloride concentrations can disrupt osmotic regulation, reduce microbial viability, and decrease iron oxidation rates. For this reason, industrial bio-oxidation processes generally operate using low-chloride process water whenever possible. In regions where saline water must be used, microbial adaptation and careful process control are required to maintain satisfactory oxidation performance [31].

The mineralogical composition of the ore also influences the formation of secondary reaction products. During prolonged oxidation, ferric iron may precipitate as jarosite or ferric hydroxides, while dissolved arsenic may form ferric arsenate phases such as scorodite. Although the formation of relatively stable ferric arsenate phases such as scorodite can contribute to arsenic immobilisation, excessive precipitation of secondary iron minerals may coat sulphide particles, restrict ferric iron diffusion, and decrease oxidation efficiency. Control of pH, redox potential, and iron concentration is therefore essential to minimise passivation while maintaining environmentally stable residues [2,22].

Recent investigations have emphasised that no single mineralogical parameter adequately predicts bio-oxidation performance. Instead, successful process design requires integrated characterisation of mineralogy, texture, liberation, trace-element distribution, and microbial response. Advances in automated mineralogy, synchrotron spectroscopy, and geometallurgical modelling have substantially improved the prediction of bio-oxidation behaviour for complex refractory gold ores and flotation tailings [9,10].

Overall, mineralogical composition and toxic elements exert a profound influence on microbial activity, oxidation kinetics, ferric iron regeneration, and reactor stability. Mineralogical characterisation therefore provides important information for evaluating and optimising bio-oxidation as a pretreatment technology for refractory gold-bearing materials, including pyritic flotation tailings.

2.3.9 Process Optimisation and Integrated Control of Bio-oxidation

Efficient bio-oxidation is achieved not by optimising individual operating parameters independently but by maintaining a balanced interaction among biological, chemical, and engineering variables. Temperature, pH, dissolved oxygen, pulp density, particle size, nutrient availability, mineralogical composition, and redox potential are closely interconnected. A change in one parameter frequently influences several others, making bio-oxidation a highly integrated process that requires continuous monitoring and control [15,18,20].

One of the primary objectives of process optimisation is to maximise sulphide oxidation while maintaining stable microbial activity throughout the residence period. Industrial experience has shown that even when all individual parameters remain within acceptable ranges, poor interaction among these variables may significantly reduce overall reactor

performance. Consequently, commercial bio-oxidation plants increasingly employ integrated process control strategies based on continuous monitoring of multiple operational parameters rather than relying on individual measurements [16,23].

Among these variables, dissolved oxygen, pH, and redox potential are generally regarded as the principal online control parameters. Variations in these measurements provide rapid information regarding microbial activity, ferric iron regeneration, oxygen transfer efficiency, and sulphide oxidation progress. Automated feedback systems therefore regulate aeration, acid addition, agitation, and slurry flow rates to maintain stable operating conditions and minimise process disturbances [16,27].

Residence time is another critical parameter influencing bio-oxidation efficiency. Insufficient residence time may result in incomplete oxidation of sulphide minerals, limiting the extent to which associated or encapsulated gold becomes accessible during subsequent leaching. Conversely, excessively long residence times increase operating costs while providing only marginal improvements in sulphide oxidation. Industrial reactor design therefore aims to identify the optimum balance between oxidation efficiency and plant productivity [2,15].

Microbial adaptation also plays an important role in process optimisation. Acidophilic microorganisms gradually develop increased tolerance to elevated concentrations of dissolved metals, arsenic, and sulphate through prolonged exposure under controlled operating conditions. Microbial adaptation can improve tolerance to the prevailing process conditions and may contribute to more stable microbial activity and sulphide oxidation performance. Consequently, many industrial operations maintain stable microbial populations over extended periods rather than repeatedly introducing fresh inocula [17,20].

Recent developments in molecular microbiology have provided new opportunities for improving industrial bio-oxidation. High-throughput sequencing, metagenomic analysis, transcriptomics, and quantitative PCR now allow operators to monitor microbial community composition and metabolic activity in near real time. These techniques enable early detection of microbial stress, population shifts, and potential process failures before significant reductions in oxidation efficiency occur [17,29].

Advances in digital process engineering have further enhanced bio-oxidation optimisation. Computational fluid dynamics (CFD), digital twins, machine learning algorithms, and predictive process models are increasingly being applied to simulate oxygen transfer, slurry mixing, microbial growth, and sulphide oxidation kinetics. These tools improve reactor design, reduce operational uncertainty, and facilitate process optimisation under changing ore characteristics [27,32].

Environmental considerations have also become an integral component of process optimisation. Modern bio-oxidation plants seek not only to maximise gold recovery but also

to minimise water consumption, reduce reagent usage, improve energy efficiency, and ensure environmentally responsible management of arsenic-bearing residues. Optimisation therefore extends beyond metallurgical performance to include sustainability, regulatory compliance, and long-term operational reliability [15,18].

Overall, successful bio-oxidation depends on the coordinated optimisation of biological activity, reactor engineering, solution chemistry, and mineralogical characteristics. Rather than treating individual operating variables separately, bio-oxidation should be considered as an integrated biogeochemical system in which coordinated monitoring and process control can support stable microbial activity and effective sulphide oxidation.

Transition to Thiourea Leaching

The primary objective of bio-oxidation pretreatment is to oxidise the sulphide matrix and thereby improve the accessibility of associated or encapsulated gold. As sulphide oxidation progresses, previously inaccessible gold may become more exposed and therefore more amenable to subsequent hydrometallurgical extraction. Traditionally, cyanidation has been the dominant leaching technology following oxidative pretreatment. However, increasing environmental concerns, restrictions on cyanide use, and the search for more selective lixiviants have stimulated considerable interest in alternative leaching systems.

Among these alternatives, thiourea leaching has emerged as one of the most promising technologies because of its rapid dissolution kinetics, effectiveness in acidic media, and ability to recover gold from certain refractory materials where cyanidation performs poorly. The following section therefore reviews the chemistry, mechanisms, operating parameters, advantages, and limitations of thiourea leaching for gold extraction.

2.4 Thiourea Leaching of Gold

2.4.1 Historical Development of Thiourea Leaching

The extraction of gold by cyanidation has dominated the gold industry for more than a century because of its simplicity, relatively low operating cost, and high recovery for free-milling ores. However, the increasing exploitation of refractory gold deposits, together with growing environmental concerns regarding cyanide toxicity, has stimulated extensive research into alternative lixiviants capable of dissolving gold under less hazardous conditions. Among these alternatives, thiourea ($\text{CS}(\text{NH}_2)_2$) has emerged as one of the most extensively investigated non-cyanide leaching reagents owing to its rapid dissolution kinetics and effectiveness in acidic environments [2,4,33].

The first systematic investigations into thiourea as a gold solvent were reported during the early twentieth century, when researchers recognised that gold could dissolve rapidly in acidic thiourea solutions in the presence of suitable oxidising agents. Unlike cyanide, which forms stable dicyanoaurate complexes under alkaline conditions, thiourea forms stable

cationic gold complexes in acidic media. This fundamental difference attracted considerable scientific interest because acidic leaching systems are often compatible with oxidised sulphide concentrates produced during bio-oxidation [33,34].

Research activity increased substantially during the 1970s and 1980s as interest grew in alternative lixiviants for gold extraction. Numerous laboratory investigations demonstrated that thiourea could dissolve gold considerably faster than cyanide under

optimised conditions. In addition, thiourea showed promising performance for certain refractory ores, carbonaceous materials, and partially oxidised sulphide concentrates where conventional cyanidation produced unsatisfactory recoveries [34,35].

Despite these advantages, large-scale industrial implementation of thiourea leaching remained limited. One of the principal obstacles was the chemical instability of thiourea under strongly oxidising conditions. Thiourea undergoes irreversible oxidation to form products such as formamidine disulphide (FDS) and other sulphur-containing compounds, resulting in high reagent consumption and increased operating costs. Consequently, maintaining an appropriate balance between gold oxidation and thiourea stability became one of the major research challenges associated with this technology [34,36].

Interest in thiourea leaching has increased again during the past two decades owing to the continued decline in free-milling gold resources and the development of more efficient pretreatment technologies such as bio-oxidation. Bio-oxidation can oxidise the sulphide matrix associated with refractory gold and operates under acidic conditions that may facilitate integration with a subsequent acidic thiourea leaching stage. This compatibility has renewed interest in integrated bio-oxidation–thiourea flowsheets as environmentally attractive alternatives to conventional bio-oxidation–cyanidation circuits [15,18,37].

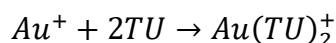
Another factor contributing to the renewed investigation of thiourea is the increasing emphasis on sustainable metallurgy. Modern research has focused on improving thiourea stability through optimisation of oxidant dosage, ferric iron concentration, pH control, and electrochemical operating conditions. Additional studies have investigated alternative oxidising agents, reagent recycling strategies, and hybrid hydrometallurgical systems to reduce thiourea consumption while maintaining high gold recoveries [36,37].

Although cyanidation remains the dominant industrial technology, thiourea continues to be regarded as one of the most promising alternative lixiviants for processing selected refractory gold ores, bio-oxidised concentrates, and materials containing cyanide-consuming minerals. Ongoing research into solution chemistry, process control, and reagent stabilisation continues to evaluate the potential of thiourea as an alternative lixiviant for selected gold-bearing materials

2.4.2 Chemistry of Thiourea

Thiourea ($\text{CS}(\text{NH}_2)_2$) is one of the most extensively investigated non- cyanide lixiviants for gold extraction because of its ability to dissolve metallic gold rapidly under acidic conditions. Unlike cyanide, which forms stable anionic complexes in alkaline media, thiourea forms a stable cationic gold(I)-thiourea complex in acidic sulphate solutions. This fundamental difference governs both the chemistry and the operating conditions of the thiourea leaching process [2,38].

The dissolution of gold in acidic thiourea solutions proceeds through the formation of the stable bis(thiourea) gold(I) complex:



where TU represents thiourea molecules. The stability constant of this complex is exceptionally high ($\beta_2 \approx 2 \times 10^{21}$), indicating a strong affinity between Au(I) and thiourea and enabling dissolved gold to remain stabilised in solution under suitable acidic conditions [38].

The effectiveness of thiourea as a lixiviant is strongly influenced by its chemical stability. Under oxidising conditions, thiourea may be converted to formamidine disulphide (FDS), which is generally regarded as the primary oxidation intermediate. Under controlled conditions, this conversion is reversible and FDS may remain involved in the overall redox chemistry of the leaching system. However, continued oxidation can lead to irreversible degradation products, including sulphur-containing species, sulphate and other compounds, thereby increasing reagent consumption and reducing leaching efficiency [38,39].

Thiourea stability is therefore closely related to the oxidation-reduction potential of the solution. Sufficiently oxidising conditions are required to support gold dissolution, whereas excessively high solution potentials accelerate thiourea oxidation and increase reagent consumption. Maintaining an appropriate balance between oxidising conditions and thiourea stability is consequently essential for effective process control [38,39].

The formation of secondary sulphur-containing products may also influence the performance of the leaching system. Under strongly oxidising or prolonged leaching conditions, such products can accumulate on reactive surfaces and contribute to passivation, thereby reducing mass transfer and slowing gold dissolution. Control of solution chemistry is therefore important not only for preserving thiourea but also for limiting the formation of passivating reaction products [39,40].

Recent studies have investigated the use of complexing additives to improve thiourea stability. Organic ligands such as citrate can modify ferric-ion activity, reduce excessive thiourea oxidation and help maintain more favourable electrochemical conditions. Such

approaches demonstrate that the performance of thiourea leaching depends on the combined control of reagent stability, oxidant activity and solution potential rather than simply on increasing reagent concentration [39,40].

Overall, the chemistry of thiourea leaching is governed by the strong complexation of Au(I) by thiourea together with the susceptibility of thiourea to oxidative degradation. Effective leaching therefore requires conditions that stabilise the Au(I)-thiourea complex while minimising irreversible thiourea oxidation and the formation of passivating products [38–40].

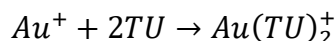
2.4.3 Mechanism of Gold Dissolution

Gold dissolution in acidic thiourea solutions is fundamentally an electrochemical process involving simultaneous anodic oxidation of metallic gold and cathodic reduction of an oxidising agent. Thiourea itself primarily acts as the complexing ligand, while an oxidant is required to convert metallic Au(0) into Au(I), which can subsequently be stabilised as the bis(thiourea) gold(I) complex [38,39].

At anodic sites on the gold surface, metallic gold is oxidised according to:

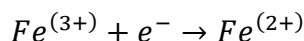


The resulting Au(I) species is rapidly complexed by thiourea:

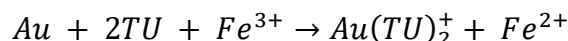


Rapid complex formation removes Au(I) from the immediate mineral-solution interface and stabilises it in the dissolved phase, thereby favouring continued oxidation of metallic gold [38].

Ferric ions are commonly used as the oxidising agent in acidic thiourea systems. At cathodic sites, ferric ions accept the electrons released during gold oxidation and are reduced to ferrous ions:



The anodic and cathodic reactions are therefore coupled. The overall gold-dissolution reaction may be represented as:



This reaction illustrates the three essential steps of the dissolution mechanism: oxidation of metallic gold, formation of the Au(I)-thiourea complex and reduction of ferric ions to ferrous ions [38,40].

The rate of gold dissolution depends strongly on the availability of ferric ions and the electrochemical conditions of the solution. If the ferric-ion concentration is too low, the oxidation of metallic gold may become insufficient to sustain rapid dissolution. Conversely, excessive ferric-ion concentrations can promote competing oxidation of thiourea and increase reagent degradation. The dissolution mechanism therefore requires a balance between sufficient oxidising power for Au oxidation and preservation of the thiourea ligand [38,39].

Surface processes can further influence the dissolution mechanism. During prolonged leaching, sulphur-containing oxidation products may accumulate on exposed gold or mineral surfaces, partially blocking active reaction sites and limiting electron transfer and reagent access. Such passivation can reduce the apparent dissolution rate even when sufficient thiourea and oxidant remain in the bulk solution [39,40].

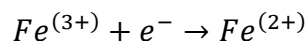
For refractory gold-bearing materials, the effectiveness of this electrochemical mechanism also depends on the accessibility of the gold surface. Bio-oxidation pretreatment may increase gold accessibility by altering the surrounding sulphide matrix and creating new pores, fractures and exposed mineral surfaces. However, the subsequent dissolution of gold remains controlled by the local electrochemical conditions and by the extent to which gold has become accessible to the thiourea solution [15,18,39].

Overall, gold dissolution in acidic thiourea solutions proceeds through coupled anodic gold oxidation, rapid complexation of Au(I) by thiourea and cathodic reduction of the oxidant. The efficiency of the mechanism is therefore determined by the combined effects of gold accessibility, ferric-ion availability, solution potential, thiourea stability and surface passivation [38–40].

2.4.4 Role of Ferric Ions and Oxidising Agents

An oxidising agent is indispensable in thiourea leaching because metallic gold cannot react directly with thiourea. The oxidant is responsible for converting elemental gold into monovalent gold (Au^+), which is subsequently stabilised through complex formation with thiourea. Among the oxidising agents investigated for acidic thiourea leaching, ferric ions (Fe^{3+}), commonly introduced as ferric sulphate, have been widely studied because they provide the oxidising conditions required for gold dissolution and are compatible with acidic leaching media [38,39].

The electrochemical basis for using ferric ions lies in the relatively high standard reduction potential of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple ($E^\circ = 0.771 \text{ V}$), which is considerably higher than that required for the oxidation of metallic gold in acidic thiourea solutions. During leaching, ferric ions accept electrons released from gold oxidation and are simultaneously reduced to ferrous ions according to:



This cathodic reaction provides the driving force necessary for continuous anodic dissolution of gold and subsequent formation of the stable gold– thiourea complex [38].

Although ferric ions promote gold dissolution, they also participate in competing reactions with thiourea. Ferric ions oxidise thiourea to formamidine disulphide (FDS), which is the principal intermediate formed during thiourea decomposition. If oxidation proceeds beyond this intermediate stage, irreversible degradation products such as sulphate, carbon dioxide, and ammonium species are produced, resulting in increased thiourea consumption. Consequently, the oxidant concentration must be carefully controlled to maximise gold oxidation while minimising reagent degradation [38].

Interestingly, the interaction between ferric ions and thiourea is not limited to oxidation reactions. Li and Miller reported that ferric ions readily form coordination complexes with thiourea in acidic sulphate solutions, including $Fe(SO_4)(TU)^+$, $Fe(TU)^{3+}$ and $Fe(TU)_2^{3+}$. These complexes reduce the direct reactivity of free ferric ions toward thiourea, thereby slowing thiourea decomposition while maintaining a sufficiently high solution potential for efficient gold dissolution. This interaction illustrates the importance of controlling ferric ion concentration and speciation to balance gold oxidation with thiourea stability [38,48].

The concentration of ferric ions strongly influences both gold extraction and reagent consumption. At low ferric concentrations, oxidation of metallic gold becomes the rate-limiting step, resulting in incomplete dissolution. Conversely, excessive ferric concentrations accelerate thiourea oxidation and increase the formation of passivating by-products without producing proportional improvements in gold recovery. Therefore, optimum ferric concentrations should be selected to balance oxidation kinetics with thiourea stability [38,39].

Recent research has focused on modifying ferric ion chemistry rather than replacing ferric ions entirely. The 2023 Hydrometallurgy study demonstrated that organic ligands such as citrate and oxalate form stable ferric complexes that decrease the effective activity of free Fe^{3+} ions. This lowers the solution redox potential sufficiently to suppress excessive thiourea oxidation while maintaining efficient gold dissolution. Among the investigated additives, citrate produced the highest gold extraction together with the lowest thiourea consumption, indicating that appropriate control of ferric ion speciation is more effective than simply reducing oxidant dosage [39].

Besides ferric sulphate, several alternative oxidising agents have been investigated, including hydrogen peroxide, dissolved oxygen, ozone, cupric ions, and electrochemically generated oxidants. Hydrogen peroxide provides rapid oxidation but decomposes readily under acidic conditions and is difficult to control during prolonged leaching. Dissolved oxygen alone

generally exhibits insufficient oxidation kinetics for efficient gold dissolution, whereas cupric ions may accelerate thiourea decomposition and promote passivation of gold surfaces. Consequently, these oxidants have not demonstrated the same balance between gold recovery and reagent stability as ferric sulphate. Bio-oxidation and thiourea leaching both involve iron redox chemistry under acidic conditions. However, the ferric ion concentration required during subsequent thiourea leaching depends on the solution composition and leaching conditions, and may therefore require separate adjustment irrespective of the ferric iron generated during the preceding bio-oxidation stage [15,18,38].

Overall, ferric ions can play an important dual role in thiourea leaching by promoting gold oxidation while also influencing thiourea stability through oxidation and complex formation. Modern research therefore emphasises controlling ferric ion speciation and solution potential rather than maximising ferric ion concentration, thereby achieving higher gold recoveries with lower thiourea consumption [38,39].

2.4.5 Factors Affecting Thiourea Leaching

The efficiency of gold extraction using thiourea is controlled by several interrelated physicochemical parameters, including solution pH, thiourea concentration, oxidant dosage, redox potential, temperature, pulp density, leaching time, and mineralogical characteristics of the feed material. Because these variables influence both gold dissolution and thiourea stability, successful process optimisation requires maintaining a balance between rapid gold extraction and minimum reagent degradation [38–40].

Effect of pH

Solution pH is one of the most critical variables affecting thiourea leaching. Thiourea leaching is generally performed under acidic conditions, commonly within a pH range of approximately 1.0–2.0, where favourable conditions for gold–thiourea complex formation and ferric ion stability can be maintained. At higher pH values, ferric ions undergo hydrolysis to form insoluble iron hydroxides, reducing the oxidising capacity of the solution. Conversely, extremely acidic conditions may accelerate thiourea decomposition and increase reagent consumption. Therefore, maintaining an optimum acidic environment is essential for achieving high gold recoveries while preserving reagent stability [38,39].

Effect of Thiourea Concentration

The concentration of thiourea directly determines the availability of ligand molecules for complex formation with dissolved gold. Insufficient thiourea concentrations limit the formation of the Au(TU)_2^+ complex, resulting in incomplete dissolution. Increasing thiourea concentration generally improves gold recovery until all accessible gold surfaces are adequately supplied with ligand molecules. Beyond this optimum level, however, additional thiourea does not proportionally increase gold extraction because reagent degradation

becomes the dominant consumption pathway. Consequently, excessive thiourea addition increases operating costs without significant metallurgical benefit [38].

Effect of Ferric Ion Concentration

Ferric ion concentration simultaneously controls gold oxidation and thiourea degradation. Low ferric concentrations reduce the oxidation rate of metallic gold and consequently decrease leaching efficiency. In contrast, excessive ferric concentrations accelerate oxidation of thiourea to formamidine disulphide and subsequent degradation products, resulting in high reagent consumption and possible surface passivation. Modern studies therefore recommend optimising ferric ion dosage rather than maximising oxidant concentration [38,39].

Effect of Redox Potential

Solution redox potential (Eh) reflects the overall oxidative capacity of the leaching system and is closely associated with the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio. Gold dissolution and thiourea stability are both influenced by solution redox potential, with the appropriate Eh depending on oxidant concentration, thiourea concentration, and the overall solution chemistry. Excessively oxidising conditions may accelerate thiourea oxidation and increase reagent consumption. The 2023 Hydrometallurgy study demonstrated that controlling ferric ion speciation through citrate addition effectively moderates solution potential, thereby reducing thiourea decomposition while maintaining high gold extraction [39].

Effect of Temperature

Temperature influences both reaction kinetics and reagent stability. Moderate increases in temperature accelerate gold dissolution by enhancing molecular diffusion and reaction rates. However, elevated temperatures also increase the oxidation rate of thiourea, leading to greater reagent consumption and formation of decomposition products. Consequently, industrial and laboratory studies generally favour moderate operating temperatures that provide rapid gold dissolution without excessive thiourea degradation [38].

Effect of Leaching Time

Gold recovery generally increases with leaching time because progressively more exposed gold particles dissolve into solution. Nevertheless, prolonged leaching also increases thiourea oxidation and promotes accumulation of sulphur-containing by-products on mineral surfaces. Several studies have shown that extending leaching beyond the optimum residence time produces only marginal improvements in gold recovery while substantially increasing reagent consumption. Therefore, optimisation of leaching duration is essential for maximising process efficiency [38,40].

Effect of Pulp Density

Pulp density affects slurry rheology, mass transfer, and reagent distribution. Higher pulp densities increase processing capacity but reduce liquid-to-solid contact, limit diffusion of

thiourea and oxidants, and may decrease gold extraction efficiency. Excessively concentrated slurries also increase the local concentration of dissolved metal ions, potentially accelerating thiourea decomposition. Appropriate pulp density therefore represents a compromise between metallurgical performance and economic productivity [38].

Effect of Mineralogical Characteristics

Mineralogy strongly influences the effectiveness of thiourea leaching. Free-milling ores containing exposed gold dissolve readily, whereas refractory ores require pretreatment to liberate encapsulated gold from sulphide minerals. For refractory gold-bearing materials in which gold is encapsulated within sulphide minerals, bio-oxidation can enhance subsequent thiourea leaching by oxidising the sulphide matrix and increasing the accessibility of previously encapsulated gold [15,18,40].

Overall, thiourea leaching efficiency is governed by the interaction among solution chemistry, oxidant dosage, reagent stability, mineralogical characteristics, and operating conditions. Optimisation of these parameters is essential to maximise gold recovery while minimising thiourea consumption and operating costs. Recent studies indicate that precise control of solution chemistry, particularly ferric ion speciation and redox potential, offers greater improvements in process performance than simply increasing reagent concentrations [38–40].

2.4.6 Advantages and Limitations of Thiourea Leaching

Thiourea has long been regarded as one of the most promising alternatives to cyanide for gold extraction because of its unique chemical properties and rapid dissolution kinetics. Although its industrial application remains limited, extensive laboratory investigations have demonstrated several important advantages that make thiourea particularly attractive for processing refractory gold ores and bio-oxidised sulphide concentrates. At the same time, significant technical and economic challenges continue to restrict its widespread commercial implementation [38–40].

One of the principal advantages of thiourea is its exceptionally rapid dissolution kinetics. Under optimised acidic conditions, the dissolution rate of gold in thiourea solutions is considerably faster than that observed during conventional cyanidation. Rapid dissolution shortens leaching times, potentially reduces reactor volume requirements, and improves overall process productivity. This characteristic is particularly beneficial for finely disseminated refractory ores where pretreatment has already exposed encapsulated gold particles [38].

Another major advantage is the compatibility of thiourea with acidic process conditions. Unlike cyanide, which requires strongly alkaline media to prevent hydrogen cyanide formation, thiourea operates efficiently at low pH values. This characteristic makes thiourea especially suitable for integration with bio-oxidation, where acidic conditions are naturally

generated during the biological oxidation of pyrite and arsenopyrite. Consequently, the acidic conditions of bio-oxidation may facilitate integration with a subsequent thiourea-leaching stage and potentially reduce the extent of pH adjustment required between the two processes [15,18,38].

Thiourea has also demonstrated excellent performance for certain refractory materials that exhibit poor cyanide recoveries. Carbonaceous ores, partially oxidised sulphide concentrates, and materials containing cyanide-consuming minerals may respond more favourably to thiourea because gold dissolution occurs through a different complexation mechanism. In addition, thiourea generally exhibits lower sensitivity to preg-robbing phenomena than cyanide under specific mineralogical conditions, making it an attractive alternative for difficult-to-process ores [38].

From an environmental perspective, thiourea is frequently considered a potential alternative to cyanide because it does not generate hydrogen cyanide gas during acidic leaching. However, this advantage should not be overstated. Thiourea itself is not environmentally benign and must be handled with appropriate safety measures. Furthermore, its oxidation products may require additional treatment before solution discharge. Therefore, the environmental benefits of thiourea depend largely on effective reagent management and waste treatment practices rather than on the intrinsic safety of the reagent alone [37,38].

Despite these advantages, thiourea possesses several important limitations. The most significant drawback is its poor chemical stability under oxidising conditions. Ferric ions, when used as the oxidant, can promote gold dissolution while simultaneously oxidising thiourea to formamidine disulphide and subsequently to further degradation products. As a result, reagent consumption is considerably higher than would be expected based solely on gold complexation stoichiometry, increasing operating costs and reducing process efficiency [38,39].

Another limitation is the relatively narrow operating window required for efficient leaching. Gold dissolution depends on maintaining an appropriate balance among pH, redox potential, ferric ion concentration, and thiourea concentration. Small deviations from optimum operating conditions may significantly reduce gold recovery or substantially increase thiourea consumption. Consequently, thiourea leaching generally requires more precise process control than conventional cyanidation [38].

Surface passivation represents an additional operational challenge. During prolonged leaching, sulphur-containing oxidation products may accumulate on gold particles, decreasing the active reaction area and slowing dissolution kinetics. Recent investigations have demonstrated that the use of complexing additives such as citrate or mechanochemical activation can substantially reduce passivation and improve reagent utilisation, although these approaches introduce additional process complexity [39,40].

Economic considerations have also limited industrial adoption. High thiourea consumption, together with the need for careful control of oxidant dosage and solution chemistry, generally results in higher reagent costs than cyanidation. Consequently, thiourea may be particularly relevant for selected gold-bearing materials where cyanidation performs poorly or where environmental or regulatory considerations favour the evaluation of alternative lixiviants [37,38].

Recent advances in reagent stabilisation, ferric ion complexation, mechanochemical activation, and process optimisation have significantly improved the competitiveness of thiourea leaching. Modern studies indicate that controlled ferric ion speciation, optimised solution chemistry, and appropriate mineral pretreatment can substantially reduce reagent consumption while maintaining high gold recoveries. These developments support continued investigation of thiourea for selected refractory gold applications, including its potential integration with bio-oxidation pretreatment [39,40].

Overall, thiourea leaching offers several significant metallurgical advantages, including rapid gold dissolution, compatibility with acidic media, and effective treatment of certain refractory ores. Nevertheless, challenges related to reagent stability, process control, and operating cost continue to limit its widespread industrial application. Ongoing research is therefore focused on improving reagent stability and reducing thiourea consumption to enhance the commercial viability of this technology [38–40].

2.4.7 Comparison Between Thiourea and Cyanide Leaching

Cyanidation has remained the dominant technology for industrial gold extraction for more than a century because of its high efficiency, relatively low reagent consumption, and well-established industrial infrastructure. Nevertheless, increasing environmental concerns, stricter regulations governing cyanide handling, and the growing importance of refractory gold resources have stimulated the investigation of alternative lixiviants. Among these, thiourea has emerged as one of the most extensively studied candidates due to its rapid dissolution kinetics and its ability to operate under acidic conditions. Despite these advantages, thiourea has not replaced cyanidation on an industrial scale because both technologies possess distinct technical and economic advantages and limitations [2,37,38].

The most significant difference between the two lixiviants is their operating chemistry. Cyanide leaching is carried out in strongly alkaline solutions, generally at pH values above 10.5, to suppress the formation of highly toxic hydrogen cyanide gas. In contrast, thiourea leaching is commonly conducted in acidic sulphate media, typically within a pH range of approximately 1–2, with ferric ions frequently used as the oxidising agent. This fundamental difference has important implications for process integration. Since bio-oxidation naturally generates acidic process solutions, thiourea can often be applied directly after biological

pretreatment without extensive neutralisation, whereas cyanidation requires pH adjustment before leaching [15,18,38].

Gold dissolution also proceeds through different chemical mechanisms. In cyanidation, metallic gold is oxidised in the presence of dissolved oxygen and stabilised as the highly stable dicyanoaurate complex, $\text{Au}(\text{CN})_2^-$. Thiourea leaching relies on ferric ions or other oxidants to oxidise gold, which subsequently forms the cationic bis(thiourea) gold(I) complex, $\text{Au}(\text{TU})_2^+$. Although both systems ultimately dissolve metallic gold, differences in complex stability and oxidant requirements strongly influence reagent consumption, reaction kinetics, and process control [2,38].

One of the principal metallurgical advantages of thiourea is its considerably faster dissolution kinetics. Numerous laboratory studies have shown that gold dissolution in thiourea solutions may occur several times faster than in cyanide under optimised conditions. Rapid kinetics can shorten the leaching time required to dissolve accessible gold following oxidative pretreatment. However, this kinetic advantage is frequently offset by the limited chemical stability of thiourea, which undergoes continuous oxidative degradation during leaching [38–40].

From an economic perspective, reagent price alone does not determine process cost. Although thiourea is generally more expensive than sodium cyanide on a unit-mass basis, the more important factor is reagent consumption. Cyanide remains relatively stable under alkaline conditions and is consumed mainly through reactions with gold and cyanide-consuming minerals. In contrast, thiourea is continuously oxidised by ferric ions to form formamidine disulphide and subsequent sulphur-containing degradation products. Consequently, actual thiourea consumption is often substantially higher than the theoretical requirement for gold complexation, significantly increasing operating costs [38].

Another important consideration is process control. Cyanidation is regarded as a relatively robust process with a broad operating window, provided that alkaline conditions are maintained. Thiourea leaching, however, requires careful control of pH, oxidation-reduction potential (Eh), ferric ion concentration, and thiourea dosage. Excessive oxidant concentration accelerates thiourea decomposition, whereas insufficient oxidant limits gold dissolution. Therefore, maintaining the optimum balance between oxidation efficiency and reagent stability remains one of the principal engineering challenges associated with thiourea leaching [38,39].

Environmental performance also differs between the two technologies. Cyanide is highly regulated because of its acute toxicity and the risk of hydrogen cyanide generation under acidic conditions. Modern cyanide destruction technologies have significantly improved environmental safety; nevertheless, public perception and regulatory requirements remain major concerns for many mining operations. Thiourea avoids cyanide-related hazards and is

therefore often considered an attractive alternative. However, thiourea should not be regarded as environmentally benign, since its degradation products also require appropriate management and treatment before discharge [37,38].

The suitability of each lixiviant largely depends on ore characteristics. Cyanidation remains the preferred option for free-milling ores because of its low operating cost and well-established industrial performance. Thiourea may offer an alternative for selected refractory sulphide ores, bio-oxidised concentrates, carbonaceous materials, and cyanide-resistant gold-bearing wastes where acidic processing conditions or mineralogical characteristics limit the effectiveness of cyanidation. Recent advances involving ferric ion complexation, citrate addition, and mechanochemical activation have further improved thiourea performance by reducing reagent degradation and increasing gold recovery [39,40].

The comparison presented above demonstrates that thiourea is unlikely to replace cyanidation as the universal gold leaching technology. Instead, current research increasingly supports the use of thiourea as a specialised lixiviant for refractory gold resources, particularly when combined with bio-oxidation pretreatment. Under suitable conditions, the compatibility between acidic bio-oxidation and acidic thiourea leaching may facilitate process integration, making the combined bio-oxidation–thiourea route a potential alternative for selected difficult-to-process gold-bearing materials [15,18,38–40].

Table 2.1 Comparison of cyanide and thiourea leaching

Parameter	Cyanide leaching	Thiourea leaching
Operating pH	Generally alkaline, commonly above 10.5	Acidic, commonly about 1–2
Main oxidant	Dissolved oxygen	Ferric iron or another suitable oxidant
Gold complex	$\text{Au}(\text{CN})_2^-$	$\text{Au}(\text{TU})_2^+$
Dissolution kinetics	Moderate under suitable conditions	Fast under optimised acidic conditions
Reagent stability	Relatively high under controlled alkaline conditions	Lower because of oxidative degradation
Reagent consumption	Usually lower	Often higher and strongly dependent on Eh and ferric iron
Process control	Relatively robust within an alkaline operating window	More sensitive to pH, Eh, ferric iron, and thiourea concentration
Neutralisation after bio-oxidation	Normally required	May be reduced or avoided, subject to slurry chemistry
Industrial maturity	Fully commercial	More limited, with important laboratory and pilot-scale development
Typical application	Free-milling and some complex ores	Selected refractory or bio-oxidised materials
Main limitation	Cyanide toxicity and regulatory requirements	Reagent degradation, consumption, and cost

2.5 Combined Bio-oxidation–Thiourea Process

The increasing exploitation of refractory gold ores has stimulated the development of integrated hydrometallurgical processes capable of overcoming the limitations associated with conventional cyanidation. Among the proposed alternatives, the combination of biological oxidation followed by thiourea leaching has emerged as a promising strategy for improving gold recovery from sulphide-rich refractory materials. In this integrated approach, bio-oxidation serves as a pretreatment stage that oxidises sulphide minerals and can increase the accessibility of encapsulated gold, while thiourea is subsequently used to dissolve accessible gold under acidic conditions. The compatibility of both processes with acidic media represents one of the principal advantages of this combined flowsheet [15,18,38].

Bio-oxidation primarily targets pyrite (FeS_2) and arsenopyrite (FeAsS), the two sulphide minerals most frequently responsible for the refractory behaviour of gold ores. During biological oxidation, acidophilic microorganisms catalyze the oxidation of ferrous iron and reduced sulphur compounds, producing ferric ions and sulphuric acid. The continuous regeneration of ferric ions sustains sulphide oxidation and can progressively alter the mineral matrix surrounding finely disseminated gold particles. As oxidation proceeds, the accessibility of gold associated with or encapsulated within these sulphide minerals may therefore increase, facilitating subsequent hydrometallurgical extraction [15,18,21,23].

Unlike conventional cyanidation, which generally requires neutralisation of acidic bio-oxidised material before leaching, thiourea operates under acidic conditions that may be compatible with those resulting from bio-oxidation. This compatibility can reduce the extent of pH adjustment required between the two stages, depending on the composition and chemistry of the bio-oxidised material [15,18,38].

The effectiveness of the combined process depends largely on the degree of sulphide oxidation achieved during biological pretreatment. Insufficient oxidation leaves a significant proportion of gold encapsulated within residual pyrite or arsenopyrite, thereby limiting subsequent thiourea extraction. Conversely, excessive oxidation may increase acid consumption or promote secondary mineral precipitation, which can reduce gold accessibility. Therefore, optimisation of the bio-oxidation stage is important for improving gold accessibility and the performance of subsequent thiourea leaching [18,23].

Following sufficient bio-oxidation, thiourea can dissolve accessible gold through oxidation by ferric ions or other suitable oxidants and subsequent complexation with thiourea to form the soluble $\text{Au}(\text{TU})_2^+$ complex. Increased exposure of gold resulting from sulphide oxidation can reduce mineralogical barriers to leaching and allow the rapid dissolution kinetics of thiourea to be utilised more effectively. Several recent investigations have demonstrated that bio-oxidised concentrates exhibit significantly higher gold recoveries during thiourea leaching than untreated refractory concentrates [38–40].

Recent research has focused on improving the efficiency of the integrated process by minimising thiourea degradation while maintaining high gold recoveries. The use of ferric-complexing additives such as citrate has been shown to reduce excessive oxidation of thiourea without impairing gold dissolution. Likewise, mechanochemical activation prior to leaching increases mineral surface area and enhances reagent accessibility, thereby reducing leaching time and improving reagent utilisation. These developments indicate that the principal limitations of thiourea can be partially mitigated through appropriate process optimisation [39,40].

From an economic perspective, the combined bio-oxidation–thiourea route should not be regarded as a universal replacement for cyanidation. Instead, its principal value lies in the treatment of refractory ores for which conventional cyanidation alone produces unsatisfactory gold recoveries. Under these circumstances, the higher reagent consumption associated with thiourea may potentially be offset by advantages such as improved gold extraction for suitable materials, compatibility with acidic bio-oxidation conditions, and reduced requirements for slurry neutralisation. Consequently, the economic feasibility of the process depends strongly on ore mineralogy, gold deportment, and the degree of refractoriness [37–40].

Overall, the integration of bio-oxidation with thiourea leaching combines the potential advantages of sulphide oxidation and acidic gold leaching. Bio-oxidation can increase the accessibility of gold associated with sulphide minerals, while thiourea provides relatively rapid gold dissolution under acidic conditions. Although this combination has shown potential for selected refractory gold-bearing materials, further improvements in reagent stability, process control, and economic performance are required to assess its suitability for wider application [15,18,38–40].

2.6 Research Gap

Bio-oxidation is well established as a pretreatment method for refractory gold-bearing materials, particularly where gold is associated with or encapsulated within sulphide minerals such as pyrite and arsenopyrite. Thiourea leaching has also been extensively investigated as an alternative gold-extraction route under acidic conditions. However, considerably less information is available on the sequential application of these two processes to flotation tailings, where complex mineralogy, relatively low gold grades, heterogeneous sulphide distribution, and secondary mineral formation may influence both bio-oxidation and subsequent gold extraction [15,18,20,21,38–41].

Previous studies have demonstrated that biological oxidation can increase gold accessibility through the oxidation of sulphide minerals, while thiourea can provide rapid gold dissolution under suitable acidic and oxidising conditions. Nevertheless, the effectiveness of the combined route is strongly dependent on the characteristics of the material and the conditions

applied during both stages. In particular, the extent to which changes occurring during bio-oxidation translate into improved subsequent thiourea extraction remains insufficiently understood for complex flotation tailings [15,18,23,38–40].

An important issue is therefore the relationship between the behaviour of sulphide-associated elements during bio-oxidation and the subsequent metallurgical response during thiourea leaching. Monitoring parameters such as pH and oxidation–reduction potential, together with the dissolution behaviour of Fe, S and other associated elements, can provide information on the progression of biological oxidation. However, changes in these parameters do not necessarily correspond directly to increased gold accessibility, because the response may also depend on mineralogical associations, the extent and location of sulphide oxidation, particle characteristics, and the formation of secondary phases.

A further research need concerns the performance of thiourea leaching after different bio-oxidation conditions. The acidic chemistry of the two processes provides a potential basis for their integration, but downstream gold extraction can also be influenced by factors such as pH, redox conditions and ferric-ion availability during thiourea leaching. Consequently, evaluating bio-oxidation solely on the basis of sulphide dissolution is insufficient; the resulting residues must also be assessed through subsequent gold-leaching tests.

The present study addresses this research gap by experimentally investigating bio-oxidation followed by thiourea leaching of sulphide-bearing flotation tailings. Bio-oxidation is evaluated through shake-flask and stirred-reactor experiments using measurements of pH, Eh and elemental dissolution, while subsequent thiourea-leaching tests are used to assess the apparent extraction of Au from the resulting residues. Direct thiourea leaching of untreated flotation tailings provides a reference for evaluating the effect of biological pretreatment, and additional tests examine the influence of ferric-ion addition during thiourea leaching. Through this experimental framework, the study aims to determine whether the investigated bio-oxidation treatments improve subsequent Au extraction and to identify the principal limitations of the combined bio-oxidation–thiourea route for the flotation tailings investigated.

2.6.1 Critical Synthesis of the Literature

The reviewed literature indicates that the potential effectiveness of a combined bio-oxidation thiourea process depends on the interaction between feed mineralogy, the extent of sulphide oxidation, and the conditions applied during subsequent gold leaching. Bio-oxidation is most relevant where sulphide minerals restrict gold accessibility, while thiourea offers rapid gold dissolution under acidic conditions when suitable solution chemistry and oxidation conditions are maintained [18,20,21,37–40].

The principal advantage of combining these processes is their potential chemical compatibility. Bio-oxidation is conducted under acidic and oxidising conditions, while

thiourea leaching also operates in acidic media. This may reduce the extent of conditioning required between pretreatment and leaching. However, compatibility of operating pH alone does not guarantee improved gold extraction. The effectiveness of the downstream leaching stage depends on whether biological oxidation has sufficiently altered the sulphide phases associated with gold and increased its accessibility.

The literature further demonstrates that indicators of bio-oxidation performance should be interpreted collectively. Changes in pH and Eh, together with the dissolution of sulphide-associated elements such as Fe and S, provide evidence of biological and chemical reactions occurring during treatment. Nevertheless, these parameters do not directly quantify gold liberation. Similar levels of elemental dissolution may produce different metallurgical responses depending on mineralogical associations, particle characteristics, and the location of gold within the feed.

Thiourea leaching introduces an additional set of controlling factors. Gold dissolution depends not only on the condition of the bio-oxidised material but also on leaching parameters including pH, redox potential, thiourea concentration and oxidant availability. Ferric iron can promote gold oxidation but may also contribute to thiourea degradation. Consequently, differences in gold extraction following bio-oxidation cannot necessarily be attributed exclusively to the extent of biological pretreatment [38–40].

Taken together, the literature supports an experimental approach in which bio-oxidation performance and subsequent thiourea-leaching behaviour are evaluated as connected but distinct stages. For flotation tailings in particular, comparison with untreated material is necessary to determine whether biological pretreatment provides a measurable improvement in subsequent Au extraction. This framework forms the basis of the experimental programme adopted in the present study.

2.7 Chapter Summary

Refractory gold ores require pretreatment because gold may be physically encapsulated, chemically associated with pyrite or arsenopyrite, or affected by preg-robbing materials. Bio-oxidation offers a lower-temperature route for degrading sulphide minerals through the activity of acidophilic microbial consortia and the regeneration of ferric iron. The result is increased accessibility of gold rather than direct biological dissolution of gold [2,15,18,20].

Thiourea is an acidic alternative lixiviant that can dissolve exposed gold rapidly, but its application is limited by oxidative degradation, high reagent consumption, sensitivity to solution chemistry, and incomplete industrial maturity. Combining bio-oxidation with thiourea leaching therefore represents a potential treatment route for selected refractory gold-bearing materials. However, its effectiveness depends on the characteristics of the feed, the extent of sulphide oxidation achieved during pretreatment, and the conditions applied during subsequent thiourea leaching [37–41].

3. CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Flotation Tailings Sample

The experimental work presented in this study was carried out using a flotation tailings sample supplied by BERALB Sh.A. Preliminary mineralogical characterisation of this material was performed by İstanbul Technical University (İTÜ) prior to the experimental programme at the University of Liège.

The flotation tailings sample was subsequently delivered to the GeMMe – Georesources, Mineral Engineering & Extractive Metallurgy Laboratory at the University of Liège for the experimental programme. Consequently, all laboratory-scale experiments described in this thesis, including particle size analysis, gravity concentration, bio-oxidation, and thiourea leaching, were performed exclusively using this flotation tailings sample. Therefore, unless otherwise stated, the term flotation tailings refers to this experimental material throughout the remainder of this thesis.

The flotation tailings originated from the processing of sulphide-bearing gold ore and contained a complex assemblage of sulphide and gangue minerals. Prior to the experimental programme, the material was homogenised and representative portions were prepared for particle size analysis, mineralogical characterisation, gravity concentration, bio-oxidation experiments, and subsequent thiourea leaching.

3.1.2 Chemical Reagents

All chemical reagents used throughout the experimental programme were of analytical grade. Distilled water was used for the preparation of all laboratory solutions unless otherwise specified.

The 9K growth medium used during the bio-oxidation experiments was prepared from ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$), dipotassium hydrogen phosphate (K_2HPO_4), magnesium sulphate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), potassium chloride (KCl), calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), and ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). Sulphuric acid (H_2SO_4) was used to adjust the pH of the culture medium to the desired operating range.

Thiourea ($\text{CS}(\text{NH}_2)_2$) was employed as the leaching reagent during gold extraction experiments. Ferric sulphate hydrate ($\text{Fe}_2(\text{SO}_4)_3 \cdot \text{H}_2\text{O}$) served as the oxidising agent required for thiourea leaching, while sulphuric acid was used for pH adjustment throughout the experiments.

3.1.3 Laboratory Equipment

The experimental programme was performed using laboratory-scale equipment available at the GeMMe – Georesources, Mineral Engineering & Extractive Metallurgy Laboratory at the University of Liège.

Particle size analysis was carried out using a mechanical sieve shaker equipped with standard laboratory sieves. The preliminary bio-oxidation tests were conducted in 250 mL Erlenmeyer flasks, while the reactor bio-oxidation experiments were performed in mechanically stirred glass reactors with working volumes of 1 L and 3 L. Thiourea leaching tests were carried out in mechanically stirred 1 L reactors. Continuous aeration during bio-oxidation was supplied using aquarium air pumps.

Routine monitoring of the experimental conditions was carried out using laboratory instruments for measuring pH, oxidation-reduction potential (Eh), and temperature. Pregnant leach solution (PLS) samples collected throughout the experimental programme were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Gold contents in selected solid samples were determined by fire assay, whereas mineralogical characterisation was performed by X-ray diffraction (XRD) and scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS).

3.2 Experimental Workflow

The experimental programme was designed to evaluate bio-oxidation as a pre-treatment prior to thiourea leaching of sulphide-bearing flotation tailings and to assess its effect on subsequent gold extraction. The work comprised sample characterisation, gravity separation, preliminary shake-flask bio-oxidation, reactor bio-oxidation, thiourea leaching, and chemical and metallurgical analyses.

The flotation tailings received at the University of Liège were homogenised and subjected to wet sieve analysis. A 200 g representative portion was separated using sieves with nominal apertures of 147, 106, 75, 53, 38, and 20 μm . After drying and weighing, three composite fractions were prepared for XRD analysis at the University of Liège: $>75\ \mu\text{m}$, 20–75 μm , and $<20\ \mu\text{m}$.

A single Falcon gravity concentration test was performed to produce a heavy-mineral concentrate for SEM/EDS characterisation. The Falcon concentrate was used only for microscopic investigation and was not used in the bio-oxidation or thiourea leaching tests. A separate pyrite-rich concentrate produced from the flotation tailings by gravity separation was prepared for Reactor Bio-oxidation Experiment 5.

The biological treatment programme comprised five preliminary shake-flask conditions, each performed in duplicate in 250 mL Erlenmeyer flasks. The duplicate pairs were designated as

numbers 11–12, 21–22, 31–32, 41–42, and 51–52. These tests were followed by five stirred-reactor bio-oxidation experiments. Experiments 1–3 were conducted at a working volume of 1 L, while Experiments 4 and 5 were conducted at a working volume of 3 L. Flotation tailings were used in Experiments 1–4, whereas the pyrite-rich concentrate was used in Experiment 5.

After bio-oxidation, a total of eight thiourea leaching tests were carried out in mechanically stirred 1 L reactors. Five tests used the residues from Reactor Experiments 1–5. The residue from Reactor Experiment 4 was divided into two subsamples and tested with and without ferric addition. A direct reference test was performed on untreated flotation tailings, and the final test used the combined residues from the duplicate shake-flask experiments.

All thiourea tests were completed over 6 h. Five solution samples were collected during each test, and pH, oxidation-reduction potential (Eh), and temperature were recorded at each sampling stage. Liquid samples from the bio-oxidation and thiourea stages were analysed chemically, while selected solid feeds and residues were analysed by fire assay and mineralogical methods.

Schematic Overview of the Experimental Workflow

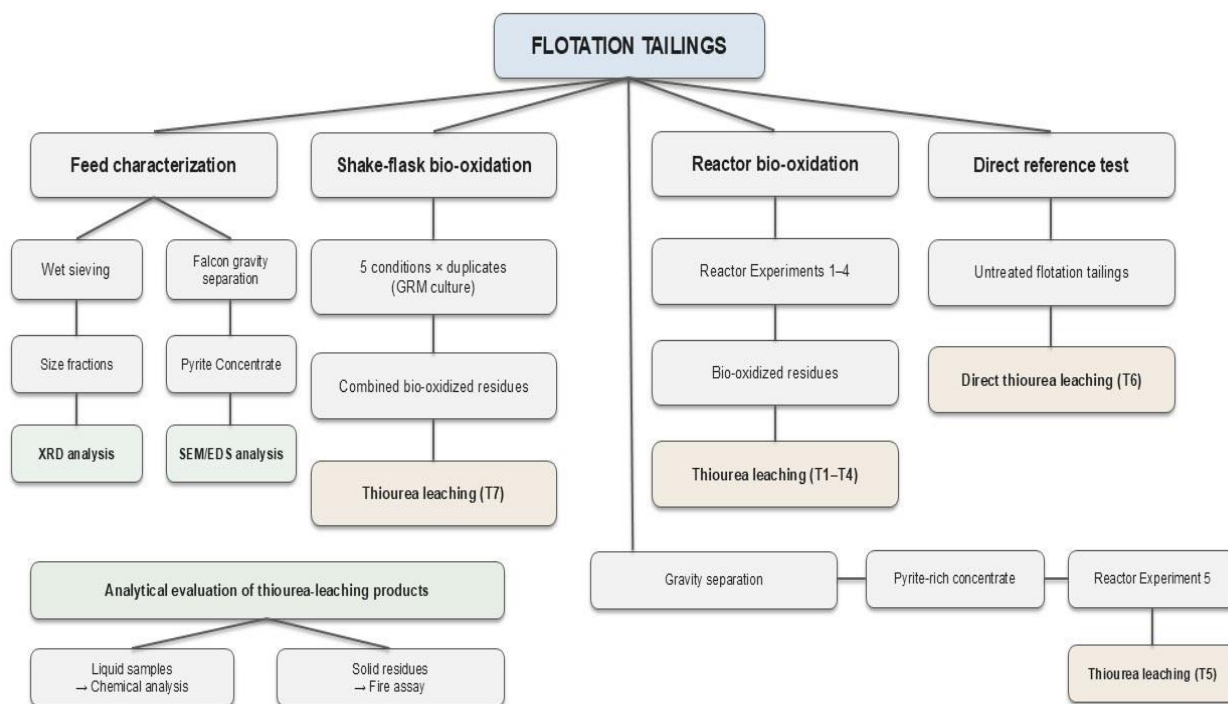


Figure 3.1 Schematic representation of the experimental workflow adopted in this study.

3.3 Sample Preparation and Particle Size Analysis

Prior to the experimental programme, the flotation tailings sample was homogenised to obtain representative subsamples for characterisation and processing tests.

Particle size analysis was carried out by wet sieving. A representative 200 g portion of flotation tailings was separated using sieves with nominal aperture sizes of 147, 106, 75, 53, 38, and 20 μm . Material passing the 20 μm sieve was collected in the bottom pan. Tap water was used during sieving to disperse agglomerated fine particles and facilitate separation.

Following sieving, the material recovered from each size interval was transferred to a labelled plastic bag and dried at 60 $^{\circ}\text{C}$. Each fraction was weighed together with its storage bag, and the corresponding empty-bag mass was subtracted to obtain the net fraction mass. The total net recovered mass was 192.12 g, corresponding to a mass recovery of 96.06% relative to the 200 g feed.

Three representative composite fractions were subsequently prepared for XRD analysis at the University of Liège. The $>75\ \mu\text{m}$ composite comprised the $+147\ \mu\text{m}$, $-147+106\ \mu\text{m}$, and $-106+75\ \mu\text{m}$ fractions. The $20-75\ \mu\text{m}$ composite comprised the $-75+53\ \mu\text{m}$, $-53+38\ \mu\text{m}$, and $-38+20\ \mu\text{m}$ fractions. The fine composite consisted of the $<20\ \mu\text{m}$ fraction.

The cumulative passing curve was used to determine d50 and d80.

3.3.1 Calculation of Particle Size Distribution

The mass percentage of each particle-size fraction was calculated using Equation (3.1):

$$\%Retained = \frac{m_i}{m_t} \times 100$$

where m_i is the net mass of the corresponding particle-size fraction and m_t is the total net recovered mass (192.12 g).

Cumulative retained and cumulative passing percentages were calculated from the normalised fraction masses. The cumulative passing curve was plotted against sieve aperture and used to determine d50 and d80 by logarithmic interpolation between adjacent sieve sizes. The full particle-size distribution, cumulative values, and characteristic sizes are presented in Chapter 4.

3.4 Mineralogical Characterisation

A comprehensive mineralogical characterisation programme was carried out to determine the mineral composition of the flotation tailings and to evaluate the distribution of sulphide minerals within the different particle-size fractions. The characterisation programme included X-ray diffraction (XRD), scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS), fire assay, and diagnostic leaching for Au deportment

analysis. These analytical techniques were selected to provide complementary information regarding mineralogical composition, particle morphology, elemental distribution, and precious metal content.

The analytical results obtained from these techniques were subsequently used to support the interpretation of the bio-oxidation and thiourea leaching experiments.

3.4.1 X-ray Diffraction (XRD)

Following wet sieve analysis, the recovered particle-size fractions were combined to prepare three representative composite samples: >75 μm , 20–75 μm , and <20 μm . These three composite samples were submitted for X-ray diffraction (XRD) analysis at the Laboratory of Mineralogy, University of Liège. The analyses were performed to identify and quantify the crystalline mineral phases present in the coarse, intermediate, and fine particle-size fractions.

In addition to the XRD analyses performed at the University of Liège, preliminary mineralogical characterisation data obtained by İstanbul Technical University (İTÜ) were used as complementary information. The İTÜ results and the XRD results obtained from the three particle-size composites prepared in the present study are presented separately in Chapter 4.

The XRD patterns and quantitative phase compositions are presented in Chapter 4.

3.4.2 Scanning Electron Microscopy and Energy-Dispersive Spectroscopy (SEM/EDS)

Scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS) was employed to investigate the morphology of the mineral particles and to determine the elemental composition of selected mineral phases.

For SEM/EDS analysis, the gravity concentrate produced during the Falcon gravity concentration test was selected because it represented a concentrated heavy-mineral fraction. The concentrate was prepared, mounted, and examined using SEM in order to identify the principal sulphide minerals and to investigate the possible occurrence of liberated gold particles.

The SEM/EDS investigation was undertaken to identify the principal sulphide and gangue phases, examine mineral associations, and assess whether liberated gold particles were present. The observations and representative spectra are presented in Chapter 4.

Elemental compositions of representative particles were determined using EDS analysis. These analyses were used to confirm the identity of the mineral phases observed during SEM examination and to support interpretation of the XRD results.

The representative SEM micrographs and corresponding EDS spectra are presented in Chapter 4.

3.4.3 Fire Assay

Fire assay analysis was employed to determine the gold content of selected solid samples throughout the experimental programme. The technique was selected because of its high analytical accuracy for precious metal determination in low-grade gold-bearing materials.

Representative samples collected during the experimental programme were submitted for fire assay analysis before and after the principal processing stages. The analytical results were subsequently used to evaluate the distribution of gold within the investigated materials and to assess the effectiveness of the applied treatment methods.

The fire assay results were used to compare the gold contents of selected feed materials and solid residues and to evaluate the apparent gold extraction associated with the investigated treatment stages.

3.5 Gravity Separation

A laboratory-scale Falcon gravity concentration test was performed on the flotation tailings to produce a heavy-mineral concentrate for subsequent SEM/EDS characterisation. The Falcon concentrate was prepared specifically for mineralogical investigation and was not used as feed material in the subsequent bio-oxidation or thiourea-leaching experiments.

Only one Falcon gravity concentration test was conducted during the experimental programme. The recovered gravity concentrate was subsequently submitted for scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS) to investigate the morphology and elemental composition of the concentrated heavy mineral fraction. Particular attention was given to the possible occurrence of liberated gold particles within the concentrate. However, SEM examination did not reveal the presence of free gold particles. The gravity concentrate produced by the Falcon concentrator was therefore used exclusively for mineralogical characterisation and was not employed in any of the subsequent bio-oxidation or thiourea leaching experiments.

The SEM/EDS observations and their implications for the subsequent treatment route are discussed in Chapter 4.

3.5.1 Preparation of the Pyrite-Rich Concentrate

In addition to the Falcon gravity concentration test, a separate pyrite-rich concentrate produced from the flotation tailings by gravity separation was prepared specifically for the

bio-oxidation programme. Unlike the Falcon concentrate, this material was produced independently and served as the feed material for Experiment 5.

The pyrite-rich concentrate was prepared to increase the proportion of sulphide minerals, particularly pyrite, relative to the original flotation tailings. The resulting pyrite-rich concentrate was subsequently characterised by chemical analysis prior to bio-oxidation in order to determine its elemental composition and gold content.

Following characterisation, the pyrite-rich concentrate was subjected to bio-oxidation under the same monitoring protocol applied to the flotation tailings. Throughout the experiment, pH, oxidation-reduction potential (Eh), temperature, and pregnant leach solution (PLS) chemistry were monitored periodically according to the experimental programme.

The analytical results obtained for the pyrite-rich concentrate are presented and discussed together with the corresponding bio-oxidation experiment in Chapter 4.

3.5.2 Purpose of Gravity Separation

The gravity separation stage fulfilled two distinct objectives within the present study.

First, the Falcon gravity concentration experiment provided a representative heavy mineral concentrate for detailed mineralogical investigation by SEM/EDS. This analysis enabled examination of the morphology of the dense mineral particles and assessment of the occurrence of liberated gold.

Second, the independently prepared pyrite-rich concentrate produced from the flotation tailings by gravity separation enabled evaluation of the influence of sulphide enrichment on the bio-oxidation process. The use of the pyrite-rich concentrate in Experiment 5 enabled the bio-oxidation and subsequent thiourea-leaching behaviour of a sulphide-enriched feed to be evaluated alongside the experiments conducted using the flotation tailings.

Accordingly, gravity separation was incorporated into the experimental programme both as a mineralogical characterisation tool and as a method for preparing a sulphide-enriched feed material for bio-oxidation experiments.

3.6 Preparation of the Growth Medium

9K medium was used throughout the bio-oxidation experiments to cultivate and maintain the acidophilic iron-oxidising microbial consortium. The composition of the medium was selected to provide the essential inorganic nutrients required for microbial growth while supplying ferrous iron as the primary energy source.

For the preparation of 1 L of 9K medium, 3.0 g $(\text{NH}_4)_2\text{SO}_4$, 0.5 g K_2HPO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g KCl, 0.01 g $\text{Ca}(\text{NO}_3)_2$, and 44.2 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were dissolved in distilled water. The solution was mixed until complete dissolution of all reagents was achieved. Subsequently, the pH of the medium was adjusted to 1.8–2.0 using concentrated sulphuric acid before use in the bio-oxidation experiments. The prepared medium was used immediately after preparation or stored for a short period under laboratory conditions until required for reactor operation.

Fresh 9K medium was prepared whenever required during the experimental programme, particularly after partial replacement of the process solution during long-term bio-oxidation experiments. The preparation procedure remained identical throughout the study to ensure consistent operating conditions among all experiments.

3.7 Bio-oxidation Experiments

3.7.1 Experimental Design

The bio-oxidation programme comprised preliminary shake-flask experiments followed by five stirred-reactor experiments.

The principal shake-flask study was conducted using the GRM mixed bacterial culture. Five experimental conditions were investigated, each performed in duplicate in 250 mL Erlenmeyer flasks. The duplicate pairs were designated 11–12, 21–22, 31–32, 41–42, and 51–52. During these experiments, pH, oxidation-reduction potential (Eh), and temperature were monitored periodically, and PLS samples were collected according to the experimental schedule. Following completion of the GRM shake-flask experiments, the bio-oxidised solid residues were preserved and subsequently combined for Thiourea Leaching Test T7.

Two additional shake-flask tests, designated Flasks 61 and 71, were conducted using the WW2 bacterial culture. These tests were monitored through periodic measurements of pH, Eh, and temperature to evaluate the behaviour of the WW2 culture under the applied conditions. However, the solid residues obtained from Flasks 61 and 71 were not subjected to subsequent thiourea leaching and were not included in the combined residue used for Test T7.

The reactor stage consisted of five independent bio-oxidation experiments. Experiments 1–3 were carried out at a working volume of 1 L, whereas Experiments 4 and 5 were conducted at a working volume of 3 L. Flotation tailings were used in Experiments 1–4, while Experiment 5 used a pyrite-rich concentrate produced from the flotation tailings by gravity separation.

Table 3.1 Overview of the bio-oxidation experimental programme and subsequent thiourea-leaching tests

Stage	Experimental system	Culture	Working volume	Feed material	Subsequent thiourea leaching
Preliminary conditions 1–5	Duplicate flasks 11–12, 21–22, 31–32, 41–42, 51–52	GRM mixed culture	250 mL	Flotation tailings	Residues combined for T7
Additional flask test	Flask 61 - 71	WW2 culture	250 mL	Flotation tailings	No
Reactor Experiment 1	Stirred reactor	GRM mixed culture	1 L	Flotation tailings	T1
Reactor Experiment 2	Stirred reactor	GRM mixed culture	1 L	Flotation tailings	T2
Reactor Experiment 3	Stirred reactor	GRM mixed culture	1 L	Flotation tailings	T3
Reactor Experiment 4	Stirred reactor	GRM mixed culture	3 L	Flotation tailings	T4A-No Fe T4B-Fe
Reactor Experiment 5	Stirred reactor	GRM mixed culture	3 L	Pyrite-rich concentrate	T5

3.7.2 Shake-Flask Bio-oxidation

The main shake-flask bio-oxidation study was performed using the GRM mixed bacterial culture. Five experimental conditions were each prepared in duplicate using 250 mL Erlenmeyer flasks. The duplicate pairs were designated 11–12, 21–22, 31–32, 41–42, and 51–52. The flasks contained flotation tailings, 9K medium, and the GRM culture and were maintained on a shaker at 165 rpm. The temperature was generally maintained at approximately 32–33 °C, while pH, Eh, and temperature were recorded periodically.

Because the sulphide-rich solids tended to settle at the bottom of the flasks, settled material was occasionally resuspended using a glass rod to improve contact between the bacterial culture and the mineral particles. PLS samples and operational observations were recorded according to the schedule of each condition.

Following completion of the GRM experiments, the bio-oxidised solid residues from Flasks 11–12, 21–22, 31–32, 41–42, and 51–52 were preserved and later combined for Thiourea Test T7.

Flasks 61 and 71 were prepared separately using the WW2 bacterial culture. Their pH, Eh, and temperature were monitored during the experimental period. These tests were monitored only through periodic measurements of pH, Eh, and temperature, and their solid residues were not used in any subsequent thiourea leaching experiment.

3.7.3 Reactor Configuration

The stirred-reactor experiments were carried out in laboratory glass reactors with working volumes of 1 L and 3 L. Continuous mixing was maintained at 175–185 rpm. Aerobic conditions were supplied continuously using aquarium air pumps connected to the reactors. pH, Eh, and temperature were monitored throughout operation.

3.7.4 Reactor Experimental Procedure

Fresh 9K medium and bacterial culture were introduced into the reactor before addition of the corresponding solid feed. Experiment 1 was conducted using 50 g of flotation tailings, whereas 100 g of solids was used in the subsequent 1 L reactor experiments. The remaining reactor conditions followed the test-specific records. Mechanical stirring and aeration were maintained throughout each experiment.

During the reactor bio-oxidation experiments, approximately 75% of the process solution was periodically withdrawn at intervals of approximately 3–4 days and replaced with fresh 9K medium prepared at the corresponding composition. This partial solution replacement was applied to simulate solution renewal during heap-leaching conditions while maintaining the solid material within the reactor.

3.7.5 Operational Monitoring and Sampling

During both flask and reactor bio-oxidation, pH, Eh, and temperature were recorded together with the date and operational observations. PLS samples were collected periodically for chemical analysis. Events such as pH adjustment, process-solution replacement, preparation of fresh medium, and addition of fresh bio-solution were documented.

3.7.6 Solid–Liquid Separation

At the end of each bio-oxidation experiment, the slurry was filtered to recover the process solution and bio-oxidised solid residue. The recovered solids were retained for the subsequent thiourea leaching tests and for additional solid analysis where required. The residues from the duplicate shake-flask experiments were preserved and later combined for Thiourea Test T7.

3.8 Thiourea Leaching

3.8.1 Experimental Design

A total of eight thiourea leaching tests were conducted in mechanically stirred 1 L reactors. The tests used different solid masses and test-specific solution and reagent quantities according to the amount and origin of the available feed. Therefore, no single solid mass, liquid volume, pulp density, or ferric-sulphate dosage was applied to all tests.

Five tests used the bio-oxidised residues obtained from Reactor Experiments 1–5. The residue from Reactor Experiment 4 was divided into two subsamples. One subsample was leached with ferric addition and the other without ferric addition to evaluate the influence of externally added ferric ions. Thiourea Test T6 used untreated flotation tailings taken directly from the original solid sample and therefore served as the non-bio-oxidised reference. Thiourea Test T7 used the combined bio-oxidised residues from the duplicate shake-flask experiments.

Table 3.2 Summary of the thiourea leaching programme.

Test	Feed material	Previous treatment	Special condition
T1	Reactor Experiment 1 residue	Reactor bio-oxidation	Test-specific conditions
T2	Reactor Experiment 2 residue	Reactor bio-oxidation	Test-specific conditions
T3	Reactor Experiment 3 residue	Reactor bio-oxidation	Test-specific conditions
T4A-No Fe	Reactor Experiment 4 residue, subsample 1	Reactor bio-oxidation	No ferric addition
T4B-Fe	Reactor Experiment 4 residue, subsample 2	Reactor bio-oxidation	Ferric addition
T5	Reactor Experiment 5 residue	Bio-oxidation of pyrite-rich concentrate	Test-specific conditions
T6	Untreated flotation tailings	None	Direct reference test
T7	Combined duplicate shake-flask residues	Shake-flask bio-oxidation	Combined-residue test

3.8.2 Test-Specific Leaching Conditions

The solid mass, liquid volume, thiourea quantity or concentration, ferric-sulphate addition, initial pH, initial Eh, and agitation conditions were recorded separately for each test. The test-specific experimental conditions for T1, T2, T3, T4-Fe, T4-NoFe, T5, T6, and T7 are reported individually with the corresponding experimental results in Chapter 4.

3.8.3 Leaching Duration and Monitoring

Each thiourea leaching test was completed over 6 h. Five liquid samples were collected during each test according to the recorded sampling schedule. At every sampling stage, pH, Eh, and temperature were measured and recorded separately for the corresponding sample. These measurements were used together with the chemical analyses to evaluate the evolution of the leaching conditions.

3.8.4 Solution Sampling

At each scheduled sampling stage, a solution aliquot was withdrawn and transferred to a labelled container for chemical analysis. Sample identifiers, leaching time, pH, Eh, and temperature were recorded in the laboratory notebook. The volume withdrawn at each sampling stage and any subsequent solution replacement were recorded separately for each test.

3.8.5 Recovery of Solid Residues

At the end of the 6 h leaching period, the slurry was filtered to separate the final solution from the solid residue. The recovered residues were retained for fire assay and additional characterisation where required.

3.9 Analytical Methods

The bio-oxidation and thiourea-leaching experiments were evaluated using a combination of mineralogical, chemical, and metallurgical analyses. Both solid and liquid samples collected throughout the experimental programme were analysed using complementary analytical techniques to characterise the materials and monitor the progress of the experiments.

The analytical methods employed in this study included inductively coupled plasma optical emission spectroscopy (ICP-OES), fire assay, X-ray diffraction (XRD), scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS), and diagnostic leaching for Au deportment analysis.

3.9.1 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Liquid samples collected during the bio-oxidation and thiourea leaching experiments were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) to determine the concentrations of dissolved elements in the pregnant leach solutions.

Immediately after sampling, the solutions were transferred into labelled polyethylene containers and preserved until analysis. Prior to ICP-OES determination, the samples were filtered when necessary to remove suspended solids. The analytical measurements were performed according to the standard operating procedures of the analytical laboratory.

The dissolved concentrations of Fe, S, and Zn were monitored at the principal bio-oxidation sampling stages, while additional elements, including Cu, Al, As, Ag, and other detectable elements, were analysed at selected stages where analytical data were available. The analytical results were subsequently used to evaluate the dissolution behaviour of the investigated mineral phases during both bio-oxidation and thiourea leaching.

3.9.2 Fire Assay Analysis

Gold concentrations in selected solid samples were determined by fire assay, which remains the reference analytical technique for precious metal determination in geological and metallurgical materials.

Selected feed materials and solid residues obtained after the principal experimental stages were submitted for fire assay. The analytical results were used to determine the Au content of these materials and to assess changes in solid-phase Au concentration following the investigated treatments.

The fire assay data provided the basis for calculating the apparent Au extraction values presented in Chapter 4.

3.9.3 X-ray Diffraction Analysis

Following wet sieving, the recovered particle-size fractions were combined to prepare three representative composite samples ($>75\ \mu\text{m}$, $20\text{--}75\ \mu\text{m}$, and $<20\ \mu\text{m}$), which were analysed by XRD at the Laboratory of Mineralogy, University of Liège. The analyses were used to identify and quantify the principal crystalline mineral phases within each particle-size class.

Preliminary mineralogical characterisation data obtained by İstanbul Technical University (İTÜ) were used as complementary information and were interpreted separately from the XRD results obtained from the three particle-size composites prepared in the present study.

3.9.4 Scanning Electron Microscopy and Energy-Dispersive Spectroscopy

Scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS) was employed to investigate the morphology and elemental composition of selected mineral particles.

The analysed material consisted of the gravity concentrate produced during the Falcon gravity separation experiment. SEM examination enabled detailed observation of particle

morphology, while EDS analyses confirmed the elemental composition of representative mineral grains.

Sulphide minerals were identified during the SEM/EDS investigation, while no liberated gold particles were observed in the analysed Falcon concentrate. The SEM/EDS results were used together with the XRD and chemical characterisation data to support interpretation of the mineralogical characteristics of the flotation tailings.

3.9.5 Diagnostic Leaching and Au Deportment Analysis

Diagnostic leaching was used as a complementary characterisation method to determine the distribution of Au among different mineralogical associations within the flotation tailings. The analysis was carried out externally by CORES Mineral Mining Consultancy Services Co. at İTÜ Teknopark. A sequential diagnostic leaching procedure was applied, beginning with direct NaCN leaching to quantify the free-milling Au fraction, followed by controlled decomposition stages targeting pyrrhotite/hematite, sphalerite, chalcopyrite, pyrite and quartz-associated Au. The diagnostic leaching results were used to evaluate the relative proportions of free, sulphide-associated and quartz-associated Au in the flotation tailings. [42]

3.10 Data Processing and Evaluation

Experimental data collected throughout the study were organised and processed using spreadsheet software. Operational parameters recorded during bio-oxidation and thiourea leaching, including pH, oxidation-reduction potential (Eh), and temperature, were arranged chronologically according to the corresponding sampling dates and experimental stages.

For the bio-oxidation experiments, pH and Eh measurements were plotted as a function of experimental time to evaluate changes in the chemical and electrochemical conditions during treatment. Temperature measurements were included in the operational data tables but were not presented graphically because only minor variations were observed during the experiments.

For each thiourea leaching test, the five sampling stages were arranged according to leaching time. pH and Eh were plotted as functions of time, while temperature was retained in the corresponding monitoring table. Each test was evaluated separately because the solid mass and reagent conditions differed among tests.

The particle-size distribution data obtained from wet sieving were used to calculate the percentage retained, cumulative percentage retained, and cumulative percentage passing for each sieve fraction. These data were subsequently employed to construct particle-size distribution curves and to determine the characteristic particle diameters (d50 and d80).

ICP-OES analytical results were organised according to sampling time for each experiment. Concentration profiles of Fe, S, and Zn were used to evaluate their dissolution behaviour during bio-oxidation, while the concentrations of other analysed elements were evaluated at the available sampling stages. The integrated evaluation of these datasets formed the basis of the Results and Discussion presented in Chapter 4.

3.11 Quality Assurance and Experimental Reliability

Experimental procedures were documented throughout the study to support consistency in reactor operation, solution preparation, sampling, and analytical measurements. Test-specific conditions were recorded where experimental parameters differed among the individual experiments.

The pH, oxidation-reduction potential (Eh), and temperature were monitored regularly during both the bio-oxidation and thiourea leaching experiments using calibrated laboratory instruments. Measurements were recorded immediately after acquisition in the laboratory notebook together with the corresponding operational observations, thereby minimising the possibility of transcription errors.

Fresh 9K growth medium was prepared according to the established formulation. Thiourea leaching solutions were prepared according to the recorded test-specific conditions because the solid masses, solution volumes, and ferric additions were not identical for all tests.

Representative liquid samples collected during reactor operation were transferred into labelled containers immediately after sampling and preserved under laboratory conditions until chemical analysis. Solid samples were similarly labelled and stored following drying to avoid cross-contamination between experiments.

Mineralogical and chemical analyses were performed using established analytical techniques, including X-ray diffraction (XRD), scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM/EDS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and fire assay. The complementary analytical methods provided different types of mineralogical and chemical information that were considered together during interpretation of the experimental results.

Throughout the study, all experimental observations, including operational interventions such as solution replacement, pH adjustment, preparation of fresh medium, and routine reactor maintenance, were documented systematically. These records provided a comprehensive operational history for each experiment and facilitated interpretation of the experimental results presented in Chapter 4.

3.12 Chapter Summary

This chapter described the materials, experimental procedures, and analytical methods used to investigate bio-oxidation and subsequent thiourea leaching of sulphide-rich flotation tailings.

The experimental sample was characterised by wet sieving, XRD, SEM/EDS, diagnostic leaching and Au deportment analysis, chemical analysis, and fire assay. Wet sieving was performed on a 200 g feed using sieves from 147 to 20 μm , yielding a total net recovered mass of 192.12 g. The $>75\ \mu\text{m}$, 20–75 μm , and $<20\ \mu\text{m}$ composite fractions were prepared from the recovered wet-sieving fractions and analysed by XRD at the Laboratory of Mineralogy, University of Liège, while the preliminary ITÜ mineralogical characterisation data were used as complementary information.

The bio-oxidation programme comprised five GRM shake-flask conditions performed in duplicate, two additional WW2 shake-flask tests, and five independent stirred-reactor experiments. The reactor residues, untreated flotation tailings, and combined flask residues were subsequently used in eight thiourea leaching tests. All thiourea tests were conducted in stirred 1 L reactors for 6 h, with five sampling stages and test-specific pH, Eh, and temperature records.

The experimental results obtained using these procedures are presented and discussed in Chapter 4.

4. CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterisation of the Flotation Tailings

Prior to the bio-oxidation and thiourea leaching experiments, the flotation tailings were characterised to assess their particle-size distribution, mineralogical composition, and selected chemical characteristics. The characterisation programme was designed to provide the necessary information for interpreting the subsequent bio-oxidation behaviour and gold extraction performance. Particle-size distribution, mineralogical composition, microstructural observations, and chemical analyses were performed using complementary analytical techniques.

The characterisation stage consisted of six main parts: (i) particle-size distribution by wet sieve analysis, (ii) X-ray diffraction (XRD), (iii) scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM-EDS), (iv) diagnostic leaching and Au deportment analysis, (v) chemical characterisation of the pyrite-rich concentrate prepared for Reactor Bio-oxidation Experiment 5, and (vi) initial Au characterisation by fire assay. The results obtained from these analyses are presented in the following sections.

4.1.1 Particle-Size Distribution

The particle-size distribution of the flotation tailings was determined by wet sieve analysis using a representative 200 g sample. Wet sieving was selected to minimise particle agglomeration and to improve the separation efficiency of the fine fractions.

The sample was separated using sieves with nominal aperture sizes of 147, 106, 75, 53, 38, and 20 μm . After sieving, the retained fractions were washed with tap water, dried in an oven at 60 $^{\circ}\text{C}$, and weighed individually. The recorded laboratory masses initially included the weight of the plastic collection bags. Therefore, the empty bag mass was subtracted from each measurement to obtain the net mass of every size fraction.

The total recovered dry mass after sieving was 192.12 g, corresponding to a mass loss of 7.88 g compared with the initial sample. During the wet sieving procedure, a small quantity of ultrafine particles was visually observed to remain suspended in the wash water and was probably lost during decantation of the excess water before drying. The particle-size distribution was calculated on the basis of the total recovered dry mass of 192.12 g, without assigning the unrecovered mass to any individual size fraction.

The particle-size distribution is presented in Table 4.1.

Table 4.1 Particle-size distribution of the flotation tailings.

Sieve aperture (μm)	Size fraction (μm)	Weight (g)	Weight (%)	Cumulative retained (%)	Cumulative passing (%)
147	+147	17.70	9.21	9.21	90.79
106	+106 -147	13.50	7.03	16.24	83.76
75	+75 -106	16.84	8.77	25.01	74.99
53	+53 -75	19.11	9.95	34.95	65.05
38	+38 -53	20.94	10.90	45.85	54.15
20	+20 -38	25.04	13.03	58.89	41.11
	-20	78.99	41.11	100.00	0.00
Total		192.12	100		

The results indicate that the flotation tailings consisted predominantly of fine particles. The <20 μm fraction represented the largest individual size class, accounting for approximately 41.11% of the total sample mass. In contrast, particles coarser than 147 μm accounted for 9.21% of the total recovered mass.

The cumulative retained and cumulative passing values calculated from the mass distribution were used to construct the particle-size distribution curves shown in Figure 4.1.

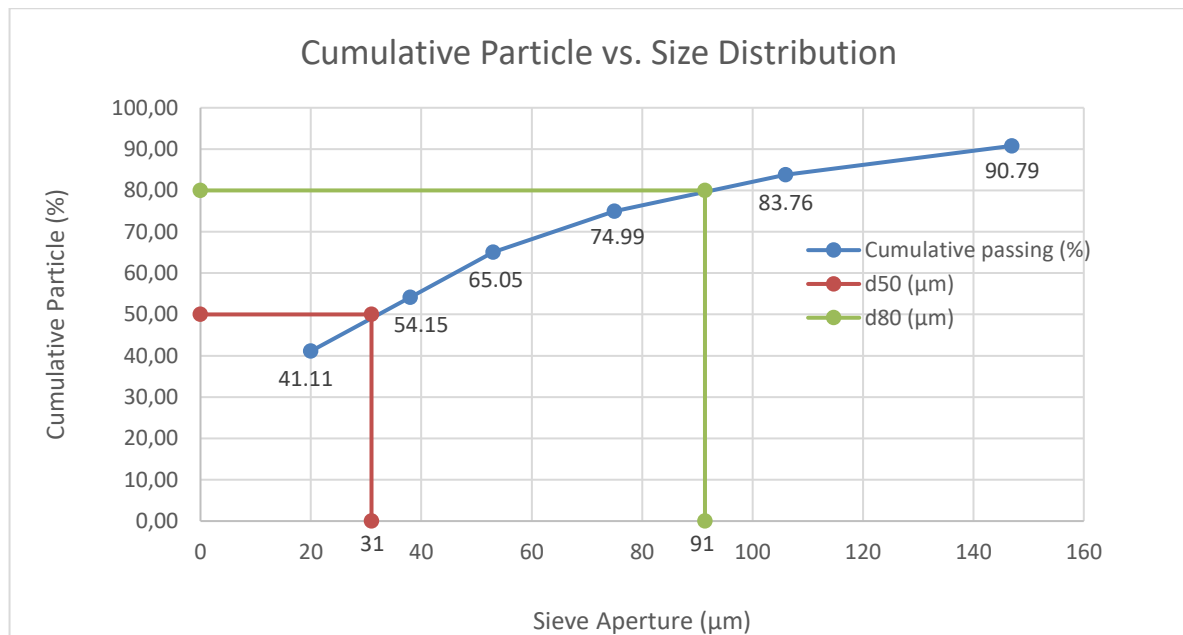


Figure 4.1 Particle-size distribution curves (cumulative passing).

From the cumulative passing curve, the characteristic particle sizes were determined by interpolation. The calculated values were approximately:

$$d_{50} \approx 31 \mu\text{m}$$

$$d_{80} \approx 91 \mu\text{m}$$

These values indicate that half of the flotation tailings consisted of particles finer than approximately 31 μm , while approximately 80% of the material was finer than 91 μm .

The predominance of fine particles increased the available mineral surface area for bio-oxidation. During the experiments, however, settling of dense sulphide-bearing particles was observed, requiring adequate agitation and occasional manual resuspension.

Following particle-size analysis, three representative particle-size fractions were prepared for mineralogical characterisation by X-ray diffraction:

$$>75 \mu\text{m}$$

$$20\text{--}75 \mu\text{m}$$

$$<20 \mu\text{m}$$

These fractions were selected to investigate the relationship between particle size and mineralogical composition before the bio-oxidation experiments.

4.1.2 X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was performed to investigate the mineralogical composition of the flotation tailings and to evaluate the distribution of the principal mineral phases within different particle-size fractions.

During this study, XRD information was obtained from two complementary sources. Initially, a representative flotation tailings sample was analysed at Istanbul Technical University (İTÜ), where a comprehensive mineralogical report was prepared. Subsequently, additional XRD analyses were carried out at the Laboratory of Mineralogy, University of Liège, using the three composite particle-size samples prepared from the wet-sieving fractions ($>75 \mu\text{m}$, $20\text{--}75 \mu\text{m}$, and $<20 \mu\text{m}$). These analyses allowed the mineralogical composition to be evaluated as a function of particle size.

Since both analyses were performed on representative samples originating from the same flotation tailings material, the results are complementary and are discussed together in this chapter.

The quantitative phase compositions obtained for the three investigated size fractions are presented in Table 4.2.

Table 4.2 Quantitative mineralogical composition of the flotation tailings determined by XRD.

Mineral phase	>75 μm (%)	20–75 μm (%)	<20 μm (%)
Quartz	75.04	65.06	28.44
Albite	11.65	6.91	4.20
Chlorite IIb	11.54	9.64	15.46
Pyrite	1.77	18.40	51.90

4.1.2.1 Coarse Fraction (>75 μm)

The coarse particle-size fraction was dominated by quartz, representing 75.04% of the crystalline phases identified by XRD. Albite and chlorite IIb accounted for 11.65% and 11.54%, respectively, whereas pyrite represented only 1.77%.

This mineralogical composition indicates that the coarse particles consisted predominantly of silicate gangue minerals, while only a limited proportion of sulphide minerals remained in this size fraction.

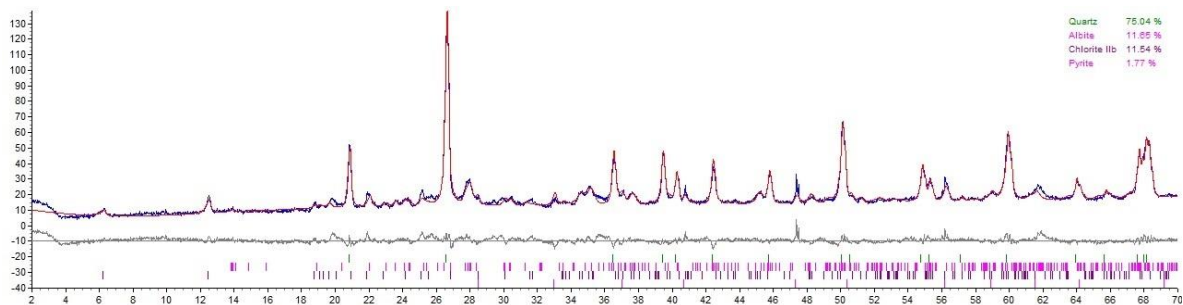


Figure 4.2 XRD diffractogram of the >75 μm fraction.

4.1.2.2 Intermediate Fraction (20–75 μm)

A significant change in mineralogical composition was observed in the intermediate particle-size fraction.

Although quartz remained the dominant mineral (65.06%), its abundance decreased compared with the coarse fraction. At the same time, the pyrite content increased markedly to 18.40%, indicating progressive enrichment of sulphide minerals as particle size decreased.

The contents of albite (6.91%) and chlorite IIb (9.64%) were slightly lower than those observed in the coarse fraction.

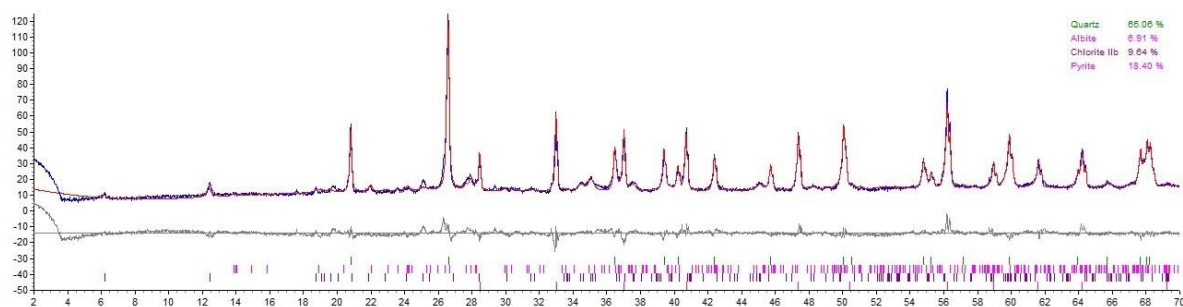


Figure 4.3 XRD diffractogram of the 20–75 µm fraction.

4.1.2.3 Fine Fraction (<20 µm)

The finest particle-size fraction exhibited a substantially different mineralogical composition.

Pyrite became the dominant crystalline phase, accounting for 51.90% of the identified minerals, whereas the quartz content decreased to 28.44%. Chlorite IIb represented 15.46%, while albite accounted for only 4.20%.

The strong enrichment of pyrite within the <20 µm fraction demonstrates that sulphide minerals were preferentially concentrated in the finest particles of the flotation tailings.

Considering that the particle-size analysis showed that the <20 µm fraction represented the largest proportion of the investigated material, these results indicate that a considerable amount of the sulphide-bearing fraction was associated with ultrafine particles.

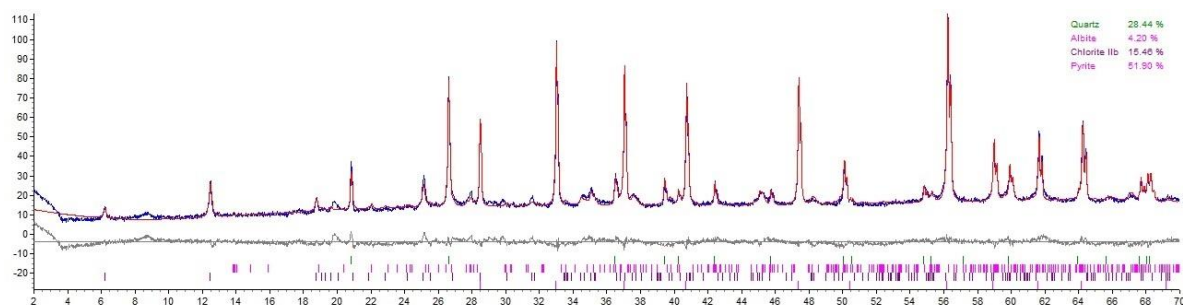


Figure 4.4 XRD diffractogram of the <20 µm fraction.

4.1.2.4 Mineralogical Distribution as a Function of Particle Size

The XRD analyses reveal a clear relationship between particle size and mineralogical composition.

Quartz showed a progressive decrease with decreasing particle size, from 75.04% in the coarse fraction to 28.44% in the finest fraction. Conversely, pyrite exhibited the opposite trend, increasing from 1.77% in the >75 µm fraction to 51.90% in the <20 µm fraction.

This enrichment of pyrite in the fine fractions is particularly significant because pyrite was the principal target mineral during the bio-oxidation stage. Bio-oxidation was therefore investigated as a pre-treatment to promote oxidation of the pyrite-rich sulphide fraction before subsequent thiourea leaching.

The mineralogical observations obtained by XRD provided supporting information for the experimental strategy adopted in this work, in which bio-oxidation was investigated prior to thiourea leaching. The flotation tailings were first subjected to bio-oxidation in order to oxidise the sulphide minerals identified by XRD, after which the bio-oxidised residues were treated by thiourea leaching to recover gold.

Furthermore, the enrichment of pyrite observed in the fine fractions supports the preparation of the pyrite-rich concentrate used in Reactor Bio-oxidation Experiment 5. The subsequent chemical characterisation of this concentrate confirmed its elevated sulphur, iron and gold contents, which are discussed in the following section.

Spectrum	Elements
Spectrum 1	Ag (77.69%), S (18.13%), Fe (4.18%)
Spectrum 2	Fe (54.85%), S (45.15%)
Spectrum 3	Ba (62.99%), O (21.42%), S (15.59%)

4.1.3 Scanning Electron Microscopy and Energy-Dispersive Spectroscopy (SEM-EDS)

Scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM-EDS) was performed to investigate the morphology and elemental composition of selected particles recovered from the gravity concentration stage. The principal objective of this analysis was to identify sulphide minerals and to investigate the possible occurrence of visible liberated gold.

The SEM-EDS investigation was carried out using a Falcon gravity concentrate prepared from the flotation tailings. This concentrate was produced solely for microscopic characterisation and was not used as feed material for the subsequent bio-oxidation experiments. The pyrite-rich concentrate employed in Reactor Bio-oxidation Experiment 5 was prepared separately.

Representative SEM micrographs together with the corresponding EDS spectra are presented in Figure 4.5.

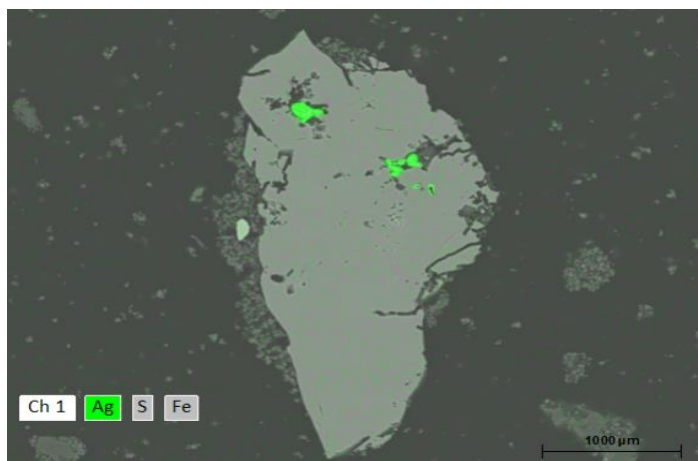


Figure 4.5 SEM images and corresponding EDS spectra.

EDS analyses identified several mineral particles with different elemental compositions.

One analysed particle exhibited predominantly iron and sulphur, consistent with an iron sulphide phase. Considering the XRD results, which confirmed the presence of pyrite in the investigated material, this particle was interpreted as pyrite. Another analysed particle showed high concentrations of silver and sulphur, while a third particle contained mainly barium, oxygen and sulphur, indicating the presence of a barium sulphate phase.

The EDS results therefore confirm the presence of sulphide minerals within the gravity concentrate and demonstrate the mineralogical complexity of the investigated material.

Despite careful examination of the analysed particles, no liberated gold particles were observed in the investigated SEM fields. Consequently, the occurrence of gold could not be confirmed directly by SEM-EDS observations.

The absence of visible liberated Au in the investigated SEM fields does not demonstrate the absence of Au from the material. Au may have been present at particle sizes or concentrations below those observable in the investigated fields, or it may have occurred in association with other mineral phases. Fire assay analyses independently confirmed measurable Au concentrations in the investigated materials.

Therefore, the SEM-EDS observations indicated that no coarse liberated Au particles were identified in the investigated fields. Although these observations do not establish the mode of Au occurrence, they are compatible with the possibility that Au occurs as very fine particles or in association with sulphide minerals.

It should be noted that the absence of visually identified liberated Au particles in the investigated SEM fields does not contradict the diagnostic leaching results, which indicated that 27.8% of the Au occurred as a free-milling fraction [42]. In the diagnostic leaching

procedure, this fraction was operationally defined by the Au extracted during the initial direct NaCN leaching stage and therefore does not necessarily correspond to coarse liberated Au particles that would be readily observable by SEM. The free-milling Au may have occurred as fine or sparsely distributed particles that were not encountered within the limited SEM fields examined. Consequently, the SEM-EDS and diagnostic leaching results should be regarded as complementary rather than contradictory observations.

4.1.4 Au Deportment by Diagnostic Leaching

Diagnostic leaching provided additional information on the operationally defined Au deportment of the flotation tailings. The results assigned 27.8% of the total Au to the free-milling fraction. The largest individual fraction, 39.2%, was assigned to the quartz-associated residual fraction. Pyrite-associated Au represented 18.9%, while 14.0% was assigned to sphalerite. The contributions attributed to chalcopyrite and pyrrhotite/hematite were negligible, at 0.2% and 0.0%, respectively.

The combined fraction attributed to sulphide minerals amounted to approximately 33.1%, indicating that bio-oxidation could potentially have its most direct effect on this part of the Au inventory. At the same time, the relatively large fraction operationally assigned to the quartz-associated residue indicates that a considerable proportion of the Au was not attributed to sulphide-bearing fractions by the diagnostic-leaching procedure. This distinction is important for interpreting the subsequent bio-oxidation and thiourea-leaching results, because oxidation of sulphide minerals would not necessarily improve the accessibility of Au assigned to the non-sulphide residual fraction.

Table 4.3 Au deportment of the flotation tailings determined by diagnostic leaching [42].

Au association	Au distribution (%)
Free Au	27.8
Pyrrhotite / Hematite	0
Sphalerite	14
Chalcopyrite	0.2
Pyrite	18.9
Quartz	39.2
Total	100

4.1.5 Chemical Characterisation of the Pyrite-rich Concentrate

To further characterise the sulphide-rich material selected for Reactor Bio-oxidation Experiment 5, the pyrite-rich concentrate obtained by gravity separation was chemically analysed prior to bio-oxidation. The objective of this analysis was to determine the elemental composition of the concentrate and to verify the effectiveness of the gravity concentration stage.

The analytical results are summarised in Table 4.4.

Table 4.4 Chemical composition of the pyrite-rich concentrate used in Reactor Bio-oxidation Experiment 5

Component	Unit	Value
Cu	wt.%	0.31
S	wt.%	40.86
Fe	wt.%	35.68
Au	g/t	1.09
Ag	g/t	3.87

The concentrate contained high concentrations of sulphur and iron, indicating that gravity separation effectively enriched the sulphide fraction of the flotation tailings. These results are consistent with the XRD analysis, which demonstrated a progressive enrichment of pyrite with decreasing particle size.

The measured Au grade of 1.09 g/t was higher than the 0.81 g/t measured for the bulk flotation tailings, indicating that the gravity concentration step also produced a moderate enrichment of Au. This sulphide-rich concentrate was therefore selected as the feed material for Reactor Bio-oxidation Experiment 5, where its bio-oxidation behaviour was compared with that of the bulk flotation tailings.

The concentrate also contained 3.87 g/t Ag.

The chemical analysis, together with the XRD and SEM-EDS observations, confirmed that the gravity concentration stage produced a sulphide-rich material suitable for investigating its behaviour during bio-oxidation and subsequent thiourea leaching

4.1.6 Summary of the Characterisation Results

The characterisation programme provided complementary information regarding the physical, mineralogical, and chemical properties of the investigated flotation tailings.

Particle-size analysis demonstrated that the material was predominantly fine-grained, with approximately 41% of the sample consisting of particles smaller than 20 µm. XRD analysis showed that pyrite became progressively enriched as particle size decreased, whereas quartz predominated in the coarse fractions.

SEM-EDS observations confirmed the presence of iron sulphide particles within the gravity concentrate. Although no liberated gold particles were identified during the SEM investigation, fire assay and chemical analyses demonstrated that measurable quantities of gold were present within the investigated materials. These results confirm the presence of

measurable Au in the investigated materials, although the mineralogical mode of Au occurrence could not be established from the SEM-EDS observations.

Diagnostic leaching further showed that 27.8% of the Au occurred as a free-milling fraction, while approximately 33.1% was associated with sulphide minerals and 39.2% with quartz. These results indicate that only part of the total Au inventory could potentially benefit directly from sulphide oxidation and provide an important mineralogical basis for interpreting the subsequent Au extraction results.

The chemical analysis of the pyrite-rich concentrate confirmed its high S and Fe contents and showed an Au grade of 1.09 g/t. Consequently, this concentrate was selected for Reactor Bio-oxidation Experiment 5 in order to investigate the bio-oxidation and subsequent thiourea-leaching behaviour of a sulphide-enriched feed material.

Overall, the characterisation results provide the mineralogical and chemical basis for interpreting the bio-oxidation behaviour and gold extraction performance discussed in the following sections.

4.2 Shake-Flask Bio-oxidation Experiments

Shake-flask bio-oxidation experiments were performed as a preliminary stage before the stirred-tank reactor experiments in order to evaluate the activity of the bacterial cultures and to determine suitable operating conditions for the subsequent scale-up studies. The experiments were designed to investigate the influence of recycled bio-solution content and solid loading on the bio-oxidation behaviour of the flotation tailings.

Two bacterial cultures were evaluated during this study. The first series of experiments was performed using the GRM mixed bacterial culture, while a second series employed the WW2 bacterial culture. The GRM experiments constituted the main preliminary bio-oxidation programme and the resulting bio-oxidised residue was subsequently subjected to thiourea leaching. In contrast, the WW2 experiments were monitored through pH, Eh, and temperature measurements, and their residues were not used in the subsequent gold leaching experiments.

Throughout the shake-flask experiments, pH, oxidation-reduction potential (Eh) and temperature were monitored periodically. At each monitoring date, pregnant leach solution (PLS) samples were collected for chemical analysis by ICP-OES. Iron (Fe), sulphur (S) and zinc (Zn) were analysed for every sampling event, whereas additional dissolved elements were determined only at selected sampling dates.

4.2.1 Experimental Design of the GRM Shake-Flask Bio-oxidation Experiments

The GRM shake-flask bio-oxidation experiments were carried out in 250 mL Erlenmeyer flasks placed on an orbital shaker operating at approximately 165 rpm. Five experimental conditions were investigated, each prepared in duplicate, resulting in ten individual shake flasks labelled as 11, 12, 21, 22, 31, 32, 41, 42, 51 and 52.

The experimental variables consisted of the proportion of recycled bio-solution, the amount of freshly prepared 9K medium and the solid loading, while the total liquid volume was maintained at 250 mL for all experiments. Three different bio-solution to fresh medium ratios (100:0, 75:25 and 50:50) and two solid loadings (25 g and 12.5 g) were evaluated. The complete experimental conditions are presented in Table 4.5.

Table 4.5 Experimental conditions of the GRM shake-flask bio-oxidation experiments.

Condition	Flask IDs	Recycled bio-solution (%)	Fresh 9K medium (%)	Total liquid volume (mL)	Solid loading (g)
1	11–12	100	0	250	25.0
2	21–22	75	25	250	25.0
3	31–32	50	50	250	25.0
4	41–42	100	0	250	12.5
5	51–52	50	50	250	12.5

Each experimental condition was prepared independently in duplicate. Individual monitoring of each flask enabled comparison of the pH, Eh, and temperature evolution between the corresponding duplicate pairs.

4.2.2 Monitoring of the GRM Shake-Flask Bio-oxidation Experiments

The progress of the GRM shake-flask bio-oxidation experiments was monitored by periodic measurements of pH, oxidation-reduction potential (Eh), and temperature. Measurements were recorded separately for each flask to follow the evolution of the individual experiments and to compare the behaviour of the corresponding duplicate pairs. The individual pH and Eh measurements recorded during the 13-day monitoring period are presented in Tables 4.6 and 4.7, respectively.

Although individual pH and Eh measurements were available for the initial 13-day monitoring period, the GRM flask systems remained in contact with the bacterial culture for a total of 104 days before the flask contents were combined. Continuous pH and Eh monitoring was not performed throughout this entire period.

Table 4.6 pH measurements recorded during the GRM shake-flask bio-oxidation experiments.

Flask	Day 0	Day 1	Day 2	Day 3	Day 7	Day 10	Day 13
11	1.98	1.89	1.81	1.81	1.70	1.61	1.49
12	1.98	1.87	1.82	1.80	1.70	1.61	1.50
21	2.06	1.96	1.87	1.85	1.68	1.59	1.48
22	2.05	1.97	1.88	1.83	1.67	1.60	1.47
31	2.13	2.04	1.89	1.85	1.69	1.60	1.48
32	2.11	2.02	1.85	1.81	1.65	1.58	1.46
41	1.90	1.86	1.78	1.80	1.69	1.58	1.54
42	1.93	1.84	1.80	1.77	1.69	1.58	1.54
51	2.08	2.05	1.92	1.87	1.70	1.57	1.53
52	2.08	2.05	1.92	1.85	1.68	1.60	1.56

Table 4.7 Oxidation-reduction potential measurements recorded during the GRM shake-flask bio-oxidation experiments.

Flask	Day 0	Day 1	Day 2	Day 3	Day 7	Day 10	Day 13
11	541	525	536	537	545	610	615
12	543	532	546	543	535	604	603
21	489	505	533	535	510	608	621
22	490	509	533	533	526	617	603
31	469	507	562	550	510	602	592
32	466	506	553	545	520	609	610
41	549	535	581	590	645	679	704
42	550	538	586	592	620	674	698
51	460	479	568	580	595	642	694
52	458	477	570	579	612	651	686

4.2.3 Evolution of pH During the GRM Shake-Flask Bio-oxidation Experiments

The evolution of pH in the individual GRM shake-flask experiments is illustrated in Figure 4.6. The corresponding numerical measurements are presented in Table 4.6.

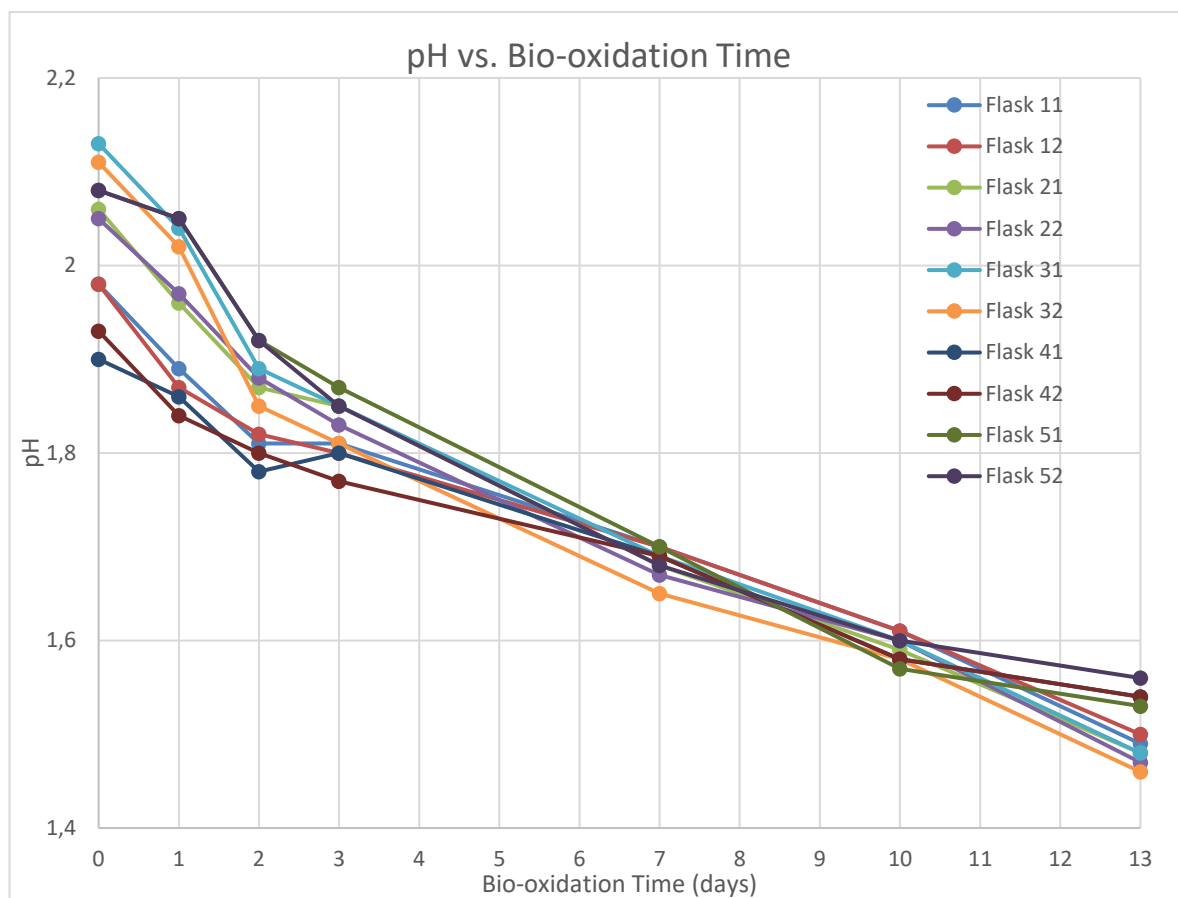


Figure 4.6 Evolution of pH during the GRM shake-flask bio-oxidation experiments.

All GRM shake-flask experiments exhibited a progressive decrease in pH over the 13-day monitoring period. Although differences were observed among the initial pH values, the measurements gradually converged towards a relatively narrow range as the experiments progressed.

The highest initial pH values were recorded in Flasks 31 and 32, at 2.13 and 2.11, respectively. In contrast, Flasks 41 and 42 showed the lowest initial values, at 1.90 and 1.93. The remaining flasks had initial pH values ranging from 1.98 to 2.08.

By Day 13, the pH values had decreased to between 1.46 and 1.56. Flask 32 reached the lowest final pH of 1.46, followed by Flask 22 at 1.47 and Flasks 21 and 31 at 1.48. The highest final pH was recorded in Flask 52 at 1.56.

The duplicate pairs exhibited closely comparable pH profiles throughout the monitoring period. Flasks 11 and 12, for example, both started at pH 1.98 and reached final values of 1.49 and 1.50, respectively. Similarly, Flasks 21 and 22 reached 1.48 and 1.47, while Flasks 41 and 42 both reached 1.54 on Day 13. The close agreement between the duplicate

measurements indicates similar pH evolution within the corresponding duplicate pairs under the investigated conditions.

Overall, the progressive decrease in pH demonstrates that acidic conditions were maintained throughout the experiments. The pH evolution is considered together with the corresponding Eh behaviour and solution chemistry in evaluating the progression of bio-oxidation.

4.2.4 Evolution of Oxidation-Reduction Potential During the GRM Shake-Flask Bio-oxidation Experiments

The evolution of oxidation-reduction potential (Eh) in the individual GRM shake-flask experiments is illustrated in Figure 4.7. The corresponding numerical measurements are presented in Table 4.7.

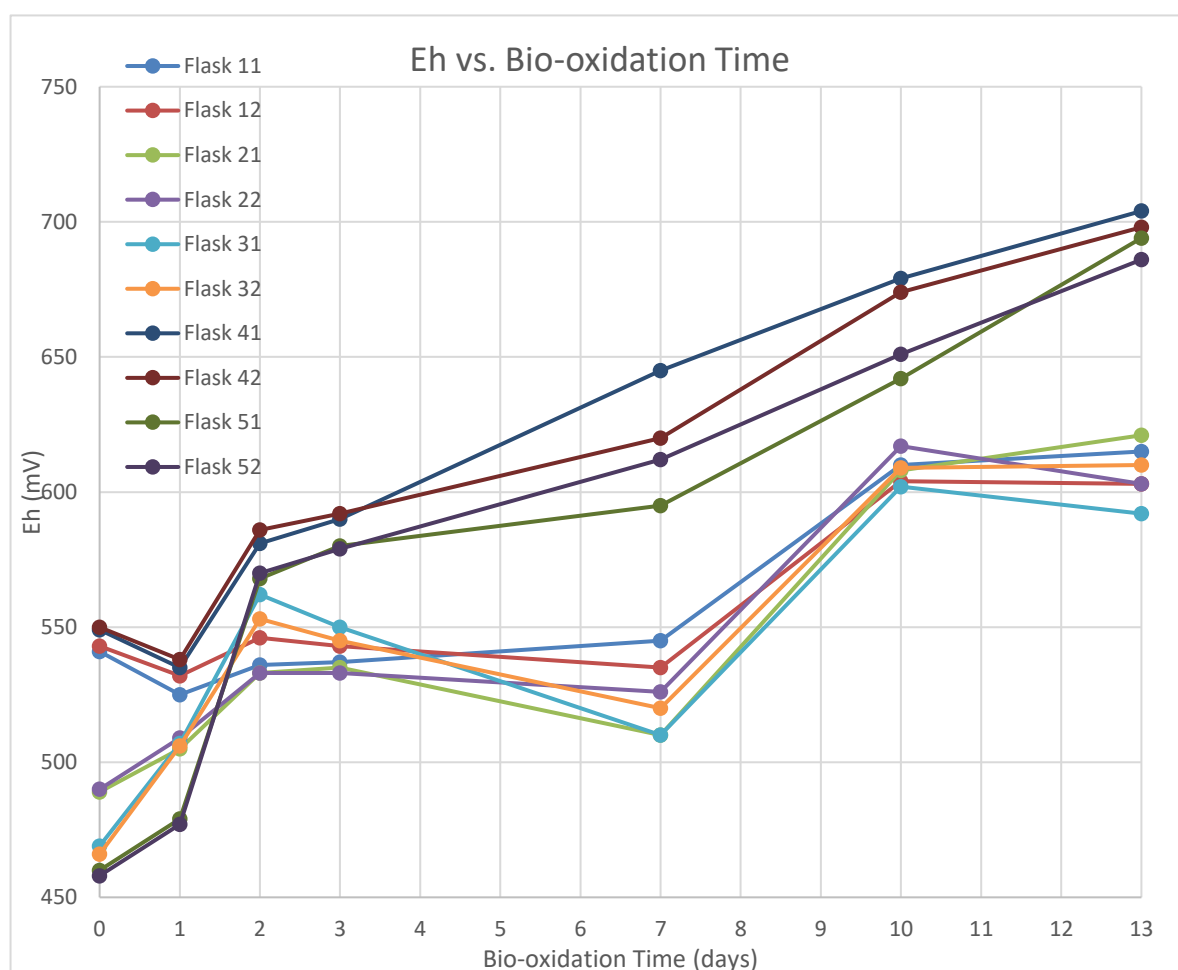


Figure 4.7 Evolution of oxidation-reduction potential during the GRM shake-flask bio-oxidation experiments.

In contrast to the decreasing pH profiles, the Eh measurements showed an overall increasing trend during the 13-day monitoring period, although temporary decreases and fluctuations occurred in several flasks during the intermediate stages.

The initial Eh values ranged from 458 to 550 mV. The lowest initial value was measured in Flask 52 at 458 mV, followed by Flask 51 at 460 mV, whereas the highest initial value was measured in Flask 42 at 550 mV.

During the first seven days, the Eh evolution differed among the experimental conditions. Some flasks exhibited relatively small changes or temporary decreases, whereas others showed a more pronounced increase. From Day 7 onwards, however, a marked increase in Eh was observed in most of the flasks.

By Day 13, the Eh values ranged from 592 to 704 mV. The highest final Eh was recorded in Flask 41 at 704 mV, followed by Flask 42 at 698 mV, Flask 51 at 694 mV, and Flask 52 at 686 mV. The lowest final Eh was recorded in Flask 31 at 592 mV.

The duplicate pairs generally exhibited similar overall trends. Flasks 41 and 42, for example, started at 549 and 550 mV and reached 704 and 698 mV, respectively. Similarly, Flasks 51 and 52 started at 460 and 458 mV and increased to 694 and 686 mV by Day 13. Greater differences were observed in some of the other duplicate pairs at individual monitoring points, but the overall evolution towards more oxidising conditions remained evident.

The simultaneous decrease in pH and general increase in Eh indicate the development and maintenance of increasingly acidic and oxidising conditions during the GRM shake-flask experiments. These physicochemical trends alone do not quantify the extent of sulphide oxidation and are therefore interpreted together with the elemental concentrations measured in the process solutions.

4.2.5 ICP-OES Monitoring of the GRM Shake-Flask Experiments

Pregnant leach solution (PLS) samples collected during the GRM shake-flask bio-oxidation experiments were analysed by ICP-OES to monitor the concentrations of Fe, S, and Zn in solution. The initial samples were collected on 2 April 2026, and a second set of samples was collected from each individual flask on 7 April 2026. After the extended bio-oxidation period, the contents of the ten GRM flasks were combined, and a final sample of the combined bio-solution was collected on 15 July 2026.

The measured Fe, S, and Zn concentrations are presented in Table 4.8. The sampling dates are reported directly rather than expressed only as experimental days because the final sample represents the combined GRM bio-solution rather than an individual flask.

Table 4.8 ICP-OES concentrations of Fe, S, and Zn measured during the GRM shake-flask bio-oxidation experiments.

Flask / Sample	Sampling date	Fe (ppm)	S (ppm)	Zn (ppm)
Flask 11	2.04.2026	9543	4544	22
Flask 11	7.04.2026	10740	5362	41
Flask 12	2.04.2026	9600	4469	22
Flask 12	7.04.2026	10450	5249	41
Flask 21	2.04.2026	8556	4114	21
Flask 21	7.04.2026	9774	4967	38
Flask 22	2.04.2026	8386	4008	19
Flask 22	7.04.2026	9626	4912	41
Flask 31	2.04.2026	7974	3836	21
Flask 31	7.04.2026	8742	4518	43
Flask 32	2.04.2026	7696	3705	21
Flask 32	7.04.2026	9213	4796	48
Flask 41	2.04.2026	9040	4395	13
Flask 41	7.04.2026	10530	5304	27
Flask 42	2.04.2026	9326	4465	14
Flask 42	7.04.2026	10340	5250	28
Flask 51	2.04.2026	7598	3558	11
Flask 51	7.04.2026	8199	4267	25
Flask 52	2.04.2026	7393	3480	11
Flask 52	7.04.2026	7965	4158	26
Combined GRM bio-solution	15.07.2026	15450	18880	77

Comparison of the individual flask analyses between 2 April and 7 April shows an increase in the dissolved concentrations of all three monitored elements in every GRM flask. The evolution of Fe, S, and Zn concentrations is presented separately in Figures 4.8, 4.9, and 4.10.

Evolution of Fe Concentration

The dissolved Fe concentration increased between 2 April and 7 April in all ten GRM shake flasks. The initial Fe concentrations ranged from 7393 to 9600 ppm, whereas the concentrations measured on 7 April ranged from 7965 to 10740 ppm.

The highest Fe concentration among the individual flask samples on 7 April was measured in Flask 11 at 10740 ppm, followed by Flask 41 at 10530 ppm and Flask 12 at 10450 ppm. The combined GRM bio-solution collected on 15 July contained 15450 ppm Fe.

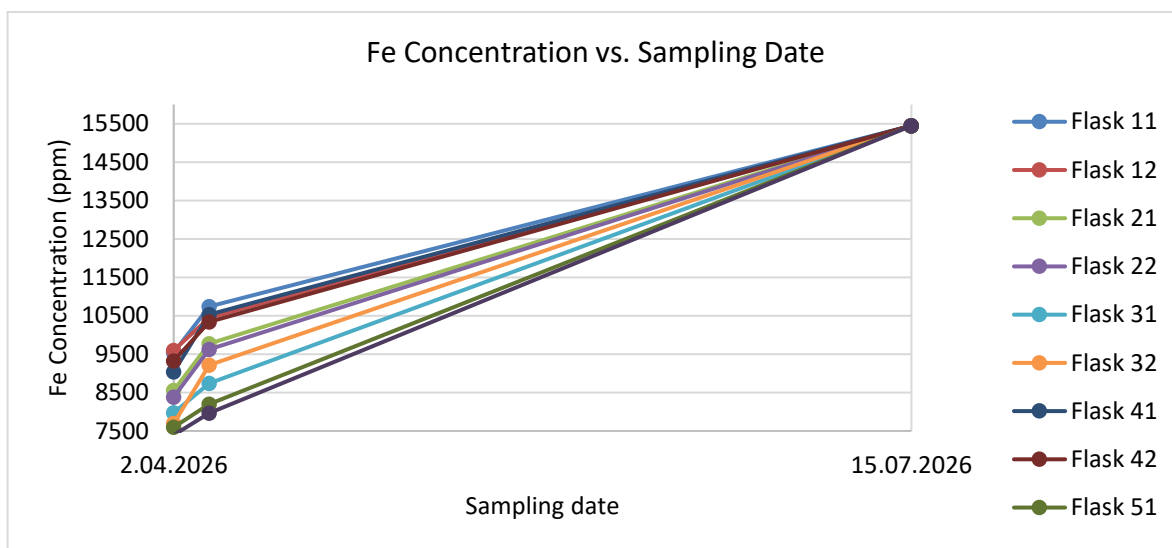


Figure 4.8 Evolution of dissolved Fe concentration during the GRM shake-flask bio-oxidation experiments.

Evolution of S Concentration

Dissolved S concentrations also increased in all individual GRM flasks between the two sampling dates. Initial concentrations ranged from 3480 to 4544 ppm, while the values measured on 7 April ranged from 4158 to 5362 ppm.

The highest S concentration among the individual flask samples on 7 April was measured in Flask 11 at 5362 ppm, followed by Flask 41 at 5304 ppm and Flask 12 at 5249 ppm. A substantially higher S concentration of 18880 ppm was measured in the combined GRM bio-solution collected on 15 July.

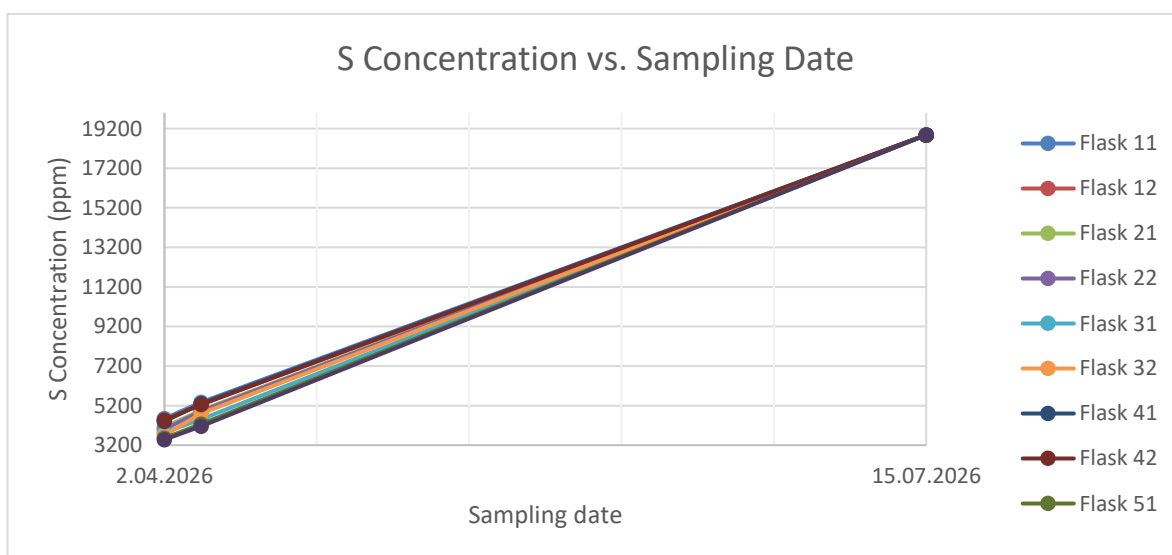


Figure 4.9 Evolution of dissolved S concentration during the GRM shake-flask bio-oxidation experiments.

Evolution of Zn Concentration

The dissolved Zn concentration increased in all GRM shake flasks between 2 April and 7 April. Initial Zn concentrations ranged from 11 to 22 ppm, while concentrations measured on 7 April ranged from 25 to 48 ppm.

The highest Zn concentration among the individual flask samples on 7 April was measured in Flask 32 at 48 ppm, followed by Flask 31 at 43 ppm and Flasks 11, 12, and 22 at 41 ppm. The combined GRM bio-solution collected on 15 July contained 77 ppm Zn.

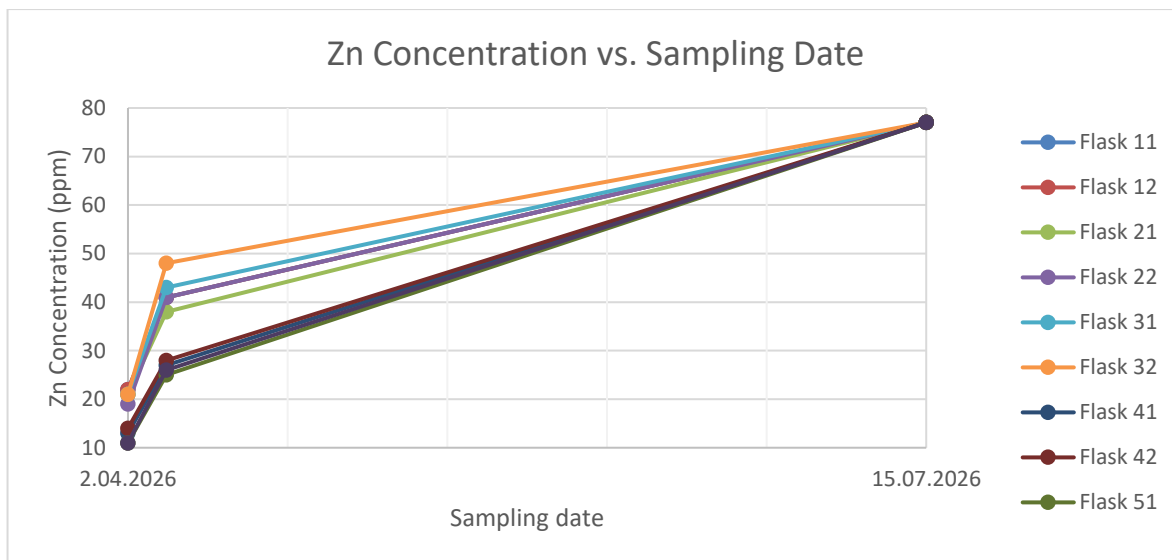


Figure 4.10 Evolution of dissolved Zn concentration during the GRM shake-flask bio-oxidation experiments.

Overall, the ICP-OES results show that the dissolved concentrations of Fe, S, and Zn increased in all ten GRM shake flasks between the individual sampling events of 2 April and 7 April. The combined GRM bio-solution collected on 15 July contained 15450 ppm Fe, 18880 ppm S, and 77 ppm Zn. Because this final sample represented the combined contents of all ten flask systems, it was treated as a separate composite measurement and was not interpreted as a direct continuation of any individual flask concentration profile.

The evolution of the dissolved elements is considered together with the pH and Eh trends presented in Sections 4.2.3 and 4.2.4 when evaluating the behaviour of the GRM bio-oxidation experiments.

4.2.6 WW2 Shake-Flask Bio-oxidation Experiments

Two additional shake-flask experiments were performed using the WW2 bacterial culture. These tests were prepared independently and on different dates. Flask 61 was initiated on 8 April 2026, whereas Flask 71 was initiated on 9 April 2026. Therefore, the monitoring results are presented according to the individual experimental timeline of each flask.

Unlike the GRM programme, the WW2 experiments were performed only to observe the behaviour of the bacterial culture under the selected bio-oxidation conditions. The solid residues obtained from Flasks 61 and 71 were not subjected to subsequent thiourea leaching.

The operating conditions are summarised in Table 4.9.

Table 4.9 Experimental conditions of the WW2 shake-flask bio-oxidation experiments.

Flask	Bio-solution (%)	Fresh 9K medium (%)	Total liquid volume (mL)	Solid loading (g)
61	100	0	250	25.0
71	75	25	250	12.5

The pH and Eh values were recorded individually for each flask.

Table 4.10 pH measurements recorded during the WW2 shake-flask bio-oxidation experiments.

Flask	Day 0	Day 1	Day 3	Day 7
61	1.99	1.99	1.98	1.75
71	1.92	1.93	1.92	1.74

Table 4.11 Oxidation-reduction potential measurements recorded during the WW2 shake-flask bio-oxidation experiments.

Flask	Day 0	Day 1	Day 3	Day 7
61	453	472	475	486
71	434	455	481	556

The pH of Flask 61 decreased from 1.99 to 1.75. Over the same period, Eh increased gradually from 453 to 486 mV. Flask 71 started at pH 1.92 and Eh 434 mV. Its pH remained nearly unchanged at 1.93 on the following day and decreased to 1.74. Eh increased more markedly, reaching 556 mV at the final measurement.

An increase in Eh was observed in both WW2 shake-flask experiments, although the magnitude of the change differed between the two systems. Because the experiments had different starting dates and operating conditions, their results were evaluated individually rather than through direct comparison. No thiourea gold-leaching tests were conducted using the WW2 residues.

4.2.7 Preparation of the Combined GRM Bio-oxidised Residue (Sample 7)

After a total contact period of 104 days, the contents of Flasks 11, 12, 21, 22, 31, 32, 41, 42, 51, and 52 were combined to prepare a single composite sample for thiourea leaching.

Both the bio-solutions and the bio-oxidised solids from the ten flasks were mixed and homogenised. Before solid-liquid separation, the physicochemical properties of the combined suspension were measured. The recorded values were:

Table 4.12 Final measurements of combined flasks samples.

Parameter	Measured value
pH	1.26
Eh (mV)	632
Temperature (°C)	31.5

Following homogenisation and measurement, the solids were separated from the combined bio-solution. The recovered solid material was designated Sample 7 and was subsequently subjected to a six-hour thiourea gold-leaching experiment.

The fire assay sample designated Initial 7 represents the combined GRM bio-oxidised residue before thiourea leaching, while Residue 7 represents the corresponding solid after thiourea leaching. The measured Au grades were 0.81 ppm for Initial 7 and 0.65 ppm for Residue 7. These results were subsequently used to evaluate the apparent Au extraction obtained from the combined GRM shake-flask residue, as discussed in Section 4.4.2.7.

The combined Sample 7 residue consisted only of material obtained from the GRM flask series. The WW2 residues from Flasks 61 and 71 were not included in this composite and were not used in the gold-leaching stage.

4.3 Stirred-Tank Reactor Bio-oxidation Experiments

Following the shake-flask bio-oxidation experiments, a series of stirred-tank reactor experiments was conducted to evaluate the bio-oxidation behaviour of the flotation tailings under controlled operating conditions and to produce sufficient bio-oxidised material for the subsequent thiourea leaching experiments.

Five independent reactor experiments were performed during this study. Experiments 1, 2 and 3 were conducted in 1 L mechanically stirred reactors, whereas Experiments 4 and 5 were carried out in 3 L reactors. Experiments 1–4 used the flotation tailings sample as the

feed material, while Experiment 5 employed a pyrite-rich concentrate produced by gravity separation of the flotation tailings.

Throughout all reactor experiments, pH, oxidation-reduction potential (Eh), and temperature were monitored periodically. Pregnant leach solution (PLS) samples were collected for ICP-OES analysis at each sampling stage. Dissolved Fe, S and Zn concentrations were monitored throughout the experiments, whereas Al, Cu and the remaining dissolved elements were analysed only at selected sampling dates.

Unlike the shake-flask experiments, the reactor tests were operated using a semi-continuous solution replacement strategy. Approximately 75% of the process solution was periodically withdrawn and replaced with recycled bio-solution and freshly prepared 9K medium while the bio-oxidised solids remained inside the reactor. This solution-renewal strategy was applied to provide renewed process solution while retaining the solids in the reactor and to approximate solution circulation conditions relevant to heap bioleaching.

4.3.1 Experiment 1

Experiment 1 was conducted in a 1 L mechanically stirred reactor using 50 g of flotation tailings. The experiment consisted of three consecutive operational stages. Continuous mechanical agitation and aeration were maintained throughout the experiment. The initial process solution contained equal proportions of recycled bio-solution and freshly prepared 9K medium. Following Stages I and II, approximately 750 mL of PLS was withdrawn, while approximately 250 mL of process solution and the bio-oxidised solids remained in the reactor. The working volume was subsequently restored to 1000 mL by adding 375 mL of recycled bio-solution and 375 mL of freshly prepared 9K medium.

The principal operating conditions and physicochemical parameters recorded during Experiment 1 are summarised in Table 4.13.

Table 4.13 Summary of the operating conditions and physicochemical parameters for Experiment 1.

Stage	Time (Days)	Solid mass (g)	Bio-solution (%)	Fresh 9K (%)	Total liquid volume (mL)	Initial Eh (mV)	Final Eh (mV)	Initial pH	Final pH	Initial T (°C)	Final T (°C)
I	4	50	50	50	1000	460	605	1.64	1.50	37.0	37.1
II	3	50	50	50	1000	465	674	1.65	1.43	37.1	36.5
III	4	50	50	50	1000	461	668	1.64	1.53	35.8	36.2

4.3.1.1 Stage I

Stage I was initiated on 3 April 2026 using 50 g of flotation tailings suspended in 1000 mL of process solution consisting of equal proportions of recycled bio-solution and freshly prepared 9K medium. During the first four days of operation, the oxidation-reduction potential increased from 460 mV to 605 mV, while the pH decreased from 1.64 to 1.50. The reactor temperature remained stable at approximately 37 °C.

At the end of Stage I, approximately 750 mL of pregnant leach solution (PLS) was withdrawn for ICP-OES analysis. The withdrawn solution was replaced with 375 mL recycled bio-solution and 375 mL freshly prepared 9K medium, restoring the reactor volume to 1000 mL. The bio-oxidised solids were intentionally retained inside the reactor before initiating Stage II.

4.3.1.2 Stage II

Stage II commenced on 7 April 2026 using the same bio-oxidised solids recovered from Stage I. The renewed process solution again consisted of equal proportions of recycled bio-solution and fresh 9K medium. During this stage, the oxidation-reduction potential increased from 465 mV to 674 mV, whereas the pH decreased from 1.65 to 1.43. Reactor temperature decreased slightly from 37.1 °C to 36.5 °C, while remaining within the desired operating range.

Following completion of Stage II, a second solution replacement was performed while retaining the bio-oxidised solids inside the reactor.

4.3.1.3 Stage III

Stage III was initiated on 10 April 2026 using the solids and approximately 250 mL of process solution retained from Stage II. Before restarting the reactor, the working volume was restored to 1000 mL by adding 375 mL of recycled bio-solution and 375 mL of freshly prepared 9K medium, resulting in an initial 50:50 bio-solution/9K medium composition. During the stage, an operational volume loss occurred and additional process solution was introduced to maintain the reactor volume. Because the exact contribution of this operational correction to the elemental inventory could not be quantified reliably, it was not incorporated into the Stage III elemental mass-balance calculation. The Stage III values were therefore evaluated from the calculated initial and measured final ICP-OES concentrations and should be interpreted as apparent net changes in dissolved elemental inventory. During the final stage, the oxidation-reduction potential increased from 461 mV to 668 mV. The pH exhibited a different behaviour compared with the previous stages, decreasing to 1.26 on 13 April 2026 before increasing to 1.53 at the final measurement on 14 April 2026.

4.3.1.4 ICP-OES Analysis of Experiment 1

Liquid samples collected during Experiment 1 were analysed by ICP-OES to monitor the concentrations of Fe, S and Zn in the process solution during bio-oxidation. Because approximately 75% of the liquid phase was withdrawn and renewed between successive stages, the measured final PLS concentrations could not be interpreted directly as elemental transfer from the flotation tailings. The elemental inventory already present in the liquid phase at the beginning of each stage was therefore taken into account.

For Stages II and III, approximately 250 mL of process solution from the preceding stage remained in the reactor following PLS withdrawal. The working volume was subsequently restored to 1000 mL by adding 375 mL of recycled bio-solution and 375 mL of freshly prepared 9K medium. Therefore, the initial elemental concentration for each stage was calculated from the volume-weighted concentrations of all liquid streams contributing to the initial reactor solution.

The initial concentration of element i in stage j was calculated using:

$$C_{initial,j} = \frac{\sum_i V_i C_i}{V_{total,j}}$$

Where V_i is the volume of each liquid stream contributing to the initial reactor solution (L), C_i is the corresponding elemental concentration of that liquid stream (mg L⁻¹), and $V_{total,j}$ is the total initial liquid volume of the stage (L).

The apparent net change in dissolved elemental concentration during each stage was then calculated as:

$$\Delta C_j = C_{final,j} - C_{initial,j}$$

Where $C_{final,j}$ is the elemental concentration measured in the PLS at the end of stage j (mg L⁻¹), and $C_{initial,j}$ is the calculated initial concentration (mg L⁻¹).

The corresponding apparent net elemental transfer to the liquid phase was calculated as:

$$m_{dissolved,j} = \frac{(C_{final,j} - C_{initial,j})V_j}{1000}$$

Where $m_{dissolved,j}$ is the apparent net elemental transfer during stage j (g), V_j is the liquid volume of the reactor during the corresponding stage (L), and the factor of 1000 converts milligrams to grams.

For comparison between experiments, the apparent net elemental transfer was normalised to the initial solid mass according to:

$$m_{normalized,j} = \left(\frac{m_{dissolved,j}}{m_{solid,j}} \right) \times 1000$$

Where $m_{normalized,j}$ is expressed as g kg⁻¹ initial solid, $m_{dissolved,j}$ is expressed in grams, and $m_{solid,j}$ is the initial solid mass expressed in grams. For Experiment 1, a solid mass of 50 g was used throughout all three stages.

The calculated initial concentrations, measured final concentrations and resulting apparent net elemental transfers for Fe, S and Zn are presented in Table 4.14.

Table 4.14 Calculated initial and measured final Fe, S and Zn concentrations during Experiment 1.

Stage	Fe initial (ppm)	Fe final (ppm)	ΔFe (ppm)	Fe dissolved (g)	Fe dissolved (g kg ⁻¹ initial solid)
I	8434.0	11760.0	3326.0	3.33	66.52
II	9797.6	15620.0	5822.4	5.82	116.45
III	12408.3	15190.0	2781.7	2.78	55.63
Total	—	—	11930.1	11.93	238.60
Stage	S initial (ppm)	S final (ppm)	ΔS (ppm)	S dissolved (g)	S dissolved (g kg ⁻¹ initial solid)
I	3399.0	6068.0	2669.0	2.67	53.38
II	5404.3	7753.0	2348.7	2.35	46.97
III	6397.2	7553.0	1155.8	1.16	23.12
Total	—	—	6173.5	6.17	123.47
Stage	Zn initial (ppm)	Zn final (ppm)	ΔZn (ppm)	Zn dissolved (g)	Zn dissolved (g kg ⁻¹ initial solid)
I	2.00	32.00	30.00	0.03	0.60
II	8.75	20.00	11.25	0.01	0.23
III	9.00	10.60	1.60	0.002	0.03
Total	—	—	42.85	0.04	0.86

The Fe concentration showed a positive apparent net increase during all three operational stages. The largest stage-wise Fe transfer was observed during Stage II, where the concentration increased from a calculated initial value of 9797.6 ppm to 15620 ppm. This corresponded to an apparent net Fe transfer of 5.82 g, equivalent to 116.45 g kg⁻¹ initial solid. Stage I and Stage III resulted in apparent net Fe transfers of 3.33 and 2.78 g, respectively.

The cumulative apparent net Fe transfer over the three stages was 11.93 g, corresponding to 238.60 g kg⁻¹ initial solid.

Sulphur also showed a positive apparent net transfer during all three stages. The calculated stage-wise values were 2.67 g in Stage I, 2.35 g in Stage II and 1.16 g in Stage III. The cumulative apparent net S transfer was 6.17 g, corresponding to 123.47 g kg⁻¹ initial solid.

The cumulative apparent net Zn transfer was approximately 0.04 g, corresponding to 0.86 g kg⁻¹ initial solid. The substantially lower Zn transfer compared with Fe and S reflected the lower dissolved Zn concentrations measured during the experiment.

The calculated Fe, S and Zn values represent apparent net changes in the dissolved elemental inventory rather than direct mineral-specific dissolution. Precipitation, adsorption, incorporation into secondary phases and redistribution between the solid and liquid phases may also have influenced the measured dissolved concentrations. Accordingly, these values are interpreted as apparent net elemental transfer to the liquid phase under the investigated experimental conditions.

4.3.1.5 Stage-Wise Elemental Mass Balance

Because approximately 75% of the process solution was withdrawn after Stages I and II, a stage-wise mass balance was used to distinguish between the apparent net elemental transfer occurring during each stage, the solid-derived elemental inventory removed with the withdrawn PLS, and the inventory retained in the reactor and carried forward to the subsequent stage.

The total elemental concentration measured in the PLS was not treated directly as material dissolved from the flotation tailings because Fe, S and Zn were already present in the process solutions introduced into the reactor. Instead, the apparent net concentration increase relative to the calculated initial liquid-phase composition was used.

Following Stage I, approximately 75% of the apparent net elemental inventory was removed with the 0.75 L PLS withdrawal, while approximately 25% remained in the reactor and was carried into Stage II. Following Stage II, the inventory retained from Stage I was combined with the apparent net elemental transfer occurring during Stage II. Approximately 75% of this combined inventory was subsequently removed with the PLS, while the remainder was carried into Stage III.

At the completion of Stage III, the remaining process solution was separated from the bio-oxidised solids. The resulting stage-wise mass balance is presented in Table 4.15.

Table 4.15 Stage-wise mass balance of Fe, S and Zn dissolved from the flotation tailings during Experiment 1.

Stage	Fe dissolved during stage (g)	Fe removed with PLS (g)	Fe remaining in reactor (g)	Cumulative Fe removed (g)
I	3.33	2.49	0.83	2.49
II	5.82	4.99	1.66	7.48
III	2.78	4.45	0.00	11.93
Total	11.93	11.93	0.00	11.93
Stage	S dissolved during stage (g)	S removed with PLS (g)	S remaining in reactor (g)	Cumulative S removed (g)
I	2.67	2.00	0.67	2.00
II	2.35	2.26	0.75	4.26
III	1.156	1.91	0.00	6.17
Total	6.17	6.17	0.00	6.17
Stage	Zn dissolved during stage (g)	Zn removed with PLS (g)	Zn remaining in reactor (g)	Cumulative Zn removed (g)
I	0.03	0.02	0.008	0.02
II	0.01	0.01	0.005	0.04
III	0.002	0.01	0.00	0.04
Total	0.04	0.04	0.0000	0.04

During Stage I, approximately 3.33 g of Fe was transferred to the liquid phase on an apparent net basis. Approximately 2.49 g of this inventory was removed with the withdrawn PLS, while approximately 0.83 g remained in the reactor and was carried forward to Stage II.

During Stage II, the retained Fe inventory from Stage I was combined with an additional apparent net transfer of 5.82 g Fe. Following the second PLS withdrawal, approximately 4.99 g Fe was removed, while approximately 1.66 g remained in the reactor and was carried forward to Stage III.

During Stage III, the Fe inventory carried over from Stage II was combined with an additional apparent net transfer of approximately 2.78 g Fe. At the end of the experiment, the remaining liquid phase was separated from the solids, giving approximately 4.45 g of solid-derived Fe inventory in the final liquid stream. The cumulative apparent net Fe transfer over the three stages was 11.93 g, corresponding to 238.60 g kg⁻¹ initial solid.

The same mass-balance procedure gave cumulative apparent net transfers of approximately 6.17 g S and 0.04 g Zn, corresponding to 123.47 g kg⁻¹ and 0.86 g kg⁻¹ initial solid, respectively.

These values represent changes in the dissolved elemental inventory after accounting for the elemental contribution of the process solutions present at the beginning of each operational stage. They should therefore be interpreted as apparent net elemental transfer to the liquid phase rather than direct mineral-specific dissolution.

4.3.1.6 Operational Observations

Experiment 1 remained under acidic and oxidising conditions throughout the three operational stages. Continuous aeration and mechanical agitation were maintained during reactor operation. Nevertheless, settling of the relatively dense sulphide-bearing particles was observed at the bottom of the reactor, and occasional manual resuspension with a glass rod was required to return the settled material to suspension.

Two planned solution-renewal events were performed following Stages I and II. During each renewal, approximately 750 mL of PLS was withdrawn while the bio-oxidised solids and approximately 250 mL of process solution remained in the reactor. The working volume was subsequently restored to 1000 mL by adding 375 mL of recycled bio-solution and 375 mL of freshly prepared 9K medium. This procedure allowed the same 50 g solid charge to remain under biological treatment throughout the complete three-stage experiment.

The progressive increases in Eh observed during all three stages, together with the acidic pH conditions and the apparent net transfer of Fe and S to the liquid phase, were consistent with oxidative alteration of the sulphide-bearing material. However, the calculated elemental transfers should not be interpreted as direct quantitative measures of individual sulphide-mineral oxidation because precipitation, adsorption and redistribution between the solid and liquid phases may also have influenced the measured dissolved concentrations.

At the completion of Stage III, the reactor slurry was filtered to separate the process solution from the bio-oxidised solids. The recovered solid residue was retained and subsequently subjected to thiourea leaching as Sample 1. The corresponding thiourea-leaching test produced an apparent Au extraction of 24.7%.

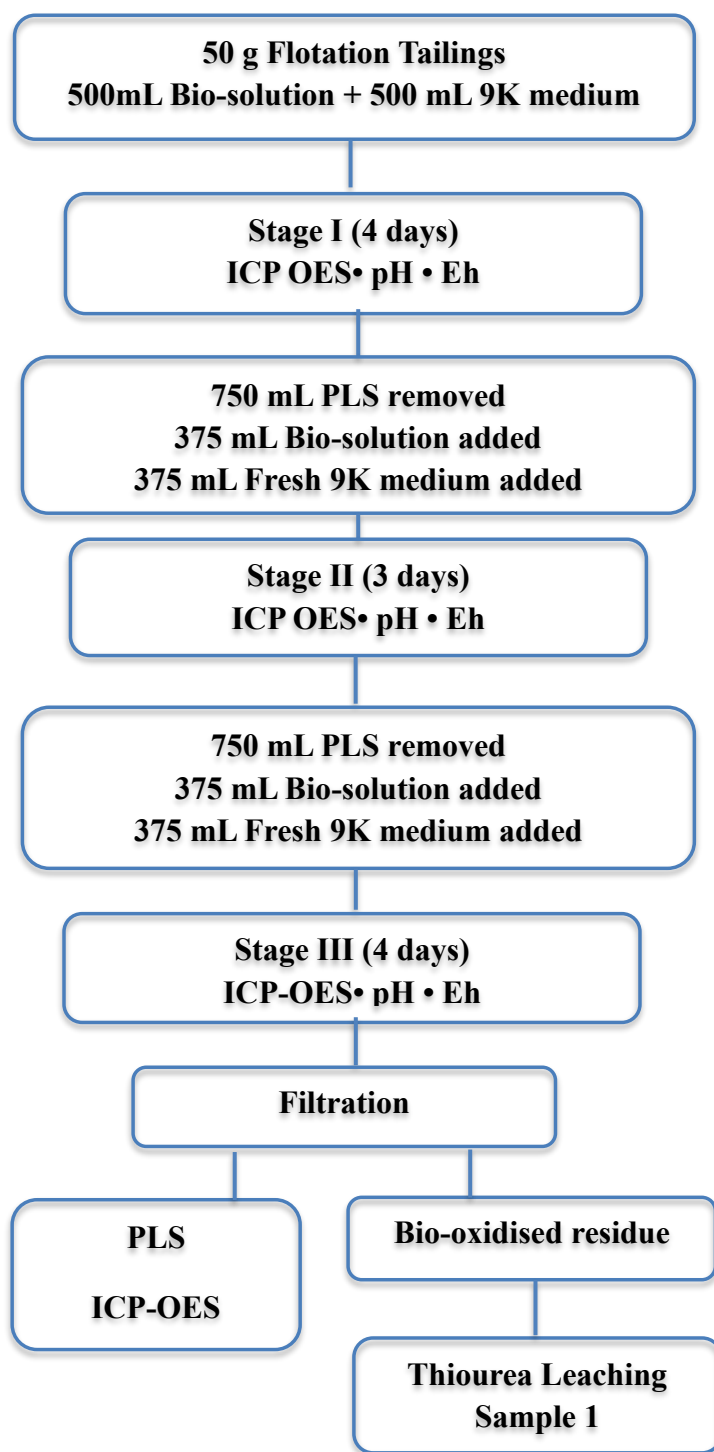


Figure 4.11 Schematic representation of the experimental sequence and solution management during Reactor Experiment 1 bio-oxidation.

4.3.2 Experiment 2

Experiment 2 was conducted in a 1 L mechanically stirred reactor using 100 g of flotation tailings. The reactor was initially charged with 1000 mL of process solution composed of 80

vol.% bio-solution and 20 vol.% freshly prepared 9K medium. The experiment consisted of three consecutive operational stages.

Unlike Experiment 1, process solution was lost from the reactor during operation. During each stage, approximately 250 mL of metal-bearing process solution was lost on three occasions. After each loss event, 250 mL of fresh 80:20 bio-solution/9K medium mixture was added to restore the working volume to 1000 mL. Consequently, both the dissolved elemental inventory removed through the daily solution losses and the elemental input introduced with the make-up solution were considered in the subsequent mass-balance calculations.

At the end of Stages I and II, 750 mL of PLS was withdrawn while 250 mL of process solution and the bio-oxidised solids remained in the reactor. The reactor was subsequently replenished with 750 mL of fresh 80:20 solution before initiating the following stage. At the completion of Stage III, the complete process solution was separated from the bio-oxidised solids by filtration.

The operating conditions and the initial and final physicochemical parameters recorded during each stage are summarised in Table 4.16.

Table 4.16 Operating conditions and physicochemical parameters of Experiment 2.

Stage	Time (Days)	Solid mass (g)	Working volume (mL)	Daily solution loss (mL)	Daily make-up addition (mL)	Initial Eh (mV)	Final Eh (mV)	Initial pH	Final pH
I	3	100	1000	250	250	473	582	1.66	1.72
II	4	100	1000	250	250	476	576	1.81	1.76
III	3	100	1000	250	250	470	541	1.75	1.64

4.3.2.1 Stage I

Stage I was initiated using 100 g of flotation tailings suspended in 1000 mL of process solution consisting of 80 vol.% bio-solution and 20 vol.% freshly prepared 9K medium. The initial pH and Eh were 1.66 and 473 mV, respectively.

During Stage I, three solution-loss events occurred. Approximately 250 mL of metal-bearing process solution was lost during each event and was replaced with an equivalent volume of fresh 80:20 bio-solution/9K medium mixture, thereby maintaining the working volume at approximately 1000 mL.

At the end of Stage I, the Eh had increased from 473 to 582 mV, whereas the pH increased slightly from 1.66 to 1.72. Subsequently, 750 mL of PLS was withdrawn, leaving approximately 250 mL of process solution together with the solids in the reactor. A total of 750 mL of fresh 80:20 solution, consisting of 600 mL bio-solution and 150 mL 9K medium, was then added before Stage II.

4.3.2.2 Stage II

Stage II was initiated using the bio-oxidised solids and approximately 250 mL of process solution retained from Stage I. The addition of 750 mL of fresh 80:20 bio-solution/9K medium mixture restored the working volume to 1000 mL.

As in Stage I, three 250 mL solution-loss events occurred during the stage, with each lost volume replaced by 250 mL of fresh 80:20 make-up solution.

The Eh increased from 476 to 576 mV during Stage II, while the pH decreased slightly from 1.81 to 1.76. At the end of the stage, 750 mL of PLS was withdrawn. The remaining 250 mL of process solution and the bio-oxidised solids were retained in the reactor, after which 750 mL of fresh 80:20 solution was introduced before Stage III.

4.3.2.3 Stage III

Stage III was initiated using the solids and process solution retained from Stage II following replenishment of the reactor to a working volume of 1000 mL.

Three additional 250 mL solution-loss events occurred during this stage. Following each event, the lost volume was replaced with 250 mL of fresh 80:20 bio-solution/9K medium mixture.

During Stage III, the Eh increased from 470 to 541 mV, while the pH decreased from 1.75 to 1.64. At the completion of the stage, the entire process solution was separated from the bio-oxidised solids by filtration. The recovered solid residue was retained for the subsequent thiourea-leaching experiment.

4.3.2.4 ICP-OES Analysis of Experiment 2

The liquid samples collected during Experiment 2 were analysed by ICP-OES to monitor the concentrations of Fe, S and Zn in the process solution during bio-oxidation. Because repeated solution losses and make-up additions occurred during each operational stage, the measured final PLS concentrations could not be interpreted directly as the quantities transferred from the flotation tailings to the liquid phase.

The initial process solution contained 7702 ppm Fe, 3277 ppm S and 2 ppm Zn. The same 80:20 bio-solution/9K medium mixture was used for the daily make-up additions, and these measured concentrations were therefore used as the elemental concentrations of the make-up solution.

For Stages II and III, the initial concentrations were reconstructed from the 250 mL of process solution retained from the preceding stage and the 750 mL of fresh 80:20 bio-solution/9K medium mixture added before the subsequent stage.

The initial concentration of each element was calculated according to:

$$C_{(initial,j)} = (V_{(retained)}C_{(final,j-1)} + V_{(make-up)}C_{(make-up)})/V_{(total)}$$

where $C_{(initial,j)}$ is the calculated initial concentration of the element in stage j, $C_{(final,j-1)}$ is the measured final concentration from the preceding stage, $C_{(make-up)}$ is the elemental concentration of the fresh make-up solution, $V_{(retained)}$ is the retained process-solution volume, $V_{(make-up)}$ is the fresh solution volume added, and V_{total} is the resulting reactor liquid volume.

The reconstructed initial concentrations were:

Table 4.17 Reconstructed initial Fe, S and Zn concentrations for each stage of Experiment 2.

Stage	Fe initial (ppm)	S initial (ppm)	Zn initial (ppm)
I	7702.0	3277.0	2.00
II	8461.5	3767.5	9.25
III	8586.5	3801.5	6.75

The corresponding measured final concentrations were 10740, 11240 and 13140 ppm Fe; 5239, 5375 and 6507 ppm S; and 31, 21 and 24 ppm Zn for Stages I, II and III, respectively

4.3.2.5 Stage-Wise Elemental Mass Balance

Because experiment 2 involved repeated solution losses and make-up additions during each operational stage, the difference between the initial and final ICP-OES concentrations could not be used directly to quantify the elemental mass transferred from the flotation tailings to the liquid phase. A stage-wise elemental mass balance was therefore established by accounting for the elemental inventory initially present in the reactor, the elemental input introduced with the make-up solution, the elemental mass removed through the daily solution losses, and the final dissolved elemental inventory.

For each element, the net mass transferred from the solid phase to the liquid phase during a stage was calculated according to:

$$m_{dissolved} = m_{final} + m_{lost} - m_{initial} - m_{make-up}$$

where:

- $m_{dissolved}$ is the net elemental mass transferred from the solid phase to the liquid phase during the stage (g);
- $m_{initial}$ is the dissolved elemental inventory present in the initial 1 L process solution (g);
- $m_{make-up}$ is the total elemental input introduced through the three 250 mL make-up additions (g);
- m_{lost} is the elemental mass removed through the three 250 mL daily solution-loss streams (g); and
- m_{final} is the dissolved elemental inventory present in the final 1 L process solution (g).

The dissolved elemental inventory corresponding to a measured concentration was calculated from:

$$m = (C \times V)/1000$$

where **m** is the elemental mass in g, **C** is the concentration in mg L⁻¹ (ppm), and **V** is the solution volume in L.

Because the daily solution-loss streams were not analysed individually by ICP-OES, their concentrations were estimated by linear interpolation between the calculated initial and measured final concentrations of each stage. The estimated concentrations of the three solution-loss streams corresponded to one-quarter, one-half and three-quarters of the concentration change between the stage endpoints. This calculation assumes a progressive change in dissolved elemental concentration during each stage and a homogeneous reactor solution at the time of each solution-loss event.

The make-up solution had the same 80:20 bio-solution/9K medium composition as the initial process solution and contained 7702 ppm Fe, 3277 ppm S and 2 ppm Zn. Three 250 mL make-up additions were performed during each stage, corresponding to a total make-up volume of 750 mL per stage. The resulting elemental inputs are summarised in Table 4.18.

Table 4.18 Elemental input introduced through the make-up solution during each stage of Experiment 2.

Element	Make-up concentration (ppm)	Input per 250 mL (g)	Total input per stage (g)
Fe	7702	1.93	5.78
S	3277	0.82	2.46
Zn	2	0.0005	0.002

The reconstructed initial concentrations, measured final concentrations, estimated elemental losses through the daily solution-loss streams, and resulting net elemental dissolution values are summarised in Table 4.19.

Table 4.19 Stage-wise elemental mass balance of Fe, S and Zn during Experiment 2.

Stage	Fe Initial (ppm)	Fe Final (ppm)	Fe Make-up Input (g)	Fe Daily Solution Losses (g)	Fe Dissolved (g)	Fe Dissolved (g kg ⁻¹ initial solid)
I	7702.00	10740	5.78	6.92	4.18	41.77
II	8461.50	11240	5.78	7.39	4.39	43.90
III	8586.50	13140	5.78	8.15	6.92	69.24
Total Fe	—	—	—	—	15.49	154.92
Stage	S Initial (ppm)	S Final (ppm)	S Make-up Input (g)	S Daily Solution Losses (g)	S Dissolved (g)	S Dissolved (g kg ⁻¹ initial solid)
I	3277.00	5239.00	2.46	3.19	2.70	26.98
II	3767.50	5375.00	2.46	3.43	2.59	25.78
III	3801.50	6507.00	2.46	3.87	4.11	41.13
Total S	—	—	—	—	9.39	93.89
Stage	Zn Initial (ppm)	Zn Final (ppm)	Zn Make-up Input (g)	Zn Daily Solution Losses (g)	Zn Dissolved (g)	Zn Dissolved (g kg ⁻¹ initial solid)
I	2.00	31.00	0.002	0.01	0.04	0.40
II	9.25	21.00	0.002	0.01	0.02	0.22
III	6.75	24.00	0.002	0.01	0.03	0.27
Total Zn	—	—	—	—	0.09	0.89

The stage-wise elemental mass balance resulted in cumulative apparent net transfers of 15.49 g Fe, 9.39 g S, and 0.09 g Zn to the liquid phase during Experiment 2. The largest stage-wise Fe and S transfers occurred during Stage III, corresponding to 6.92 g Fe and 4.11 g S. When normalised to the initial solid mass of 0.100 kg, the cumulative apparent net transfers corresponded to 154.92 g kg⁻¹ Fe, 93.89 g kg⁻¹ S, and 0.89 g kg⁻¹ Zn.

Table 4.20 Distribution and cumulative removal of solid-derived Fe, S and Zn during Experiment 2.

Stage	Fe dissolved during stage (g)	Fe removed through daily solution losses (g)	Fe removed with PLS/final filtrate (g)	Fe remaining in reactor (g)	Cumulative Fe removed (g)
I	4.18	1.32	2.14	0.71	3.46
II	4.39	1.80	2.48	0.83	7.74
III	6.92	2.67	5.08	0.00	15.49
Total	15.49	5.79	9.70	0.00	15.49
Stage	S dissolved during stage (g)	S removed through daily solution losses (g)	S removed with PLS/final filtrate (g)	S remaining in reactor (g)	Cumulative S removed (g)
I	2.70	0.85	1.38	0.46	2.24
II	2.58	1.08	1.47	0.49	4.79
III	4.11	1.58	3.02	0.00	9.39
Total	9.39	3.52	5.87	0.00	9.39
Stage	Zn dissolved during stage (g)	Zn removed through daily solution losses (g)	Zn removed with PLS/final filtrate (g)	Zn remaining in reactor (g)	Cumulative Zn removed (g)
I	0.04	0.01	0.02	0.007	0.03
II	0.02	0.01	0.01	0.004	0.06
III	0.03	0.01	0.02	0.00	0.09
Total	0.09	0.04	0.05	0.00	0.09

During Stage I, 4.18 g Fe was transferred from the solid phase to the liquid phase. Of this amount, 1.32 g was removed through the daily solution losses and 2.14 g was subsequently removed with the stage end PLS, while 0.71 g remained in the reactor and was transferred to Stage II.

During Stage II, the retained Fe inventory from Stage I was combined with an additional 4.39 g Fe transferred from the solid phase. The daily solution losses removed 1.80 g Fe, while 2.48 g was removed with the stage-end PLS. A dissolved solid-derived Fe inventory of 0.83 g remained in the reactor and was transferred to Stage III.

During Stage III, the 0.83 g Fe inventory retained from Stage II was combined with an additional 6.92 g Fe transferred from the solid phase. Of the resulting solid-derived dissolved Fe inventory, 2.67 g was removed through the daily solution losses, while 5.08 g was recovered in the final filtrate. The cumulative solid-derived Fe removal was 15.49 g.

The corresponding cumulative removals were 9.39 g S and 0.09 g Zn. Following final filtration, no calculated solid-derived dissolved elemental inventory remained in the reactor solution.

4.3.2.6 Operational Observations

Experiment 2 was operated over three consecutive stages using 100 g of flotation tailings in a 1 L mechanically stirred reactor. Continuous aeration and mechanical agitation were maintained throughout the bio-oxidation experiment.

A distinctive operational feature of Experiment 2 was the repeated loss of process solution during reactor operation. During each stage, approximately 250 mL of process solution was lost on three occasions. Each lost volume was replaced with 250 mL of fresh 80:20 bio-solution/9K medium mixture, restoring the working volume to approximately 1000 mL.

The Eh increased during each stage. It increased from 473 to 582 mV during Stage I, from 476 to 576 mV during Stage II, and from 470 to 541 mV during Stage III. The pH remained acidic throughout the experiment, with initial and final values of 1.66 and 1.72 in Stage I, 1.81 and 1.76 in Stage II, and 1.75 and 1.64 in Stage III.

At the end of Stages I and II, 750 mL of PLS was withdrawn while approximately 250 mL of process solution and the bio-oxidised solids were retained in the reactor. The working volume was subsequently restored by adding 750 mL of fresh 80:20 bio-solution/9K medium mixture before initiation of the following stage.

At the completion of Stage III, the entire process solution was separated from the bio-oxidised solids by filtration. The recovered solid residue was retained for the subsequent thiourea-leaching experiment.

4.3.3 Experiment 3

Experiment 3 was conducted in a 1 L mechanically stirred reactor using 100 g of flotation tailings. Prior to reactor operation, the flotation tailings were ground for 10 min to obtain a particle size below approximately 100 μm .

The experiment consisted of three consecutive operational stages. Stage I was operated for 7 days, Stage II for 4 days, and Stage III for 3 days. The solids were retained throughout the

complete bio-oxidation experiment, while the process solution was renewed between successive stages.

A distinctive feature of Experiment 3 was that the initial process solution of each stage was analysed directly by ICP-OES. Consequently, the initial Fe, S and Zn concentrations did not require reconstruction from solution-mixing calculations.

During Stage I, the reactor was operated with a process solution consisting of 20 vol.% bio-solution and 80 vol.% 9K medium. Three additional 100 mL portions of 9K medium were introduced during this stage. Stage II was also operated using a 20:80 bio-solution/9K medium ratio, and an additional 100 mL of 9K medium was introduced during this stage. These additions were made to compensate for process-solution losses and to maintain the reactor working volume at approximately 1 L. Because the added 9K medium introduced additional Fe and S into the system, these inputs were taken into account when determining the net elemental transfer from the flotation tailings.

The principal operating conditions and physicochemical parameters are summarised in Table 4.21.

Table 4.21 Summary of the operating conditions and physicochemical parameters for Experiment 3.

Stage	Time (Days)	Solid mass (g)	Working volume (mL)	Bio-solution (%)	9K (%)	Additional 9K	Initial Eh (mV)	Final Eh (mV)	Initial pH	Final pH
I	7	100	1000	20	80	3×100 mL	410	513	1.67	1.48
II	4	100	1000	20	80	100 mL	480	538	1.74	1.42
III	3	100	1000	20	80	None	476	600	1.55	1.21

4.3.3.1 Stage I

Stage I was initiated using 100 g of ground flotation tailings suspended in 1000 mL of process solution consisting of 20 vol.% bio-solution and 80 vol.% 9K medium. The initial pH and Eh were 1.67 and 410 mV, respectively.

During Stage I, three additional 100 mL portions of 9K medium were introduced, corresponding to a total additional volume of 300 mL. These additions were subsequently considered in the elemental mass-balance calculation because the added 9K medium contributed Fe and S to the liquid phase.

At the end of Stage I, the Eh had increased from 410 to 513 mV, while the pH had decreased from 1.67 to 1.48. The final process solution was collected as PLS 1 for ICP-OES analysis, while the bio-oxidised solids were retained for Stage II.

4.3.3.2 Stage II

Stage II was initiated using the bio-oxidised solids retained from Stage I and 1000 mL of process solution consisting of 20 vol.% bio-solution and 80 vol.% 9K medium. The initial pH and Eh were 1.74 and 480 mV, respectively.

During Stage II, an additional 100 mL of 9K medium was introduced. As in Stage I, the elemental contribution associated with this additional solution must be considered when calculating the net elemental transfer from the flotation tailings.

At the end of Stage II, the Eh had increased from 480 to 538 mV, while the pH had decreased from 1.74 to 1.42. The final process solution was collected as PLS 2, while the bio-oxidised solids were retained for Stage III.

4.3.3.3 Stage III

Stage III was conducted for 3 days using the bio-oxidised solids retained from Stage II. The initial pH and Eh were 1.55 and 476 mV, respectively.

No additional 9K medium addition was recorded during Stage III.

At the completion of Stage III, the Eh had increased substantially from 476 to 600 mV, while the pH decreased from 1.55 to 1.21, representing the lowest final pH recorded during Experiment 3.

Following the final measurements, the reactor slurry was filtered. The final process solution was collected as PLS 3, and the bio-oxidised solid residue was recovered for the subsequent thiourea-leaching experiment.

4.3.3.4 ICP-OES Analysis of Experiment 3

The initial process solution and final PLS of each operational stage were analysed directly by ICP-OES to determine the dissolved Fe, S and Zn concentrations. Unlike Experiments 1 and 2, the initial solution of each stage was measured directly and therefore did not require reconstruction from the composition of retained and newly added process solutions. The recorded initial and final ICP-OES concentrations were 8500 and 11390 ppm Fe, 3382 and 6613 ppm S, and 2 and 87 ppm Zn during Stage I; 10170 and 14080 ppm Fe, 5269 and 8051

ppm S, and 48 and 77 ppm Zn during Stage II; and 11020 and 17100 ppm Fe, 5593 and 9654 ppm S, and 33 and 54 ppm Zn during Stage III.

Additional 9K medium was introduced during both Stages I and II. During Stage I, three 100 mL additions were made, corresponding to a total additional volume of 300 mL. During Stage II, one additional 100 mL portion of 9K medium was introduced. These additions contributed dissolved Fe and S to the reactor and were therefore excluded from the quantities attributed to transfer from the flotation tailings.

Based on the 9K medium composition used in the experiment, each 100 mL addition introduced **0.89 g Fe, 0.59 g S**, and no measurable Zn. Consequently, the total Stage I input from 300 mL 9K medium was **2.66 g Fe and 1.77 g S**, while the Stage II addition contributed **0.89 g Fe and 0.59 g S**.

4.3.3.5 Stage-Wise Elemental Mass Balance

Because additional 9K medium was introduced during Stages I and II, the difference between the measured initial and final elemental inventories could not be attributed entirely to dissolution from the flotation tailings.

The elemental mass corresponding to an ICP-OES concentration was calculated as:

$$m = (C \times V)/1000$$

where m is the dissolved elemental mass in g, C is the measured concentration in mg/L (ppm), and V is the solution volume in L.

The gross change in dissolved elemental inventory was calculated as:

$$m_{(gross)} = m_{(final)} - m_{(initial)}$$

For Stages I and II, the elemental mass introduced through the additional 9K medium was subsequently subtracted:

$$m_{(dissolved)} = m_{(final)} - m_{(initial)} - m_{(9K)}$$

For Stage III, no additional 9K medium was introduced during reactor operation. Therefore:

$$m_{(dissolved)} = m_{(final)} - m_{(initial)}$$

For the calculation, a liquid volume of 1 L was used for the directly measured initial and final analytical points. Minor volume changes associated with analytical sampling and physicochemical measurements were considered negligible

Table 4.22 Stage-wise elemental mass balance of Fe, S and Zn during Experiment 3.

Stage	Fe initial (ppm)	Fe final (ppm)	Fe input with 9K (g)	Fe dissolved (g)	Fe dissolved (g kg ⁻¹ initial solid)
I	8500	11390	2.66	0.23	2.27
II	10170	14080	0.89	3.02	30.22
III	11020	17100	0.00	6.08	60.80
Total	—	—	3.55	9.33	93.29
Stage	S initial (ppm)	S final (ppm)	S input with 9K (g)	S dissolved (g)	S dissolved (g kg ⁻¹ initial solid)
I	3382	6613	1.77	1.46	14.64
II	5269	8051	0.59	2.19	21.93
III	5593	9654	0.00	4.06	40.61
Total	—	—	2.36	7.72	77.18
Stage	Zn initial (ppm)	Zn final (ppm)	Zn input with 9K (g)	Zn dissolved (g)	Zn dissolved (g kg ⁻¹ initial solid)
I	2	87	0.00	0.09	0.85
II	48	77	0.00	0.03	0.29
III	33	54	0.00	0.02	0.21
Total	—	—	0.00	0.14	1.35

The corrected mass balance therefore gives a cumulative net transfer of **9.33 g Fe**, **7.72 g S**, and **0.14 g Zn** from the flotation tailings to the liquid phase during Experiment 3.

For Fe, the net dissolved quantities were 0.23 g, 3.02 g and 6.08 g during Stages I, II and III, respectively. Stage III therefore produced the greatest Fe transfer, corresponding to 60.80 g kg⁻¹ initial solid. The total net Fe dissolution was 9.33 g, equivalent to **93.29 g kg⁻¹ initial solid**.

For S, net dissolution amounted to 1.46 g during Stage I, 2.19 g during Stage II and 4.06 g during Stage III. The cumulative net S dissolution was therefore 7.72 g, equivalent to **77.18 g kg⁻¹ initial solid**.

Zn was not introduced with the additional 9K medium and therefore required no correction for these additions. The cumulative net Zn dissolution remained **0.14 g**, corresponding to **1.35 g kg⁻¹ initial solid**.

To determine the distribution of this solid-derived dissolved inventory between the stage-end PLS withdrawals and the liquid retained for subsequent stages, a removal balance was applied. Following Stages I and II, 75% of the process solution was removed as PLS, whereas 25% of the solid-derived dissolved inventory remained in the reactor and was transferred to the subsequent stage. At the completion of Stage III, the complete remaining liquid phase was recovered by filtration. The Fe and S introduced through the additional 9K medium were excluded from this removal balance.

Table 4.23 Distribution and cumulative removal of solid-derived Fe, S and Zn during Experiment 3.

Stage	Fe dissolved during stage (g)	Fe removed with PLS/final filtrate (g)	Fe remaining in reactor (g)	Cumulative Fe removed (g)
I	0.23	0.17	0.06	0.17
II	3.02	2.31	0.77	2.48
III	6.08	6.85	0.00	9.33
Total	9.33	9.33	0.00	9.33
Stage	S dissolved during stage (g)	S removed with PLS/final filtrate (g)	S remaining in reactor (g)	Cumulative S removed (g)
I	1.46	1.10	0.37	1.10
II	2.19	1.92	0.64	3.02
III	4.06	4.70	0.00	7.72
Total	7.72	7.72	0.00	7.72
Stage	Zn dissolved during stage (g)	Zn removed with PLS/final filtrate (g)	Zn remaining in reactor (g)	Cumulative Zn removed (g)
I	0.09	0.06	0.02	0.06
II	0.03	0.04	0.01	0.10
III	0.02	0.03	0.00	0.14
Total	0.14	0.14	0.00	0.14

During Stage I, 0.23 g Fe was attributed to net transfer from the flotation tailings after correction for the Fe introduced with the additional 9K medium. Approximately 0.17 g was removed with PLS 1, while 0.06 g remained in the reactor.

During Stage II, the retained 0.06 g Fe from Stage I was combined with an additional **3.02 g Fe** transferred from the flotation tailings. The subsequent 75% PLS withdrawal removed approximately **2.31 g Fe**, leaving **0.77 g** in the reactor for transfer to Stage III.

During Stage III, the retained Fe inventory was combined with an additional 6.08 g Fe transferred from the solid phase. Since the entire remaining liquid phase was recovered

during final filtration, **6.85 g Fe** was removed in the final liquid stream. The cumulative solid-derived Fe removal was therefore **9.33 g**.

The corresponding cumulative removals were **7.72 g S** and **0.14 g Zn**. No calculated solid-derived dissolved elemental inventory remained in the reactor solution following final filtration.

4.3.3.6 Operational Observations

Experiment 3 was conducted over three consecutive operational stages using 100 g of ground flotation tailings in a 1 L mechanically stirred reactor.

The initial and final physicochemical measurements indicate increasingly oxidising and acidic conditions over the course of the experiment. During Stage I, Eh increased from 410 to 513 mV while pH decreased from 1.67 to 1.48. Stage II showed an increase in Eh from 480 to 538 mV accompanied by a decrease in pH from 1.74 to 1.42. During Stage III, Eh increased from 476 to 600 mV while pH decreased from 1.55 to 1.21.

Additional 9K medium was required during the first two stages. Stage I received three 100 mL additions, corresponding to a total of 300 mL, while Stage II received one additional 100 mL portion. No additional 9K medium was recorded during Stage III. These additions were explicitly considered in the elemental mass balance to prevent Fe and S introduced with the 9K medium from being incorrectly attributed to dissolution from the flotation tailings.

Another important feature of Experiment 3 was the direct ICP-OES analysis of the initial process solution for each operational stage. This allowed the mass-balance calculations to use experimentally measured initial concentrations rather than reconstructed values.

At the end of Stages I and II, PLS was separated while the bio-oxidised solids were retained for continuation of the experiment. Following Stage III, the complete reactor slurry was filtered. The final process solution was collected and the bio-oxidised solid residue was retained for the subsequent thiourea-leaching experiment.

4.3.4 Experiment 4

Experiment 4 was conducted in a 3 L mechanically stirred reactor using 300 g of flotation tailings. Prior to bio-oxidation, the material was ground for 10 min to obtain a particle size below approximately 100 µm. The reactor was operated with a working liquid volume of 3000 mL using a process solution containing 80 vol.% bio-solution and 20 vol.% 9K medium.

The experiment consisted of four consecutive operational stages. The flotation tailings remained in the reactor throughout the complete bio-oxidation sequence. At the end of Stages

I, II and III, approximately 75% of the process solution, corresponding to 2250 mL, was withdrawn as PLS, while approximately 750 mL of process solution and the bio-oxidised solids remained in the reactor. The working liquid volume was subsequently restored to 3000 mL before the following stage.

No additional solution was introduced during the operational periods to compensate for evaporation, leakage, or other volume losses. Therefore, unlike Experiments 2 and 3, no additional make-up correction was required within the individual stage-wise elemental mass balances.

At the end of Stage IV, the complete reactor slurry was filtered and the bio-oxidised solid residue was recovered for the subsequent thiourea-leaching experiment.

The principal operating conditions and initial and final physicochemical parameters are summarised in Table 4.24.

Table 4.24 Summary of the operating conditions and physicochemical parameters for Experiment 4.

Stage	Time (Days)	Solid mass (g)	Working volume (mL)	Bio- solution (%)	9K (%)	Initial Eh (mV)	Final Eh (mV)	Initial pH	Final pH
I	4	300	3000	80	20	480	532	1.74	1.66
II	3	300	3000	80	20	499	537	1.60	1.44
III	3	300	3000	80	20	485	581	1.61	1.42
IV	4	300	3000	80	20	502	610	1.70	1.16

The physicochemical measurements used in this table correspond to the recorded initial and final values for the four operational stages

4.3.4.1 Stage I

Stage I was initiated using 300 g of ground flotation tailings suspended in 3000 mL of process solution consisting of 80 vol.% bio-solution and 20 vol.% 9K medium. The initial Eh and pH were 480 mV and 1.74, respectively.

No additional solution was introduced during the operational stage. At the end of Stage I, the Eh had increased from 480 to 532 mV, while the pH decreased from 1.74 to 1.66.

Following the final physicochemical measurements, 2250 mL of PLS was withdrawn. Approximately 750 mL of process solution and the bio-oxidised solids remained in the reactor and were retained for Stage II.

4.3.4.2 Stage II

Stage II was initiated after renewal of the process solution while retaining the bio-oxidised solids produced during Stage I. The initial Eh was 499 mV and the initial pH was 1.60.

During Stage II, no additional solution was introduced during reactor operation. At the completion of the stage, the Eh had increased to 537 mV and the pH had decreased to 1.44.

A further 2250 mL of PLS was withdrawn, while the remaining process solution and bio-oxidised solids were retained for continuation of the experiment.

4.3.4.3 Stage III

Stage III was conducted using the bio-oxidised solids retained from Stage II. The initial Eh and pH values were 485 mV and 1.61, respectively.

No additional process solution was introduced during the operational stage. At the end of Stage III, the Eh increased to 581 mV, while the pH decreased to 1.42.

Following the final measurements, 2250 mL of PLS was withdrawn and approximately 750 mL of process solution remained in the reactor together with the bio-oxidised solids before Stage IV.

4.3.4.4 Stage IV

Stage IV was initiated using the bio-oxidised solids retained from Stage III. The initial Eh and pH were 502 mV and 1.70, respectively.

During the final operational stage, the Eh increased substantially to 610 mV, while the pH decreased to 1.16. This represented both the highest final Eh and the lowest final pH recorded during Experiment 4.

At the completion of Stage IV, the entire reactor slurry was filtered. The final process solution was collected as PLS 4 and the bio-oxidised solid residue was recovered for the subsequent thiourea-leaching experiment.

4.3.4.5 ICP-OES Analysis of Experiment 4

The initial process solution and final PLS of each operational stage were analysed by ICP-OES to determine the dissolved Fe, S and Zn concentrations.

During review of the analytical sequence, the Fe and S results originally assigned to PLS 1 and the Stage II initial solution were identified as having been interchanged during sample

labelling. Their assignments were therefore corrected according to the experimental sequence. The Zn assignments were retained without modification.

The corrected ICP-OES dataset used for the Experiment 4 calculations is summarised in Table 4.25.

Table 4.25 Initial and final Fe, S and Zn concentrations during Experiment 4.

Stage	Fe initial (ppm)	Fe final (ppm)	S initial (ppm)	S final (ppm)	Zn initial (ppm)	Zn final (ppm)
I	8519	8974	3618	4380	14	53
II	7150	8741	4075	4841	14	29
III	9290	10670	4187	11570	8	20
IV	9797	13030	9152	13990	6	21

Because the initial process solution of each stage was analysed directly and no additional solution was introduced during stage operation, the net elemental concentration change could be determined directly from the measured initial and final concentrations:

$$\Delta C = C_{(final)} - C_{(initial)}$$

where $C_{(initial)}$ is the elemental concentration measured at the beginning of the stage and $C_{(final)}$ is the concentration measured in the final PLS.

The elemental mass transferred to the liquid phase was subsequently calculated from:

$$m_{(dissolved)} = (\Delta C \times V)/1000$$

where $m_{(dissolved)}$ is expressed in g, ΔC is expressed in mg/L, and V is the reactor liquid volume in L.

For Experiment 4: V=3 L

The dissolved mass was normalised to the initial solid mass according to:

$$m_{(normalized)} = m_{(dissolved)}/m_{(solid)}$$

where the initial solid mass was 0.3 kg.

Minor volume changes associated with analytical sampling, pH and Eh measurements, evaporation and filtration were considered negligible for the calculation.

The resulting stage-wise elemental dissolution values are presented in Table 4.26.

Table 4.26 Stage-wise elemental mass balance of Fe, S and Zn during Experiment 4.

Stage	Fe initial (ppm)	Fe final (ppm)	Δ Fe (ppm)	Fe dissolved (g)	Fe dissolved (g kg ⁻¹ initial solid)
I	8519	8974	455	1.37	4.55
II	7150	8741	1591	4.77	15.91
III	9290	10670	1380	4.14	13.80
IV	9797	13030	3233	9.70	32.33
Total	—	—	6659	19.98	66.59
Stage	S initial (ppm)	S final (ppm)	Δ S (ppm)	S dissolved (g)	S dissolved (g kg ⁻¹ initial solid)
I	3618	4380	762	2.29	7.62
II	4075	4841	766	2.30	7.66
III	4187	11570	7383	22.15	73.83
IV	9152	13990	4838	14.51	48.38
Total	—	—	13749	41.25	137.49
Stage	Zn initial (ppm)	Zn final (ppm)	Δ Zn (ppm)	Zn dissolved (g)	Zn dissolved (g kg ⁻¹ initial solid)
I	14	53	39	0.12	0.39
II	14	29	15	0.05	0.15
III	8	20	12	0.04	0.12
IV	6	21	15	0.05	0.15
Total	—	—	81	0.24	0.81

The values are consistent with the ICP-OES mass-balance calculations previously obtained for Experiment 4.

Fe showed a positive net transfer to the liquid phase during every operational stage. Stage I resulted in 1.37 g Fe dissolution, followed by 4.77 g during Stage II and 4.14 g during Stage III. The largest stage-wise Fe dissolution occurred during Stage IV, corresponding to 9.70 g or 32.33 g kg⁻¹ initial solid.

The cumulative net Fe dissolution over the four stages was **19.98 g**, equivalent to **66.59 g kg⁻¹ initial solid**.

Sulphur dissolution was substantially greater during the later stages. Stages I and II resulted in 2.29 and 2.30 g S dissolution, respectively, whereas Stage III produced a marked increase to 22.15 g. Stage IV resulted in an additional 14.51 g S dissolution. The cumulative net S dissolution was therefore **41.25 g**, corresponding to **137.49 g kg⁻¹ initial solid**.

Zn dissolution remained substantially lower. The calculated stage-wise quantities were 0.12, 0.05, 0.04 and 0.05 g during Stages I–IV, respectively. The cumulative net Zn dissolution was **0.24 g**, equivalent to **0.81 g kg⁻¹ initial solid**.

4.3.4.6 Stage-Wise Elemental Mass Balance

Because 75% of the process solution was withdrawn after Stages I, II and III, an additional stage-wise removal balance was required to distinguish between the elemental mass newly transferred from the flotation tailings, the quantity removed with each PLS withdrawal, and the quantity retained in the reactor and transferred to the following stage.

Only the net elemental inventory attributed to transfer from the flotation tailings was included in this calculation.

After Stage I, 75% of the newly dissolved elemental inventory was removed with the 2.25 L PLS withdrawal, while 25% remained in the reactor.

For subsequent stages, the inventory retained from the preceding stage was combined with the amount newly dissolved during the current stage:

$$m_{(available,j)} = m_{(retained,j-1)} + m_{(dissolved,j)}$$

For Stages I–III, the mass removed with the PLS was:

$$m_{(PLS)} = 0.75 \times m_{(available)}$$

and the mass retained in the reactor was:

$$m_{(retained)} = 0.25 \times m_{(available)}$$

At the end of Stage IV, the entire remaining liquid phase was recovered by final filtration. Therefore, no calculated dissolved solid-derived inventory remained in the reactor.

Table 4.27 Distribution and cumulative removal of solid-derived Fe, S and Zn during Experiment 4.

Stage	Fe dissolved during stage (g)	Fe removed with PLS/final filtrate (g)	Fe remaining in reactor (g)	Cumulative Fe removed (g)
I	1.37	1.02	0.34	1.03
II	4.77	3.84	1.28	4.86
III	4.14	4.06	1.36	8.92
IV	9.70	11.05	0.00	19.98
Total	19.98	19.98	0.00	19.98

Stage	S dissolved during stage (g)	S removed with PLS/final filtrate (g)	S remaining in reactor (g)	Cumulative S removed (g)
I	2.29	1.72	0.57	1.72
II	2.30	2.15	0.72	3.87
III	22.15	17.15	5.72	21.02
IV	14.51	20.23	0.00	41.25
Total	41.25	41.25	0.00	41.25
Stage	Zn dissolved during stage (g)	Zn removed with PLS/final filtrate (g)	Zn remaining in reactor (g)	Cumulative Zn removed (g)
I	0.12	0.09	0.03	0.09
II	0.05	0.06	0.02	0.14
III	0.04	0.04	0.01	0.18
IV	0.05	0.06	0.00	0.24
Total	0.24	0.24	0.00	0.24

These values correspond to the recorded Experiment 4 stage-wise removal balance.

During Stage I, a net **1.37 g Fe** was transferred from the solid phase to the liquid phase. Of this amount, approximately **1.02 g** was removed with PLS 1, while **0.34 g** remained in the reactor solution and was carried forward to Stage II.

During Stage II, the 0.34 g Fe retained from Stage I was combined with an additional **4.77 g Fe** transferred from the flotation tailings. The resulting dissolved solid-derived inventory was approximately 5.11 g. The subsequent 75% PLS withdrawal removed **3.84 g Fe**, while approximately **1.28 g** remained in the reactor for transfer to Stage III.

During Stage III, the retained Fe inventory was combined with an additional **4.14 g Fe** transferred from the solid phase, resulting in approximately 5.42 g of solid-derived dissolved Fe. The PLS withdrawal removed **4.06 g**, leaving **1.35 g Fe** in the reactor for Stage IV.

During Stage IV, the retained 1.36 g Fe was combined with an additional **9.70 g Fe** transferred during the final operational stage. Because the entire remaining liquid phase was recovered by filtration, approximately **11.05 g Fe** was removed in the final liquid stream.

The cumulative net solid-derived Fe removal over the complete Experiment 4 was therefore **19.98 g**.

The same mass-balance procedure gave cumulative removals of **41.25 g S** and **0.24 g Zn**. Because the complete remaining process solution was separated from the solids following

Stage IV, no calculated solid-derived dissolved elemental inventory remained in the reactor after final filtration.

These calculated quantities represent net changes in the dissolved elemental inventory attributed to the solid phase. They should therefore be interpreted as net elemental transfer to the liquid phase rather than as direct mineral-specific dissolution, since precipitation, adsorption or redistribution between solid and liquid phases may also have occurred during bio-oxidation.

4.3.4.7 Operational Observations

Experiment 4 was conducted over four consecutive operational stages in a 3 L mechanically stirred reactor using 300 g of ground flotation tailings. Continuous aeration and mechanical agitation were applied throughout the bio-oxidation experiment.

The reactor remained under acidic conditions during all four stages. The pH decreased from 1.74 to 1.66 during Stage I, from 1.60 to 1.44 during Stage II, from 1.61 to 1.42 during Stage III, and from 1.70 to 1.16 during Stage IV. The lowest pH observed during Experiment 4 was therefore recorded at the end of Stage IV.

The oxidation-reduction potential increased during every operational stage. Eh increased from 480 to 532 mV during Stage I, from 499 to 537 mV during Stage II, from 485 to 581 mV during Stage III, and from 502 to 610 mV during Stage IV. The highest final Eh was obtained at the completion of Stage IV.

No additional solution additions were required during the operational periods. Consequently, the elemental mass-balance calculations did not require correction for additional Fe, S or Zn introduced during the stages.

At the end of Stages I, II and III, 2250 mL of PLS was withdrawn while approximately 750 mL of process solution and the bio-oxidised solids remained in the reactor. The retained dissolved solid-derived elemental inventory was therefore carried forward to the subsequent stage and explicitly considered in the stage-wise removal balance.

A substantial increase in dissolved S was observed during Stage III, when the calculated net transfer reached 22.15 g. Stage IV showed the greatest stage-wise Fe transfer, corresponding to 9.70 g.

At the completion of Stage IV, the complete reactor slurry was filtered to separate the process solution from the bio-oxidised solids. The recovered solid residue was retained for the subsequent thiourea-leaching experiment.

4.3.5 Experiment 5

Experiment 5 was conducted in a 3 L mechanically stirred reactor using 300 g of a pyrite-rich concentrate produced from the flotation tailings by gravity separation. Unlike Experiments 1–4, which used the bulk flotation tailings as feed material, Experiment 5 was designed to evaluate the bio-oxidation behaviour of a sulphide-enriched feed.

The reactor was initially operated with a working liquid volume of 3000 mL using a process solution consisting of 80 vol.% bio-solution and 20 vol.% 9K medium. The experiment consisted of three consecutive operational stages.

At the end of Stages I and II, approximately 75% of the process solution, corresponding to 2250 mL, was withdrawn as PLS. The remaining 750 mL of process solution and the bio-oxidised solids were retained in the reactor, and the working volume was restored before initiation of the following stage.

No additional within-stage solution additions were recorded for Experiment 5. Consequently, no additional make-up correction was required when calculating net elemental transfer during the individual stages.

At the completion of Stage III, the entire reactor slurry was filtered and the bio-oxidised pyrite-rich concentrate was recovered for the subsequent thiourea-leaching experiment.

The principal operating conditions and initial and final physicochemical parameters are summarised in Table 4.28.

Table 4.28 Summary of the operating conditions and physicochemical parameters for Experiment 5.

Stage	Time (Days)	Solid mass (g)	Working volume (mL)	Bio- solution (%)	9K (%)	Initial Eh (mV)	Final Eh (mV)	Initial pH	Final pH
I	4	300	3000	80	20	490	723	1.60	1.50
II	3	300	3000	80	20	490	630	1.85	1.55
III	7	300	3000	80	20	495	650	1.60	1.27

The physicochemical measurements correspond to the recorded initial and final values for the three operational stages.

4.3.5.1 Stage I

Stage I was initiated using 300 g of pyrite-rich concentrate suspended in 3000 mL of process solution containing 80 vol.% bio-solution and 20 vol.% 9K medium. The initial Eh and pH were 490 mV and 1.60, respectively.

During Stage I, the oxidation-reduction potential increased markedly from 490 to 723 mV, while the pH decreased from 1.60 to 1.50.

At the end of the stage, 2250 mL of PLS was withdrawn for ICP-OES analysis. Approximately 750 mL of process solution and the bio-oxidised solids remained in the reactor and were retained for Stage II.

4.3.5.2 Stage II

Stage II was initiated using the bio-oxidised concentrate retained from Stage I following renewal of the process solution. The initial Eh and pH were 490 mV and 1.85, respectively.

At the completion of Stage II, the Eh had increased to 630 mV, while the pH decreased to 1.55.

Following the final measurements, 2250 mL of PLS was withdrawn. The remaining process solution and bio-oxidised concentrate were retained in the reactor, and the working volume was restored before Stage III.

4.3.5.3 Stage III

Stage III was conducted using the bio-oxidised concentrate retained from Stage II. The initial Eh and pH were 495 mV and 1.60, respectively.

During the final operational stage, the Eh increased to 650 mV, while the pH decreased to 1.27.

At the completion of Stage III, the entire reactor slurry was filtered. The final process solution was recovered and the bio-oxidised pyrite-rich concentrate was retained for the subsequent thiourea-leaching experiment.

4.3.5.4 ICP-OES Analysis of Experiment 5

The initial process solution and the final PLS collected from each operational stage were analysed by ICP-OES to determine the dissolved Fe, S and Zn concentrations.

Because the initial process solution of each stage was analysed directly and no additional within-stage solution additions were recorded, the difference between the measured initial and final elemental concentrations was used to determine the net elemental concentration change during each stage.

The measured ICP-OES concentrations are summarised in Table 4.29.

Table 4.29 Initial and final Fe, S and Zn concentrations during Experiment 5.

Stage	Fe initial (ppm)	Fe final (ppm)	S initial (ppm)	S final (ppm)	Zn initial (ppm)	Zn final (ppm)
I	8281	9173	6725	9366	2	44
II	9198	10910	8180	11180	13	61
III	9202	14820	8271	16150	19	75

For each element, the net concentration change was calculated according to:

$$\Delta C = C_{(final)} - C_{(initial)}$$

where ($C_{initial}$) is the ICP-OES concentration measured at the beginning of the stage and (C_{final}) is the concentration measured in the final PLS.

The corresponding elemental mass transferred to the liquid phase was calculated as:

$$m_{(dissolved)} = (\Delta C \times V)/1000$$

where ($m_{(dissolved)}$) is expressed in g, (ΔC) is expressed in mg/L (ppm), and (V) is the liquid volume in L.

For Experiment 5: $V = 3.000$ L

The dissolved mass was normalised to the initial solid mass according to:

$$m_{(normalized)} = m_{(dissolved)}/m_{(solid)}$$

where the initial solid mass was 0.3 kg.

The resulting stage-wise elemental dissolution values are summarised in Table 4.30.

Table 4.30 Stage-wise elemental mass balance of Fe, S and Zn during Experiment 5.

Stage	Fe initial (ppm)	Fe final (ppm)	Δ Fe (ppm)	Fe dissolved (g)	Fe dissolved (g kg ⁻¹ initial solid)
I	8281	9173	892	2.68	8.92
II	9198	10910	1712	5.14	17.12
III	9202	14820	5618	16.85	56.18
Total	—	—	8222	24.67	82.22

Stage	S initial (ppm)	S final (ppm)	ΔS (ppm)	S dissolved (g)	S dissolved (g kg ⁻¹ initial solid)
I	6725	9366	2641	7.92	26.41
II	8180	11180	3000	9.00	30.00
III	8271	16150	7879	23.64	78.79
Total	—	—	13520	40.56	135.20
Stage	Zn initial (ppm)	Zn final (ppm)	ΔZn (ppm)	Zn dissolved (g)	Zn dissolved (g kg ⁻¹ initial solid)
I	2	44	42	0.13	0.42
II	13	61	48	0.14	0.48
III	19	75	56	0.17	0.56
Total	—	—	146	0.44	1.46

The dissolved Fe concentration increased during all three operational stages. Stage I resulted in an apparent net Fe transfer of 2.68 g, followed by 5.14 g during Stage II. The greatest Fe transfer occurred during Stage III, corresponding to 16.85 g or 56.18 g kg⁻¹ initial solid. The cumulative apparent net Fe transfer during Experiment 5 was 24.67 g, equivalent to 82.22 g kg⁻¹ initial solid.

Sulphur exhibited a similar trend. The apparent net S transfer increased from 7.92 g during Stage I to 9.00 g during Stage II and reached 23.64 g during Stage III. The cumulative apparent net S transfer was 40.56 g, corresponding to 135.20 g kg⁻¹ initial solid.

Zn transfer remained substantially lower, with stage-wise values of 0.13, 0.14 and 0.17 g during Stages I–III, respectively. The cumulative apparent net Zn transfer was 0.44 g, equivalent to 1.46 g kg⁻¹ initial solid.

4.3.5.5 Stage-Wise Elemental Mass Balance

Because 75% of the process solution was withdrawn following Stages I and II, a stage-wise removal balance was established to distinguish between the elemental inventory newly transferred from the pyrite-rich concentrate, the quantity removed with each PLS withdrawal, and the dissolved inventory retained in the reactor for the subsequent stage. Only the net elemental inventory attributed to transfer from the pyrite-rich concentrate was included in this calculation.

For each stage, the available solid-derived dissolved inventory was calculated as:

$$m_{(available,j)} = m_{(retained,j)} + m_{(dissolved,j)}$$

For Stages I and II, 75% of the available inventory was removed with the 2250 mL PLS withdrawal:

$$m_{(PLS)} = 0.75 \times m_{(available)}$$

The remaining 25% was retained in the reactor:

$$m_{(retained)} = 0.25 \times m_{(available)}$$

At the completion of Stage III, the entire remaining process solution was recovered during final filtration. Therefore, no calculated solid-derived dissolved inventory remained in the reactor.

Table 4.31 Distribution and cumulative removal of solid-derived Fe, S and Zn during Experiment 5.

Stage	Fe dissolved during stage (g)	Fe removed with PLS/final filtrate (g)	Fe remaining in reactor (g)	Cumulative Fe removed (g)
I	2.68	2.01	0.67	2.01
II	5.14	4.35	1.45	6.36
III	16.85	18.31	0.00	24.67
Total	24.67	24.67	0.00	24.67
Stage	S dissolved during stage (g)	S removed with PLS/final filtrate (g)	S remaining in reactor (g)	Cumulative S removed (g)
I	7.92	5.94	1.98	5.94
II	9.00	8.24	2.75	14.18
III	23.64	26.38	0.00	40.56
Total	40.56	40.56	0.00	40.56
Stage	Zn dissolved during stage (g)	Zn removed with PLS/final filtrate (g)	Zn remaining in reactor (g)	Cumulative Zn removed (g)
I	0.13	0.10	0.03	0.10
II	0.14	0.13	0.04	0.23
III	0.17	0.21	0.00	0.44
Total	0.44	0.44	0.00	0.44

The stage-wise removal values correspond to the existing Experiment 5 mass-balance calculations.

During Stage I, a net **2.68 g Fe** was transferred from the pyrite-rich concentrate to the liquid phase. Of this quantity, **2.01 g** was removed with PLS 1, while **0.67 g** remained in the reactor and was carried forward to Stage II.

During Stage II, the 0.67 g Fe retained from Stage I was combined with an additional **5.14 g Fe** transferred from the solid phase, giving a total available solid-derived dissolved Fe

inventory of 5.81 g. The subsequent PLS withdrawal removed **4.35 g Fe**, while **1.45 g** remained in the reactor for transfer to Stage III.

During Stage III, the retained Fe inventory was combined with an additional **16.85 g Fe** transferred from the pyrite-rich concentrate. This resulted in a final dissolved solid-derived Fe inventory of **18.31 g**. Since the entire remaining liquid phase was recovered during final filtration, this quantity was completely removed in the final liquid stream.

The cumulative net solid-derived Fe removal during Experiment 5 was therefore **24.67 g**.

The corresponding cumulative removals were **40.56 g S** and **0.44 g Zn**. Following final filtration, no calculated solid-derived dissolved elemental inventory remained in the reactor solution.

These values represent net changes in the dissolved elemental inventory attributed to the pyrite-rich concentrate. They should therefore be interpreted as net elemental transfer to the liquid phase rather than as direct mineral-specific dissolution, since precipitation, adsorption or redistribution between solid and liquid phases may also have occurred during bio-oxidation.

4.3.5.6 Operational Observations

Experiment 5 was conducted over three consecutive operational stages in a 3 L mechanically stirred reactor using 300 g of pyrite-rich concentrate produced from the flotation tailings by gravity separation.

The oxidation-reduction potential increased during every operational stage. Stage I showed the largest increase, from 490 to **723 mV**, which was the highest final Eh recorded for Experiment 5. Eh subsequently increased from 490 to 630 mV during Stage II and from 495 to 650 mV during Stage III.

The reactor remained strongly acidic throughout the experiment. The pH decreased from 1.60 to 1.50 during Stage I, from 1.85 to 1.55 during Stage II, and from 1.60 to 1.27 during Stage III. The lowest pH was therefore recorded at the completion of Stage III.

At the end of Stages I and II, 2250 mL of PLS was withdrawn while approximately 750 mL of process solution and the bio-oxidised solids remained in the reactor. The retained solid-derived dissolved elemental inventory was carried forward to the subsequent stage and was explicitly accounted for in the stage-wise removal balance.

The ICP-OES results showed that the largest stage-wise transfers of Fe, S and Zn all occurred during Stage III. The calculated Stage III net dissolved quantities were **16.85 g Fe, 23.64 g S and 0.17 g Zn**.

At the completion of Stage III, the complete reactor slurry was filtered. The final process solution was separated and the bio-oxidised pyrite-rich concentrate was recovered for the subsequent thiourea-leaching experiment.

4.4 Thiourea Leaching of Bio-oxidised Residues

Following the bio-oxidation experiments, thiourea leaching was performed to evaluate the extraction of gold from the bio-oxidised solids. Thiourea was selected as the leaching reagent because of its rapid gold dissolution kinetics under acidic conditions and its lower environmental impact compared with conventional cyanidation. The principal objective of this stage was to determine the influence of the different bio-oxidation conditions on the subsequent dissolution of gold and to compare the leaching behaviour of the bio-oxidised residues with that of untreated flotation tailings.

Eight independent thiourea-leaching experiments were conducted. Six thiourea-leaching tests were performed using residues from the five reactor bio-oxidation experiments, because the residue from Reactor Experiment 4 was divided into two subsamples tested with and without ferric-ion addition. Two additional tests were conducted using untreated flotation tailings and the combined GRM shake-flask residue, giving a total of eight tests.

The operating conditions were selected to maintain broadly comparable liquid-to-solid ratios and thiourea concentrations where practicable, although solid mass, solution volume, agitation speed, and feed material varied among individual tests. Samples 4A and 4B constituted a specifically paired comparison, in which the same bio-oxidised residue, solid mass, solution volume, thiourea concentration, agitation speed, and leaching duration were used, while ferric sulphate addition was the intentional experimental difference.

4.4.1 Experimental Procedure

All thiourea-leaching experiments were performed in a 1L mechanically stirred glass reactor operating under atmospheric pressure. Continuous mechanical agitation was applied throughout the experiments to ensure homogeneous suspension of solid particles and adequate contact between the leaching solution and the bio-oxidised residue.

A stock thiourea solution was prepared by dissolving 12.2 g of thiourea in 1L of deionised water. Immediately before each experiment, equal volumes of deionised water and thiourea stock solution were mixed, producing a working solution containing 6.1 g L^{-1} thiourea.

Most experiments were carried out using 55 g of solid and 220 mL of leaching solution consisting of 110 mL of deionised water and 110 mL of thiourea stock solution. Sample 1 was performed using 41.35 g of residue and 160 mL of solution, whereas Sample 5 employed 130 g of residue with a total solution volume of 520 mL. The solution volumes were adjusted proportionally to maintain approximately the same liquid-to-solid ratio throughout the experimental programme.

Ferric sulphate hydrate ($\text{Fe}_2(\text{SO}_4)_3 \cdot \text{H}_2\text{O}$) was used as the oxidising agent. According to the total solution volume, 1.35 g, 1.84 g, or 4.35 g of ferric sulphate hydrate was added to maintain comparable initial ferric-ion concentrations in all experiments. Sample 4A was intentionally performed without ferric-ion addition in order to evaluate the effect of Fe^{3+} on gold dissolution under otherwise identical experimental conditions.

The initial pH of each experiment was adjusted using sulphuric acid prior to the addition of the solid sample. Each leaching experiment was conducted for 6 h under continuous agitation.

Liquid samples were collected after 0.5, 1, 2, 4 and 6 h. During every sampling event, 10 mL of pregnant leach solution (PLS) was withdrawn for chemical analysis. Immediately after sampling, the removed volume was replaced with 5 mL of deionised water and 5 mL of freshly prepared thiourea stock solution, thereby maintaining a constant liquid volume throughout the experiment.

Following each sampling event, the solution pH was manually adjusted, when necessary, by the addition of sulphuric acid in order to maintain the acidic conditions required for thiourea leaching. Consequently, the recorded pH and oxidation-reduction potential (Eh) values primarily represent controlled operating conditions rather than the natural evolution of the leaching reactions. Throughout the experiments, pH, Eh and temperature were measured at each sampling interval to verify that the desired leaching conditions were maintained.

After completion of the experiments, the entire slurry was filtered to separate the pregnant leach solution from the solid residue. The recovered solids were washed with deionised water, dried at 60 °C and submitted for fire-assay analysis. The collected liquid samples were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) for dissolved elemental concentrations, including Au.

Table 4.32 Operating conditions of the thiourea-leaching experiments.

Sample	Feed material	Solid mass (g)	Water (mL)	Thiourea stock solution (mL)	Total solution (mL)	Ferric sulphate hydrate (g)	Stirring speed (rpm)	Leaching time (h)
Sample 1	Bio-oxidised residue from Experiment 1	41.35	80	80	160	1.35	185	6
Sample 2	Bio-oxidised residue from Experiment 2	55	110	110	220	1.84	250	6
Sample 3	Bio-oxidised residue from Experiment 3	55	110	110	220	1.84	250	6
Sample 4A	Bio-oxidised residue from Experiment 4, without Fe ³⁺	55	110	110	220	-	200	6
Sample 4B	Bio-oxidised residue from Experiment 4, with Fe ³⁺	55	110	110	220	1.84	200	6
Sample 5	Bio-oxidised residue from Experiment 5	130	260	260	520	4.35	250	6
Sample 6	Untreated flotation tailings	55	110	110	220	1.84	185	6
Sample 7	Combined GRM shake-flask residue	55	110	110	220	1.84	250	6

4.4.1.1 Evaluation of Gold Extraction

Five PLS samples were collected during each 6 h thiourea-leaching experiment at 0.5, 1, 2, 4 and 6 h. However, Au was reported as not detected (N/D) in the analysed PLS samples. Consequently, the liquid-phase results could not be used to establish a quantitative time-dependent Au dissolution profile or a complete solution-based Au mass balance.

Gold extraction was therefore evaluated from the difference between the initial Au grade of the relevant feed material and the Au grade remaining in the final solid residue after thiourea leaching. Because this approach is based on solid-phase grades rather than a closed Au mass balance, the calculated values are reported as apparent Au extraction.

$$R_{(Au)} = (C_{(initial)} - C_{(residue)})/C_{(initial)} \times 100$$

where $C_{(initial)}$ is the initial Au grade of the relevant material and $C_{(residue)}$ is the Au grade measured in the final solid residue after thiourea leaching. For the flotation-tailings-based tests, the reference initial Au grade was 0.81 ppm. For Sample 5, which used the pyrite-rich concentrate, the initial Au grade was 1.09 ppm.

Because this calculation is based on changes in solid-phase Au grade rather than a complete mass balance incorporating both the initial and final dry solid masses and quantitatively measured dissolved Au, the calculated values are reported as **apparent Au extraction** rather than absolute Au recovery.

4.4.2 Thiourea Leaching Results

The thiourea-leaching experiments were conducted under the operating conditions described in Section 4.4.1. During each 6 h experiment, pH, oxidation-reduction potential (Eh), and temperature were monitored at five sampling points to verify that the required acidic and oxidising leaching conditions were maintained.

Because the pH was manually readjusted with sulphuric acid after sampling whenever required, the measured pH and Eh values are interpreted primarily as operational control parameters rather than as direct indicators of the intrinsic leaching kinetics.

Five PLS samples were collected from each experiment at 0.5, 1, 2, 4 and 6 h and analysed by ICP-OES. However, Au was reported as not detected (N/D) in the analysed liquid samples. Consequently, quantitative time-dependent Au dissolution curves and a complete liquid-phase Au mass balance could not be established. The quantitative comparison of the thiourea-leaching tests was therefore based on the apparent Au extraction calculated from the initial and final solid-phase fire-assay results, as described in Section 4.4.1.1.

4.4.2.1 Sample 1

Sample 1 was prepared using the bio-oxidised residue obtained from Reactor Bio-oxidation Experiment 1. The experiment was performed using 41.35 g of residue and 160 mL of leaching solution containing thiourea and ferric sulphate under the operating conditions summarised in Table 4.33.

Table 4.33 Physicochemical measurements during thiourea leaching of Sample 1

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.68	416	28.4
1	1.80	428	28.8
2	1.68	423	29.0
4	1.73	473	29.3
6	1.66	475	29.5

As explained previously, the pH was periodically readjusted after sampling to maintain the desired acidic leaching conditions. Consequently, the measured pH and Eh values confirm that stable operating conditions were maintained throughout the experiment. The temperature remained between 28.4 °C and 29.5 °C, indicating negligible thermal variation during leaching. The experiment was conducted for 6 h under mechanical agitation at 185 rpm.

Au was reported as N/D in all five analysed PLS samples. Therefore, no quantitative solution-based Au recovery profile could be calculated for Sample 1.

Sample 1 produced an apparent Au extraction of 24.7%. This value was higher than the 18.5% obtained from direct thiourea leaching of the untreated flotation tailings, corresponding to an improvement of approximately 6.2 percentage points after the bio-oxidation treatment.

4.4.2.2 Sample 2

Sample 2 consisted of the bio-oxidised residue recovered from Reactor Experiment 2. A total of **55 g** of residue was leached using **220 mL** of leaching solution, consisting of 110 mL deionised water and 110 mL thiourea stock solution. Ferric sulphate hydrate was used as the oxidising agent, and the test was conducted for 6 h under continuous mechanical agitation at 250 rpm.

The physicochemical parameters measured during Sample 2 are presented in Table 4.34.

Table 4.34 Physicochemical measurements during thiourea leaching of Sample 2

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.70	480	27.3
1	1.72	479	27.5
2	1.79	483	27.6
4	1.74	492	27.7
6	1.77	511	28.0

The pH remained between 1.70 and 1.79 during the complete test, reflecting the controlled acidic conditions. Eh increased gradually from approximately 480 mV during the first hour to 511 mV at the end of the 6 h leaching period. Temperature variation was limited, ranging from 27.3 to 28.0 °C

Au was reported as N/D in all analysed PLS samples from this test. Accordingly, no solution-based cumulative Au recovery was calculated.

The solid-phase fire-assay results gave an apparent Au extraction of 22.2%. This value was approximately 3.7 percentage points higher than that obtained for the untreated flotation-tailings reference, indicating only a modest apparent improvement under the investigated conditions.

4.4.2.3 Sample 3

Sample 3 was prepared using the bio-oxidised solid residue obtained from Reactor Experiment 3. The thiourea-leaching experiment was conducted using **55 g of residue and 220 mL of total leaching solution** under the standard experimental conditions described in Section 4.4.1.

Table 4.35 Physicochemical measurements during thiourea leaching of Sample 3

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.72	470	27.5
1	1.60	473	27.6
2	1.73	476	27.7
4	1.63	485	27.8
6	1.84	512	28.0

The pH varied between 1.60 and 1.84 as a result of periodic manual adjustment following sampling. Eh increased progressively from 470 mV at 0.5 h to 512 mV after 6 h. The temperature remained nearly constant, increasing only from 27.5 to 28.0 °C during the complete experiment. The experiment was conducted for 6 h under mechanical agitation at 250 rpm.

Au was reported as N/D in all analysed liquid samples, and therefore no solution-based cumulative Au recovery could be quantified for this experiment.

According to the solid-phase fire-assay results, Sample 3 produced an **apparent Au extraction of 27.2%**, which was the highest value obtained among the completed thiourea-leaching tests for which fire-assay results were available.

Compared with the untreated flotation-tailings reference value of 18.5%, Sample 3 showed an apparent Au extraction that was approximately 8.7 percentage points higher. This result suggests a modest improvement following the bio-oxidation treatment under the investigated conditions. Nevertheless, the grade-based apparent extraction remained below 30%.

4.4.2.4 Samples 4A and 4B: Effect of Ferric-Ion Addition

Samples 4A and 4B were prepared from the bio-oxidised solid residue obtained from Reactor Experiment 4. These two tests were conducted to evaluate the effect of ferric-ion addition on Au extraction during thiourea leaching.

Both tests were performed using **55 g of bio-oxidised residue and 220 mL of total leaching solution**, consisting of 110 mL of deionised water and 110 mL of thiourea stock solution. Sample 4A was conducted **without ferric sulphate addition**, whereas **1.84 g of ferric sulphate hydrate** was added to Sample 4B. The remaining principal operating conditions were maintained as consistently as possible between the two tests.

The experimental records confirm that both tests were monitored for pH, Eh and temperature during leaching. The measured pH values remained within the acidic range, while Eh values were approximately 431–511 mV.

4.4.2.4.1 Sample 4A

Sample 4A was prepared using the bio-oxidised residue obtained from Reactor Experiment 4. This test was performed without ferric-ion addition in order to evaluate the role of Fe^{3+} during thiourea leaching by comparison with Sample 4B.

A total of **55 g of bio-oxidised residue** was leached using **220 mL of total leaching solution**, consisting of 110 mL of deionised water and 110 mL of thiourea stock solution. No ferric sulphate hydrate was added. The experiment was conducted for 6 h under mechanical agitation at **200 rpm**. The omission of ferric sulphate was the intentional experimental variation used to assess the influence of Fe^{3+} on Au extraction.

Table 4.36 Physicochemical measurements during thiourea leaching of Sample 4A

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.64	429	26.2
1	1.70	431	26.2
2	1.70	434	26.3
4	1.79	448	26.3
6	1.75	460	26.4

The pH remained within a relatively narrow acidic range of **1.64–1.79** throughout the experiment. The Eh initially increased from 429 mV at 0.5 h to 460 mV after 6 h of leaching. The temperature remained stable throughout the experiment, increasing slightly from 26.2 °C at 0.5 h to 26.4 °C after 6 h.

As in the preceding tests, pH was manually adjusted with sulphuric acid when required. Therefore, the recorded pH and Eh values are considered primarily as indicators of the controlled operating conditions rather than direct indicators of the intrinsic Au dissolution kinetics.

Au was reported as N/D in all analysed PLS samples. Consequently, no quantitative solution-based cumulative Au recovery could be calculated.

Based on the solid-phase fire-assay results, Sample 4A produced an **apparent Au extraction of 13.6%**. This value was lower than the **18.5%** obtained from direct thiourea leaching of the untreated flotation tailings, corresponding to a difference of approximately **4.9 percentage points**.

Thus, under the investigated conditions, Sample 4A did not show an apparent Au extraction higher than that of the untreated reference.

4.4.2.4.2 Sample 4B

Sample 4B was prepared using the same bio-oxidised residue obtained from Reactor Experiment 4. The test was performed under comparable conditions to Sample 4A, with the principal difference being the addition of ferric sulphate hydrate as the oxidising agent.

A total of **55 g of bio-oxidised residue** was leached using **220 mL of total leaching solution**, consisting of 110 mL of deionised water and 110 mL of thiourea stock solution. A total of **1.84 g of ferric sulphate hydrate** was added, and the experiment was conducted for 6 h at **200 rpm**.

Table 4.37 Physicochemical measurements during thiourea leaching of Sample 4B

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.70	459	26.5
1	1.66	462	26.6
2	1.68	465	25.6
4	1.80	481	26.7
6	1.90	511	26.7

The pH remained between **1.66 and 1.90** throughout the leaching experiment. Eh showed a progressive increase from 459 mV at 0.5 h to **511 mV after 6 h**, indicating increasingly oxidising conditions during the test. The recorded temperature varied between 25.6 and 26.7 °C during the experiment.

The progressive increase in Eh was more pronounced than in Sample 4A, consistent with the presence of ferric ions in the leaching system. However, because pH was manually controlled during the experiment, the physicochemical measurements are interpreted as operational parameters.

Au was reported as N/D in all analysed PLS samples. Therefore, no quantitative solution-based Au recovery profile could be established. Based on the solid-phase fire-assay results, Sample 4B produced an **apparent Au extraction of 19.8%**. This value was slightly higher than the 18.5% obtained from direct thiourea leaching of untreated flotation tailings.

4.4.2.4.3 Comparison of Samples 4A and 4B

Samples 4A and 4B provide a direct assessment of the influence of ferric-ion addition because both tests used the same bio-oxidised residue, solid mass, thiourea concentration, liquid volume, agitation speed and leaching duration. The intentional difference was the omission of ferric sulphate from Sample 4A.

Table 4.38 Comparison of the principal operating conditions of Samples 4A and 4B.

Parameter	Sample 4A	Sample 4B
Solid mass (g)	55	55
Total solution volume (mL)	220	220
Ferric sulphate hydrate (g)	0	1.84
Stirring speed (rpm)	200	200
Final pH	1.75	1.90
Final Eh (mV)	460	511
Apparent Au extraction (%)	13.6	19.8

The addition of ferric sulphate increased the apparent Au extraction from **13.6% to 19.8%**, corresponding to an increase of **6.2 percentage points**.

The difference in the final Eh values was also notable. Sample 4A reached 460 mV after 6 h, whereas Sample 4B reached **511 mV**. The higher oxidation-reduction potential in the ferric-containing system was accompanied by the higher apparent Au extraction.

Under the investigated conditions, Fe^{3+} addition was associated with a 6.2 percentage-point increase in apparent Au extraction. Nevertheless, the apparent Au extraction remained below 20%, indicating that the overall improvement was limited.

Because the extraction values were obtained from solid-phase fire-assay grades rather than a complete solution-based Au mass balance, they are reported as **apparent Au extraction**.

4.4.2.5 Sample 5

Sample 5 was prepared using the bio-oxidised solid residue obtained from Reactor Experiment 5. Unlike Samples 1–4, Reactor Experiment 5 used a **pyrite-rich concentrate produced from the flotation tailings by gravity separation** as the feed material.

A total of **130 g of bio-oxidised residue** was leached using **520 mL of total leaching solution**, consisting of 260 mL of deionised water and 260 mL of thiourea stock solution. A total of **4.35 g of ferric sulphate hydrate** was added as the oxidising agent. The experiment was conducted for 6 h under mechanical agitation at **250 rpm**.

Table 4.39 Physicochemical measurements during thiourea leaching of Sample 5.

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.68	440	28.3
1	1.70	454	28.4
2	1.75	454	28.6
4	1.80	454	28.8
6	1.70	455	29.0

The pH remained between **1.68 and 1.80** throughout the 6 h leaching experiment. Eh increased from 440 mV at 0.5 h to approximately 454 mV after 1 h and subsequently remained nearly constant, reaching 455 mV at the final sampling point.

Temperature increased gradually from **28.3 to 29.0 °C**, indicating only minor thermal variation during the experiment.

As in the preceding tests, the measured pH values reflect periodic manual adjustment with sulphuric acid and are therefore considered operational control parameters.

Au was reported as N/D in all analysed PLS samples. Consequently, no quantitative time-dependent Au recovery profile could be established.

The initial Au grade of the pyrite-rich concentrate was **1.09 ppm**, which differs from the 0.81 ppm reference grade used for the flotation-tailings-based experiments.

Based on the solid-phase fire-assay results, the final thiourea-leached residue contained **0.84 ppm Au**, corresponding to an apparent Au extraction of:

$$R_{(Au)} = \left((C_{(initial)} - C_{(residue)}) / C_{(initial)} \right) \times 100$$

$$R_{(Au)} = ((1.09 - 0.84) / 1.09) \times 100 = 22.9\%$$

Sample 5 therefore produced an **apparent Au extraction of 22.9%**.

Although this value is comparable to those obtained for Samples 1 and 2, direct ranking should be made cautiously because Sample 5 originated from a pyrite-rich concentrate with a different mineralogical composition and initial Au grade.

4.4.2.6 Sample 6

Sample 6 was conducted using **untreated flotation tailings without prior bio-oxidation**. The purpose of this experiment was to provide a direct thiourea-leaching reference against which the effect of the different bio-oxidation pretreatments could be evaluated.

A total of **55 g of untreated flotation tailings** was leached using **220 mL of total leaching solution**, consisting of 110 mL of deionised water and 110 mL of thiourea stock solution. A total of **1.84 g of ferric sulphate hydrate** was added as the oxidising agent. The experiment was conducted for 6 h under mechanical agitation at **185 rpm**.

Table 4.40 Physicochemical measurements during thiourea leaching of Sample 6.

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.75	453	25.2
1	1.75	450	25.3
2	1.75	447	25.4
4	1.71	443	25.5
6	1.74	442	25.6

The pH remained highly stable throughout the experiment, varying only between **1.71 and 1.75**. In contrast to Samples 1–5, Eh showed a gradual decrease during the leaching period, declining from 453 mV at 0.5 h to **442 mV after 6 h**.

The recorded temperature increased slightly from **25.2 °C at 0.5 h to 25.6 °C at 6 h**.

Au was reported as N/D in all analysed PLS samples. Therefore, no quantitative liquid-phase cumulative Au recovery could be calculated.

The untreated flotation tailings had an initial Au grade of **0.81 ppm**. The final solid-phase fire-assay result corresponded to an apparent Au extraction of **18.5%**.

4.4.2.7 Sample 7

Sample 7 was prepared using the combined bio-oxidised solid residue obtained from the ten GRM shake-flask experiments, namely Flasks 11, 12, 21, 22, 31, 32, 41, 42, 51 and 52. The WW2 residues from Flasks 61 and 71 were not included in this composite sample.

Before solid-liquid separation, the contents of the ten GRM flasks were combined and homogenised. The combined suspension had a measured pH of **1.26**, Eh of **632 mV**, and temperature of **31.5 °C**. The solids were subsequently separated from the combined bio-solution and recovered for the thiourea-leaching experiment.

A total of **55 g of the combined GRM bio-oxidised residue** was leached using **220 mL of total leaching solution**, consisting of 110 mL of deionised water and 110 mL of thiourea stock solution. A total of **1.84 g of ferric sulphate hydrate** was added as the oxidising agent. The experiment was conducted for 6 h under mechanical agitation at **250 rpm**.

Table 4.41 Physicochemical measurements during thiourea leaching of Sample 7.

Leaching time (h)	pH	Eh (mV)	Temperature (°C)
0.5	1.46	460	29.8
1	1.42	454	29.5
2	1.40	447	29.2
4	1.37	433	28.7
6	1.34	420	28.3

Au was reported as N/D in the analysed PLS samples. Therefore, no quantitative liquid-phase Au recovery profile could be established.

The fire-assay result for Initial 7 showed an Au grade of 0.81 ppm, while the final thiourea-leached residue contained 0.65 ppm Au. Based on the difference between the initial and final solid-phase Au grades, the apparent Au extraction was calculated as:

$$R_{(Au)} = ((0.81 - 0.65)/0.81) \times 100 = 19.8\%$$

Sample 7 therefore produced an apparent Au extraction of 19.8%. This value was approximately 1.3 percentage points higher than the 18.5% obtained by direct thiourea leaching of the untreated flotation tailings (Sample 6). The difference was small and remained below the apparent Au extractions obtained for Samples 1, 2 and 3. Therefore, the combined GRM shake-flask treatment was associated with only a limited apparent improvement under the investigated conditions.

4.4.3 Overall Comparison of Thiourea-Leaching Results

The apparent Au extraction values obtained from the completed thiourea-leaching tests are summarised in Table 4.42. As dissolved Au was reported as N/D in the analysed PLS samples, the quantitative comparison was based on the solid-phase fire-assay results. Consequently, the calculated values are reported as apparent Au extraction rather than as absolute Au recovery.

Table 4.42 Comparison of apparent Au extraction obtained from the thiourea-leaching experiments.

Sample	Feed to thiourea leaching	Initial Au grade (ppm)	Final residue Au grade (ppm)	Apparent Au extraction (%)
Sample 1	Bio-oxidised residue from Experiment 1	0.81	0.61	24.7
Sample 2	Bio-oxidised residue from Experiment 2	0.81	0.63	22.2
Sample 3	Bio-oxidised residue from Experiment 3	0.81	0.59	27.2
Sample 4A	Bio-oxidised residue from Experiment 4, without Fe ³⁺	0.81	0.70	13.6
Sample 4B	Bio-oxidised residue from Experiment 4, with Fe ³⁺	0.81	0.65	19.8
Sample 5	Bio-oxidised residue from Experiment 5	1.09	0.84	22.9
Sample 6	Untreated flotation tailings	0.81	0.66	18.5
Sample 7	Combined GRM shake-flask residue	0.81	0.65	19.8

It should be noted that Sample 5 originated from a pyrite-rich concentrate with a different initial Au grade (1.09 ppm) and mineralogical composition; therefore, its apparent extraction value is not directly comparable to those of Samples 1-4 and 6-7, which were derived from the bulk flotation tailings.

The apparent Au extraction values varied between 13.6% and 27.2% across the thiourea-leaching tests. The highest apparent extraction was obtained for Sample 3, corresponding to the bio-oxidised residue from Reactor Experiment 3, with a value of 27.2%. Samples 1, 2 and 5 produced apparent Au extractions of 24.7%, 22.2% and 22.9%, respectively.

Direct thiourea leaching of the untreated flotation tailings (Sample 6) resulted in an apparent Au extraction of 18.5%. The combined GRM shake-flask residue (Sample 7) produced a slightly higher value of 19.8%, corresponding to an improvement of approximately 1.3 percentage points relative to the untreated reference. This indicates that the shake-flask bio-oxidation treatment provided only a limited improvement in subsequent thiourea leaching under the investigated conditions.

The paired Experiment 4 tests indicated a positive effect of ferric-ion addition on apparent Au extraction under the investigated conditions. Sample 4A, prepared without Fe^{3+} addition, produced the lowest apparent extraction of 13.6%, whereas Sample 4B, with Fe^{3+} addition, reached 19.8%. The difference of 6.2 percentage points indicates that ferric-ion addition improved Au extraction under the specific conditions applied in these paired tests.

Overall, the bio-oxidised residues did not show a uniform improvement relative to the untreated flotation tailings. Samples 1, 2, 3, 4B, 5 and 7 produced apparent extraction values equal to or higher than the untreated reference, whereas Sample 4A produced a lower value. The highest improvement was observed for Sample 3, but even this test remained below 30% apparent Au extraction. These results indicate that bio-oxidation alone was not sufficient to ensure high subsequent Au extraction and that thiourea leaching performance remained strongly dependent on the specific pretreatment and leaching conditions.

4.4.4 Optical Microscopy Assessment of the Bio-oxidation Pre-treatment

The apparent Au extraction obtained during the thiourea-leaching experiments remained relatively low, ranging from 13.6% to 27.2%, despite the preceding bio-oxidation treatment. The highest apparent extraction was obtained for Sample 3 at 27.2%, indicating that a substantial proportion of the initial Au remained in the final solid residues. Following these results, an additional optical microscopy examination was performed to qualitatively compare the appearance of the flotation tailings before and after bio-oxidation and to investigate whether incomplete alteration of the sulphide-bearing particles could have contributed to the limited subsequent Au extraction [49].

Representative optical microscopy images obtained before and after bio-oxidation are presented in Figures 4.12 and 4.13.

Comparison of the images revealed visible differences in particle morphology following bio-oxidation. Prior to bio-oxidation, the particles generally exhibited relatively well-defined boundaries, angular morphologies and compact reflective surfaces. Following bio-oxidation, the material appeared more heterogeneous, with a greater occurrence of smaller and irregular particles and locally rougher or altered particle surfaces. These observations suggest that the biological treatment affected the morphology of the solid material. Nevertheless, numerous well-defined reflective mineral grains remained visible after treatment, indicating that the observed alteration was not uniform throughout the sample. The optical observations are

therefore consistent with partial or heterogeneous alteration during bio-oxidation rather than complete alteration of the sulphide-bearing material [50].

These observations provide qualitative support for considering incomplete sulphide oxidation as one possible factor contributing to the limited Au extraction obtained during the subsequent thiourea-leaching experiments. If part of the Au was associated with or encapsulated within sulphide minerals, incomplete oxidation of these mineral phases could have restricted Au accessibility to the thiourea solution.

However, optical microscopy alone cannot conclusively identify the remaining particles as pyrite, quantify the degree of sulphide oxidation, or demonstrate that the residual Au was encapsulated within sulphide minerals. The microscopy observations should therefore be regarded as qualitative supporting evidence rather than direct confirmation of the mechanism responsible for the relatively low Au extraction. Other factors related to the thiourea-leaching conditions may also have contributed to the observed extraction behaviour.

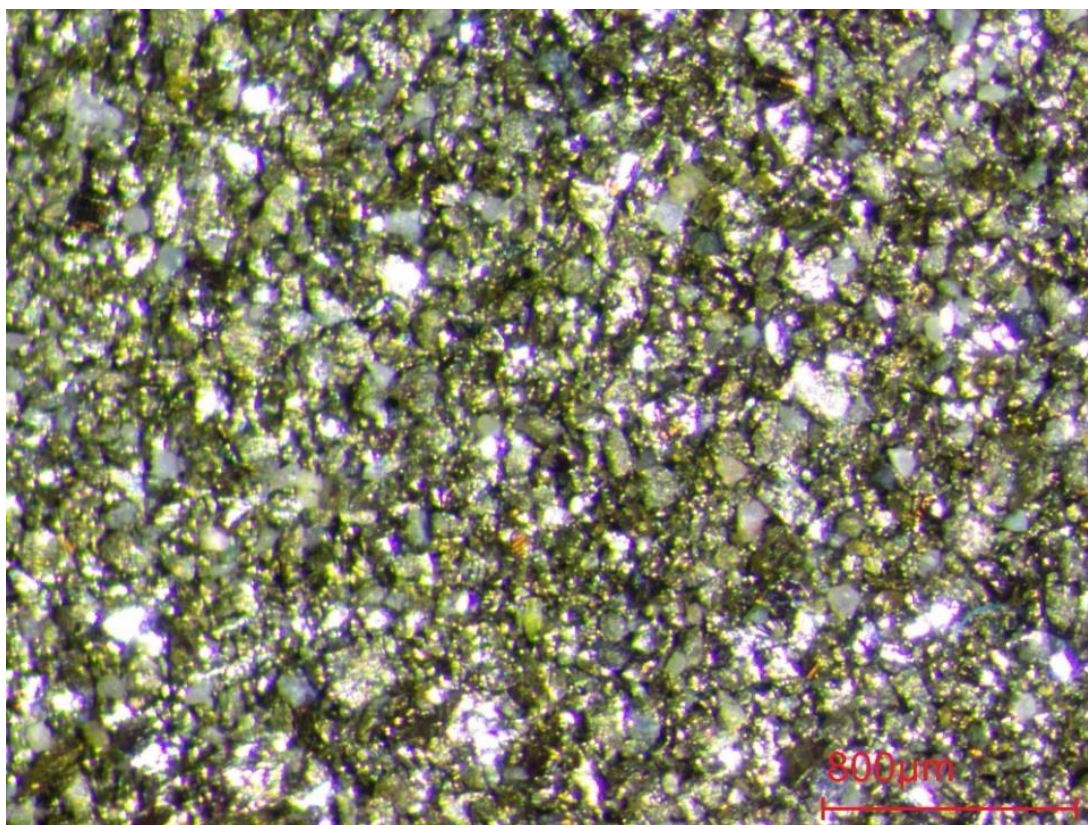


Figure 4.12 Representative optical microscopy images of the flotation tailings before bio-oxidation

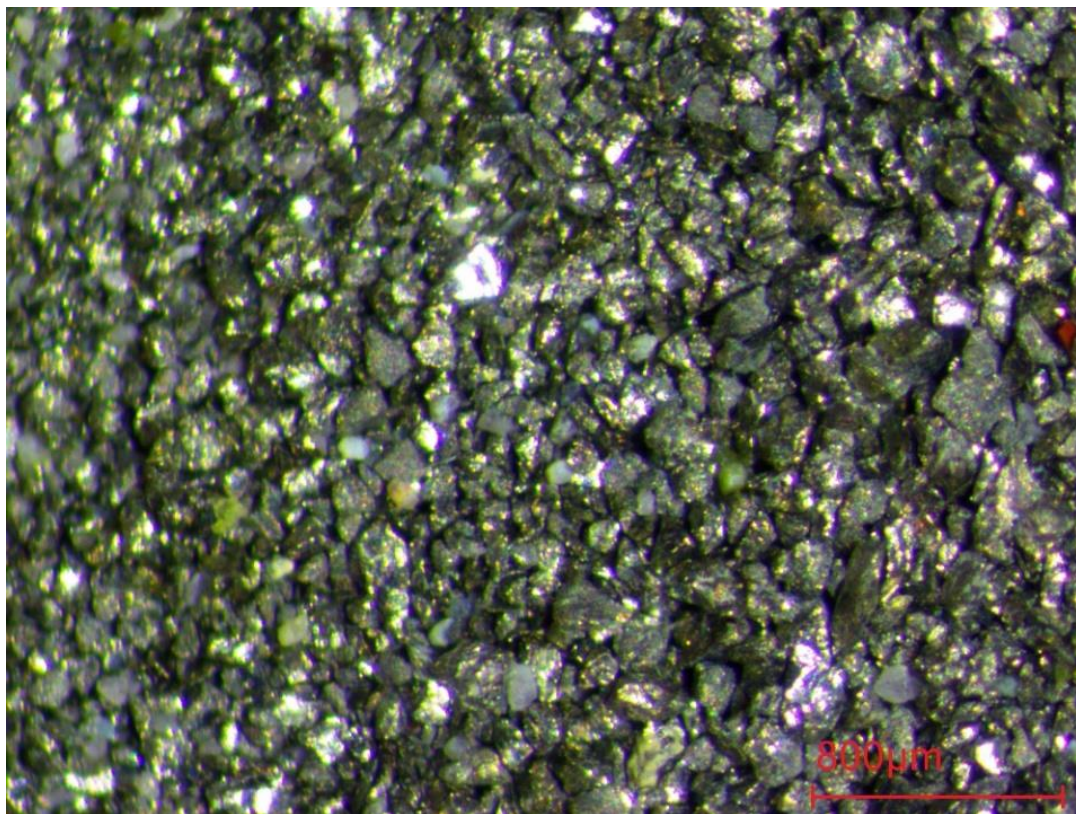


Figure 4.13 Representative optical microscopy images of the flotation tailings after bio-oxidation.

4.5 Overall Discussion

The combined results of the characterisation, bio-oxidation, thiourea-leaching, and post-experimental microscopy investigations provide an overall assessment of the treatment route investigated in this study. The bio-oxidation experiments produced measurable changes in pH and Eh and were accompanied by changes in the dissolved inventories of Fe, S, and Zn. These observations are consistent with elemental transfer to the liquid phase during treatment. However, the magnitude of the calculated apparent net elemental transfer varied considerably among the individual reactor experiments, demonstrating that the response of the flotation tailings was strongly dependent on the experimental conditions.

No direct proportional relationship was observed between the apparent transfer of Fe or S to the liquid phase during bio-oxidation and the subsequent apparent Au extraction obtained by thiourea leaching. Experiment 1 showed the highest normalised net Fe transfer whereas the highest apparent Au extraction was obtained from Sample 3, corresponding to the residue from Reactor Experiment 3. Similarly, Experiment 4 exhibited relatively high normalised S transfer but did not produce the highest subsequent Au extraction. These results show that bulk changes in dissolved Fe and S alone were not sufficient to predict the accessibility of Au during subsequent leaching. The diagnostic leaching results provide additional

mineralogical context for this behaviour. The Au deportment analysis showed that 27.8% of the Au occurred as a free-milling fraction, while 18.9% was associated with pyrite, 14.0% with sphalerite, 0.2% with chalcopyrite and 39.2% with quartz [42]. The combined sulphide-associated Au fraction was therefore approximately 33.1%. These results indicate that only part of the total Au inventory was directly associated with sulphide phases and could potentially benefit from sulphide oxidation. In contrast, the large quartz-associated fraction represents a mineralogical limitation that would not necessarily be overcome by bio-oxidation of the sulphide phases alone. Consequently, the relatively modest improvement in apparent Au extraction after bio-oxidation should not be interpreted solely as evidence of insufficient sulphide oxidation; the initial Au deportment of the flotation tailings also imposed an important constraint on the overall treatment response. The mineralogical location of Au, the particular sulphide phases affected during bio-oxidation, particle-scale heterogeneity, and possible formation of secondary surface products may also have influenced the final leaching response.

Comparison with the untreated flotation tailings provides a direct reference for evaluating the effect of the bio-oxidation pre-treatment. Direct thiourea leaching of the untreated flotation tailings (Sample 6) resulted in an apparent Au extraction of 18.5%. Samples 1, 2, and 3, obtained after stirred-reactor bio-oxidation, produced apparent Au extractions of 24.7%, 22.2%, and 27.2%, respectively. These values correspond to improvements of approximately 6.2, 3.7, and 8.7 percentage points relative to the untreated material. Although these results indicate that bio-oxidation improved subsequent Au extraction under some of the investigated conditions, the magnitude of the improvement remained modest and even the highest apparent extraction remained below 30%, indicating that the overall improvement in Au extraction was limited under the investigated conditions.

The combined GRM shake-flask residue (Sample 7) produced an apparent Au extraction of 19.8%, compared with 18.5% for the untreated flotation tailings. The difference of only 1.3 percentage points indicates that the combined shake-flask bio-oxidation treatment produced little improvement in subsequent thiourea leaching under the conditions applied. Importantly, the flotation tailings remained in contact with the bacterial culture in the shake flasks for a total of 104 days before the flask contents were combined and the resulting solid residue was subjected to thiourea leaching. Despite this prolonged contact period, the apparent Au extraction obtained from Sample 7 was not substantially different from those obtained from the reactor-derived residues and was only slightly higher than that obtained from the untreated flotation tailings. This observation indicates that prolonging the bacterial treatment to 104 days did not provide a corresponding improvement in thiourea-leachable Au under the conditions investigated. However, since pH and Eh were not continuously monitored throughout the entire 104-day period, the progression and extent of bio-oxidation during this prolonged contact period cannot be quantitatively established. Therefore, the result should

not be interpreted as evidence that oxidation ceased or remained constant, but rather that the prolonged shake-flask treatment did not translate into a substantial improvement in subsequent apparent Au extraction.

The paired tests performed using the bio-oxidised residue from Reactor Experiment 4 provided information on the influence of ferric-ion addition during thiourea leaching. Sample 4A, tested without Fe^{3+} addition, produced an apparent Au extraction of 13.6%, whereas Sample 4B, tested with Fe^{3+} addition, reached 19.8%. The increase of 6.2 percentage points indicates a limited positive effect of ferric-ion addition under the specific conditions of these paired experiments. However, the apparent extraction remained below 20% even with Fe^{3+} addition. Ferric-ion availability was therefore one factor influencing the thiourea-leaching response, but it was not sufficient to overcome the overall limitations affecting Au extraction.

The pyrite-rich concentrate used in Reactor Experiment 5 requires separate consideration because its feed composition differed from that of the bulk flotation tailings. The concentrate contained an initial Au grade of 1.09 ppm, which decreased to 0.84 ppm following thiourea leaching, corresponding to an apparent Au extraction of 22.9%. Although this value was comparable with those obtained for Samples 1 and 2, direct ranking against the flotation-tailings-based experiments is not appropriate because of the different feed composition, sulphide enrichment, and initial Au grade.

Overall, the thiourea-leaching results show that the bio-oxidation pre-treatment did not produce a uniform improvement in Au extraction. The most favourable result was obtained for Sample 3 at 27.2%, while the majority of the initial Au remained in the final solid residues in all tests. The relatively limited difference between the bio-oxidised samples and the untreated reference suggests that either the preceding bio-oxidation treatment did not sufficiently increase the accessibility of a large proportion of the Au, the subsequent thiourea-leaching conditions were not sufficiently favourable for its dissolution, or both effects contributed simultaneously.

Following the relatively low apparent Au extraction obtained during thiourea leaching, an additional optical microscopy investigation was undertaken to qualitatively compare the flotation tailings before and after bio-oxidation. Visible changes in particle appearance were observed after treatment; however, particles exhibiting relatively well-defined and apparently intact morphologies remained present in the bio-oxidised material. The microscopy observations therefore did not indicate complete physical alteration of the particle population during bio-oxidation.

The persistence of apparently intact particles is consistent with incomplete alteration of the sulphide-bearing material as one possible explanation for the limited improvement in subsequent Au extraction. If a proportion of the Au was associated with or encapsulated within sulphide minerals, incomplete oxidation of these phases could have restricted its

accessibility to the thiourea solution. This interpretation is also compatible with the absence of a direct relationship between bulk Fe or S transfer and subsequent Au extraction, since dissolution of part of the sulphide inventory does not necessarily imply effective alteration of the particular mineral domains containing Au. This interpretation should also be considered together with the diagnostic leaching results, which showed that a substantial proportion of the Au was associated with quartz rather than sulphide minerals [42]. Therefore, incomplete sulphide oxidation represents only one possible limitation, while the initial Au deportment itself also constrained the maximum benefit that could reasonably be expected from a sulphide-oxidation pretreatment.

Nevertheless, the optical microscopy observations cannot establish the mineralogical identity of the remaining particles, quantify the degree of pyrite or sulphide oxidation, or demonstrate that the residual Au remained encapsulated within sulphide minerals. Incomplete sulphide oxidation should therefore be regarded as a plausible contributing factor rather than a confirmed explanation for the limited Au extraction. The paired Experiment 4 results further demonstrate that the thiourea-leaching chemistry itself also affected the final outcome. Consequently, the relatively low Au extraction observed in this study most likely reflects a combination of limitations associated with the bio-oxidation pre-treatment, Au mineralogical occurrence, and the subsequent thiourea-leaching conditions.

Interpretation of the calculated Au extraction values is also subject to an important analytical limitation. Au was reported as N/D in the analysed thiourea PLS samples, and a complete solution-phase Au mass balance could therefore not be established. The quantitative assessment was based on differences between the initial and final solid-phase Au grades rather than directly measured dissolved Au. For this reason, the calculated values are reported as apparent Au extraction rather than absolute Au recovery. At the low Au concentrations investigated, analytical variability and incomplete solid mass balance may also influence the calculated percentages.

Taken together, the experimental results indicate that bio-oxidation produced measurable changes in the sulphide-bearing flotation tailings and provided a modest improvement in subsequent Au extraction under some conditions, particularly for Reactor Experiments 1–3. However, the treatment did not achieve consistently high Au extraction, and bio-oxidation alone was not sufficient to ensure effective subsequent thiourea leaching of the investigated flotation tailings. Further optimisation of both the biological pre-treatment and the thiourea-leaching stage, together with mineral-specific examination of the residual solids, would be required to identify the principal limitation controlling Au recovery.

4.6 Chapter Summary

This chapter presented the characterisation of the flotation tailings, the shake-flask and stirred-reactor bio-oxidation experiments, and the subsequent thiourea-leaching results. The characterisation results showed a fine-grained, sulphide-bearing material with increasing pyrite abundance toward the finer particle-size fractions. During bio-oxidation, changes in pH, Eh, and dissolved Fe, S, and Zn were observed, although the magnitude of these changes varied considerably among the individual experiments.

Thiourea leaching produced apparent Au extraction values between 13.6% and 27.2%, with only modest improvements over direct leaching of the untreated flotation tailings under the investigated conditions. The results showed that neither bulk elemental transfer during bio-oxidation nor ferric-ion addition during thiourea leaching alone was sufficient to explain the final Au extraction behaviour. The post-experimental optical microscopy comparison further suggested that incomplete alteration of the sulphide-bearing material may have contributed to the limited Au accessibility, although this could not be confirmed mineralogically. Because Au was reported as N/D in the analysed PLS samples, the calculated values were interpreted as apparent Au extraction rather than absolute Au recovery.

5. CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study investigated the treatment of sulphide-bearing flotation tailings through a combined bio-oxidation and thiourea-leaching route to determine whether biological oxidation of sulphide minerals could improve Au accessibility and subsequent extraction by acidic thiourea leaching.

Characterisation of the flotation tailings by particle-size analysis, XRD and SEM/EDS confirmed the presence of sulphide minerals, including pyrite, together with gangue phases. No liberated Au particles were observed by SEM/EDS; however, the mineralogical mode of Au occurrence could not be established from these observations because of the detection limitations of the technique.

Diagnostic leaching provided important information on Au deportment. The analysis indicated that 27.8% of the Au occurred as a free-milling fraction, while 18.9% was associated with pyrite, 14.0% with sphalerite, 0.2% with chalcopyrite and 39.2% with quartz [42]. The combined sulphide-associated Au fraction was approximately 33.1%. This distribution indicates that the potential direct benefit of bio-oxidation was principally associated with the sulphide-hosted fraction, while the substantial quartz-associated fraction represented an important mineralogical constraint on the combined treatment route.

The pH, Eh and dissolved-element trends observed during the monitored period of the shake-flask experiments were consistent with bio-oxidation activity under acidic conditions. Settling of the relatively dense sulphide particles occurred and required occasional manual resuspension. The flotation tailings remained in contact with the bacterial culture in the GRM shake flasks for a total of 104 days. After the flask contents were combined, the resulting residue produced an apparent Au extraction of 19.8%, compared with 18.5% for the untreated flotation tailings. Thus, the prolonged shake-flask treatment did not result in a substantial improvement in subsequent thiourea-leachable Au under the investigated conditions.

The stirred-reactor experiments demonstrated measurable transfer of Fe, S and Zn from the solid phase to the process solution, consistent with sulphide oxidation during biological treatment. Experiment 1 showed the highest calculated normalised net Fe transfer at approximately 238.60 g kg⁻¹, while Experiment 4 showed the highest normalised net S transfer at approximately 137.49 g kg⁻¹, followed closely by Experiment 5 at 135.20 g kg⁻¹. Experiment 5 also showed the highest normalised net Zn transfer and the highest recorded Eh value of the reactor programme. However, because the degree of direct analytical measurement and the mass-balance approach differed among the experiments, these values

should be regarded as semi-quantitative rather than as a strict ranking of sulphide-oxidation performance.

Increasing the reactor volume from 1 to 3 L did not produce a consistent improvement in normalised net elemental transfer or subsequent apparent Au extraction. Because reactor volume changed together with other operating variables, its independent effect could not be established. Similarly, the use of ground flotation tailings in Experiments 3 and 4 did not produce a consistent improvement in subsequent apparent Au extraction. Although Sample 3 produced the highest apparent extraction, the Experiment 4 residue did not show a similarly high result, indicating that grinding alone was not sufficient to determine the overall process performance.

Au was reported as N/D in all PLS samples collected during the thiourea-leaching tests. Consequently, time-dependent Au dissolution curves and a complete solution-phase Au mass balance could not be established. The quantitative evaluation was therefore based on differences between the initial solid Au grade and the Au grade remaining in the final residue. Because these calculations were not supported by quantifiable solution-phase Au concentrations or a fully closed solid mass balance, the resulting values were reported as apparent Au extraction.

For the flotation-tailings-based tests, the highest apparent Au extraction was obtained for Sample 3 at 27.2%, followed by Sample 1 at 24.7% and Sample 2 at 22.2%, compared with 18.5% for direct thiourea leaching of the untreated flotation tailings (Sample 6). Reactor bio-oxidation therefore produced modest improvements of approximately 3.7–8.7 percentage points for Samples 1–3. In contrast, the combined GRM shake-flask residue (Sample 7) produced 19.8%, corresponding to an improvement of only 1.3 percentage points relative to the untreated reference. Even the highest apparent extraction remained below 30%, indicating that most of the initial Au remained in the final residues.

The paired Sample 4 tests demonstrated a beneficial effect of ferric-ion addition under the investigated thiourea-leaching conditions. The Fe^{3+} -free test produced an apparent Au extraction of 13.6%, compared with 19.8% when ferric sulphate was added, corresponding to an increase of approximately 6.2 percentage points.

The pyrite-rich concentrate used in Experiment 5 contained 1.09 ppm Au initially and produced an apparent Au extraction of 22.9%. Although this result was comparable to Samples 1 and 2, the different feed composition and initial Au grade prevent direct ranking against the bulk flotation-tailings tests.

No direct proportional relationship was observed between calculated net Fe or S transfer during bio-oxidation and subsequent apparent Au extraction. Experiment 1 showed the highest normalised net Fe transfer, whereas Sample 3 produced the highest apparent Au

extraction. Experiment 4 showed the highest normalised net S transfer but did not produce the highest Au extraction. Bulk elemental transfer alone was therefore insufficient to predict subsequent apparent Au extraction, suggesting that mineralogical association, particle-scale heterogeneity, the location of altered sulphide phases and possible secondary surface products may also have influenced the response.

Optical microscopy showed visible changes in particle appearance after bio-oxidation, while relatively intact particle morphologies remained observable. These observations are consistent with incomplete alteration of the sulphide-bearing material as a possible contributor to the limited Au extraction. However, optical microscopy could neither quantify sulphide oxidation nor establish the mineralogical occurrence of residual Au. Incomplete sulphide oxidation should therefore be regarded as a plausible contributing factor rather than a confirmed cause.

Overall, bio-oxidation produced physicochemical and dissolved-element changes consistent with sulphide oxidation and modest improvements in subsequent apparent Au extraction for some reactor-treated samples. However, Au extraction remained limited under the investigated conditions, and the analytical uncertainty associated with the low Au grades prevented establishment of a fully closed Au balance. Further optimisation of both the biological pre-treatment and thiourea-leaching stages would therefore be required to achieve higher Au extraction. These conclusions are specific to the investigated flotation tailings and experimental conditions.

5.2 Recommendations for Future Work

Future studies should prioritise the establishment of a complete Au mass balance. In the present study, Au was reported as not detected (N/D) in the thiourea-leaching solutions analysed by ICP-OES. Therefore, the detection limit of the selected analytical method should be confirmed before testing, and a technique with sufficient sensitivity for the expected low dissolved Au concentrations should be employed. Suitable approaches may include preconcentration prior to analysis or the use of a more sensitive technique such as ICP-MS.

The dry masses of both the initial feed and final leach residues should be recorded accurately. This would allow Au extraction to be calculated using complete solid-phase inventories rather than grade differences alone.

Replicate fire-assay measurements should also be performed because the investigated Au grades were low and small analytical variations could strongly affect the calculated extraction percentages [43].

The bio-oxidation stage should be optimised with respect to solid loading, particle size, agitation, aeration and residence time. Improved reactor design may be required to prevent

settling of dense sulphide particles and ensure more uniform contact between the bacteria, oxygen and mineral surfaces.

Further bio-oxidation experiments should also investigate the WW2 bacterial culture, which was included in the preliminary shake-flask programme but was not evaluated through subsequent thiourea leaching in the present study. Comparative experiments using the GRM and WW2 cultures under identical operating conditions would help determine whether the bacterial culture influences sulphide oxidation and subsequent thiourea-leachable Au. These tests should include consistent monitoring of pH, Eh, dissolved Fe and S, together with Au analysis before and after treatment.

The effect of bio-oxidation duration should also be evaluated through controlled time-series experiments, with residues collected at defined intervals and subjected to identical thiourea-leaching conditions. This would help determine whether prolonged biological treatment provides any additional benefit in terms of subsequent Au extraction.

Future work should also examine whether continuous or staged air and oxygen supply can improve sulphide oxidation. Dissolved oxygen should be monitored directly, particularly in larger reactors where gas transfer may become limiting.

A broader range of thiourea concentrations should be tested. The influence of ferric-ion concentration should also be examined systematically, since the Sample 4 comparison indicated a positive effect of ferric sulphate addition, while excessive ferric ion may increase thiourea decomposition.

Thiourea consumption should be measured during leaching. This would help determine whether low Au extraction resulted from reagent degradation, adsorption onto mineral surfaces, oxidation by ferric ions or insufficient available thiourea.

The pH and Eh should be controlled through a standardised dosing procedure rather than intermittent manual correction. Automatic pH control and regular measurement of ferric and ferrous ion concentrations would provide a clearer understanding of the chemical conditions governing Au dissolution.

Additional tests should include comparison with a conventional cyanide-leaching reference under appropriate laboratory and safety conditions. Such a comparison would help distinguish between limitations arising from incomplete Au liberation during bio-oxidation and limitations associated specifically with the thiourea system.

The qualitative optical microscopy observations obtained in the present study indicated that relatively intact particle morphologies remained after bio-oxidation, raising the possibility of incomplete alteration of the sulphide-bearing material. Future work should therefore include detailed mineralogical comparison of the feed, bio-oxidised residues and

final thiourea-leached residues. XRD, SEM/EDS and, where available, automated mineralogy should be used to identify and quantify remaining sulphide phases, investigate secondary reaction products, and evaluate changes in mineral associations during treatment. Particular attention should be given to determining the mineralogical occurrence of the residual Au and whether it remains associated with incompletely oxidised sulphide phases. Such analyses would allow the qualitative optical microscopy observations obtained in the present study to be evaluated using mineral-specific evidence.

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