Sustainable gold leaching from waste printed circuit boards using biogenic thiosulfate produced by *Acidithiobacillus thiooxidans*

Abstract

This study investigated biogenic thiosulphate as an environmentally friendly alternative to cyanide for gold leaching from waste printed circuit boards. Biogenic thiosulphate, an unstable intermediate formed during sulphur oxidation by *Acidithiobacillus thiooxidans*, was stabilised by pH adjustment and use of inhibitors; the effects of sodium fluoride and sodium diethyldithiocarbamate were evaluated. The inhibitory effects of sodium diethyldithiocarbamate and sodium fluoride at concentrations up to 10^{-2} M on thiosulphate oxidation were not sufficiently strong, which was attributed to differences in metabolic pathways during bacterial growth. The highest thiosulphate concentration, 219 mg/L, was achieved without inhibitors at pH 6. The maximum gold leaching rate reached 10% after 3 days under conditions of 30 °C, 120 mg/L biogenic thiosulphate, 0.02 M Cu^{2+}, 1 M ammonia, and 1% pulp density. Ammonia and copper were added as stabiliser and catalyst, respectively. Kinetic and thermodynamic analyses supported these findings and guided the routes for biogenic thiosulphate production as a sustainable leaching agent for gold leaching from electronic waste. Overall, this study demonstrates that biogenic thiosulphate produced by *Acidithiobacillus thiooxidans* is a promising alternative to chemical thiosulphate for sustainable gold recovery from electronic waste, offering a viable route toward efficient and green microbial-based processing.

Keywords: Bioleaching, Electronic waste, Metal recycling, Precious metals

1. Introduction

The growing reliance on short-lived electrical devices has increased electronic waste (e-waste), with global e-waste production reaching 52 Mt in 2021 and projected to reach 75 Mt by 2030 (Esmaeili et al., 2022). However, only 10–15% of e-waste is recycled, and the rest ends up in landfills, causing environmental and health problems. (Arshadi and Yaghmaei, 2020). In 2019, the value of raw materials recovered from e-waste was about 57 billion USD, with printed circuit boards (PCBs) the most valuable component. PCBs contain 28 % of metals, including copper (10-20%), aluminium (5%), iron (7%), nickel (1-3%) and trace amounts of precious metals such as gold (20-250 ppm) and silver (200-3000 ppm), which makes them a promising

secondary source for metal recycling. According to data from the U.S. Geological Survey, China is the leading country in gold recovery (420 tons), followed by Australia and Russia. Thus, the growing demand for gold across sectors and the depletion of natural resources make the recovery of gold from alternative sources, such as PCBs, essential (Wang et al., 2021). Conventional metal recovery methods include pyrometallurgy, which relies on high-temperature processing is therefore energy-intensive and costly, and hydrometallurgy, which uses acidic or alkaline solutions to dissolve metals, generating toxic secondary wastes that contaminate the environment (Sadeghi et al., 2024). Gold has been extracted from ores by chemical cyanide for centuries. However, the environmental and safety concerns over the use of cyanide have gained attention toward thiosulphate as a non-toxic, low corrosive, and safe alternative. The dissolution of gold by thiosulphate is represented by Eq. (1) (Ilkhani and Aiouache, 2025):

$$4Au + 8S_2O_3^{2-} + O_2 + 2H_2O \rightarrow 4Au(S_2O_3)_2^{3-} + 4OH^- \qquad \Delta G° = -97.9\ \text{kJ/mol} \qquad (1)$$

In thiosulphate-based leaching, Cu^{2+} ion acts as an oxidising agent that increases the dissolution of gold and thiosulphate consumption at the same time. The consumption of thiosulphate can be controlled by adding ammonia to the solution, forming a cupric ammonia complex that reduces the interaction between Cu^{2+} ions and thiosulphate and increases the interaction between gold and thiosulphate (Ilkhani and Aiouache, 2025). Ammonia also prevents the formation of passivation layers on the gold surface and enhances the leaching kinetics. Camelino et al. (2015) reported a 70% gold leaching rate from waste PCBs using 0.08 to 0.12 M ammonium thiosulphate, 0.1 to 0.2 M ammonia, and 15 mM Cu^{2+} ions at pH 10.5 within 2 h. In another study, approximately 57% of gold was leached under the optimum conditions of 0.1 M ammonium thiosulphate, 40 mM Cu^{2+}, pulp density of 1%, pH 10-10.5, at room temperature and a stirring speed of 250 rpm for 8 h (Tripathi et al., 2012). However, the high consumption of thiosulphate and the formation of by-products such as sulphate, tetrathionate, and trithionate are the main challenges of this process, which can be mitigated by controlling key factors such as temperature, agitation speed, pH, Cu^{2+} ions and ammonia concentration (Ilkhani and Aiouache, 2025). Also, using additives such as ethylenediaminetetraacetic acid (EDTA), humic acid (HA), ethylenediamine (EDA), triethanolamine (TEA), ammonium alcohol polyvinyl phosphate, citric acid, carboxymethyl cellulose (CMC), and amino acids can help reduce thiosulphate consumption by decreasing the direct interaction between thiosulphate and Cu^{2+} ions (Breuer and Jeffrey, 2003). For example, Zhao et al. (2020) reported that adding TEA increased the gold leaching rate in a thiosulphate–copper–ammonia system by 50%. It

also reduced thiosulphate consumption by 10%. Thiosulphate has shown strong potential as a leaching agent for gold leaching from waste PCBs. Replacing the commercial thiosulphate with biogenic thiosulphate produced by microorganisms via bioleaching would be a breakthrough, as it would consume fewer toxic substances and less energy for thiosulphate synthesis. This approach would help develop an environmentally friendly and sustainable technology (McNeice et al., 2022a; Pourhossein and Mousavi, 2022). Bioleaching relies on microorganisms' ability to produce leaching agents as metabolites, allowing metals to dissolve from solid waste into the leaching solution. At present, Nevada Gold Mines utilises an industrial thiosulphate leaching process with treatment of sulphur and calcium hydroxide at 90 °C under high pressure (550 kPa) (Soleymani et al., 2021). Advancing a biologically based leaching process that operates under mild conditions, such as room temperature and atmospheric pressure, offers a promising and environmentally friendly alternative. Cyanobacteria and proteobacteria have been among the primary microbial groups known to produce thiosulphate. For instance, *Microcoleus chthonoplastes* produced up to 695 mg/L of thiosulphate by utilising light, sulphide, and hydrogen as energy sources, while relying on carbonate as a carbon source at 22 °C and pH 8 (de Wit and van Gemerden, 1987). *Methylophaga sulfidovorans* produced approximately 672 mg/L thiosulphate by utilising methanol or dimethyl sulphide (DMS) as carbon sources at a temperature ranging from 22 to 30 °C and alkaline conditions (pH > 7.5) (McNeice et al., 2022b). *Acidithiobacillus thiooxidans* can also produce thiosulphate as an intermediate metabolite. As thiosulphate is unstable under acidic conditions, it is stabilised by adjusting pH and adding inhibitors. Previous studies have reported that inhibitors such as potassium cyanide, sodium azide, 2-Heptyl-4-hydroxy-quino, sodium fluoride (NaF), sodium diethyldithiocarbamate (DDC), and 2-Heptyl-4-hydroxy-quino (CCCP) have the potential to reduce the activity of sulphur, thiosulphate, sulphite, and tetrathionate oxidising enzymes (Iwatsuka et al., 1962; Kamimura et al., 2005; Takakuwa, 1975).

The present study investigates biogenic thiosulphate produced by the bacterium *A. thiooxidans* as a safe and sustainable alternative to bio-cyanidation of gold leaching process from waste PCBs, with reduced toxicity risks. One of the main challenges of cyanidation is the need for complete removal of copper from the material, as even small amounts can interfere with the gold leaching process and increase reagent consumption. In thiosulphate leaching, a small amount of copper not only hinders the process, but also can act as a catalyst to enhance the

gold leaching process. The recovery of copper (~96%) as the first stage was reported in the previous research (Ilkhani et al., 2025).

The biochemical process, particularly from kinetic and thermodynamic perspectives, has not yet been sufficiently elaborated. This study discusses these aspects and examines why the bioprocess did not achieve a sufficient gold leaching rate comparable to the chemical leaching, indicating opportunities for further research. Although a few studies exist, the development of biogenic thiosulphate for gold leaching from e-waste remains limited (McNeice et al., 2022a; Pourhossein and Mousavi, 2022). From a biological perspective, the impact of inhibitors such as NaF and DDC on reducing thiosulphate oxidation during the growth of *A. thiooxidans* was evaluated. *Acidithiobacillus* species have a complex thiosulphate-oxidising system for rapid oxidation of thiosulphate and sulphur-transfer enzymes to convert oxidised thiosulphate into other sulphur compounds. The addition of inhibitors can help preserve it by reducing the activity of thiosulphate-oxidising enzymes. Among the range of inhibitors reported in the literature, NaF and DDC were selected in this study due to their relative safety, efficiency, and cost-effectiveness (Iwatsuka et al., 1962; Iwatsuka and Mori, 1960). The effects of leaching time, ammonia, and Cu^{2+} concentration are investigated, and the possible mechanism of biogenic thiosulphate leaching is discussed.

2. Methods and materials

2.1 Pre-processing of printed circuit boards

Fig. 1 illustrates the schematic diagram of the overall process used in this study. Waste computer PCBs supplied by ICT Reverse© were manually dismantled, cut into small pieces, and soaked overnight in a 5M NaOH solution to remove the protective coating. The mixture was then blended and sieved through a 200-mesh sieve to obtain a fine powder with an average particle size of approximately 78 μm. Following copper removal using biogenic ferric iron (Fe^{3+}) described in detail in (Ilkhani et al., 2025), the residual powder was collected, washed, dried, and prepared for gold leaching. The composition of the PCB powder before and after copper removal is shown in Table 1.

2.2 Bacterial culture conditions

The sulphur-oxidising bacterium *A. thiooxidans* (DSMZ 9463) was cultivated in a medium containing: $(NH_4)_2SO_4$ (2 g/L), $MgSO_4.7H_2O$ (0.25 g/L), K_2HPO_4 (0.1 g/L), KCl (0.1 g/L),

supplemented with 10 g powdered elemental sulphur as the main energy source in 1 L of deionised water. Sulphuric acid (40%) was used to adjust the medium pH to 3.5. The culture was incubated at 28 °C with a rotation speed of 140 rpm for 7-10 days. During incubation, the production of biogenic sulphate (g/L), pH, bacterial cell concentration (cells/mL), and the rate of sulphur oxidation to sulphate (%) were monitored daily.

2.3 Thiosulphate production

Incubation was carried out until the middle of the logarithmic growth phase. Then, the bacteria were transferred to a 2-litre stirred-tank reactor (STR) (Armfield Ltd, CEM MKII, United Kingdom) equipped with a temperature controller and heating tape to maintain an optimal temperature of 28 °C for bacterial activity. Aeration was provided at 1–2 L/min using an Interpet™ air pump to maintain sufficient oxygen for bacterial growth. The pH of the culture medium was adjusted to 6–7 by adding 0.1 N NaOH and was automatically maintained at that level using a pH controller. To study the impact of inhibitors, various concentrations of NaF and DDC at 10^{-4}, 10^{-3}, and 10^{-2} M were added to the media after pH adjustment. Samples were collected at specific times to measure biogenic thiosulphate (mg/L) and bacterial cell density (cells/mL).

2.4 Bioleaching of gold

Once the maximum thiosulphate concentration was reached, bacterial cells were removed using a 0.2 µm Corning® bottle-top vacuum filter, and the leaching experiments were conducted in a cell-free medium. Biogenic thiosulphate was used to leach gold from the residue powder at a pulp density of 1%. The effects of Cu^{2+} ions (0.02, 0.04, and 0.05 M) and ammonia (0.5 and 1 M) were investigated at 30 °C with a rotation speed of 160 rpm for 3 days. The gold concentration in the leachate was measured by inductively coupled plasma mass spectrometry (ICP-MS). pH and redox potential (Eh) were monitored daily. As a control experiment, chemical leaching was performed using commercial sodium thiosulphate at a concentration similar to that generated by bacteria under the best conditions.

2.5 Analytical method

Sulphate ion concentration was determined by adding 100 µL of culture sample to 2 mL of a buffer solution (30 g/L $MgCl_2 \cdot 6H_2O$; 5 g/L $CH_3COONa \cdot 3H_2O$; 1 g/L KNO_3; 20 mL CH_3COOH (99%) in 1 L deionised water, followed by the addition of 0.06 g $BaCl_2$ and made up to 10 mL. Then, the absorbance of the solution was measured at 420 nm using an Ultraviolet–visible

(UV–Vis) spectrometer (Evolution 220, Thermo Fisher Scientific, USA) (Rossum and Villarruz, 1961). Iodine solution (0.05 M) was titrated with samples until the colour changed from brown to pale yellow, then 2-3 mL of starch was added as an indicator, and the colour changed to blue instantly due to the presence of iodine. The titration was then continued until the solution became colourless, indicating the endpoint. pH was monitored and controlled by a pH controller (Bluelab Corporation Limited, New Zealand). The temperature was controlled using a temperature controller (RS PRO/RS Components Ltd, Corby, United Kingdom). An aquarium air pump (Airvolution AV3, Interpet Ltd, United Kingdom) was used to supply oxygen for bacterial growth in the reactor. The Eh was measured with an ORP meter (HI-991003, Hanna Instruments, United Kingdom). The metal content of powder was measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent 5100 VDV, Agilent Technologies Inc, Australia) and gold content in the leachate was measured using ICP-MS (Agilent 7700, Santa Clara, CA, USA). The metal leaching rate was calculated by the following Eq. (2):

$$Metal\ leaching\ (\%) = C \times V/M \times m \quad (2)$$

Where C is the concentration of leached metal (mg/L), V is the volume of leaching solution (L), m is the mass of PCB powder (g), and M is the metal content in the residue PCB powder (mg/g), respectively.

3. Results and discussions

3.1 Production of biogenic sulphate

Sulphur oxidation mechanism by the bacterium *A. thiooxidans* follows a series of reactions (Eqs. (3)-(8)) via cleavage of elemental sulphur to produce sulphate (SO_4^{2-}) as the final product. In bacterial systems, elemental sulphur is first adsorbed to the cell surface and then transported into the periplasmic space by outer membrane proteins (OMPs). After a series of biooxidation reactions, it is converted into sulphate ions and exported from the cell (Yang et al., 2019). During this process, some intermediate sulphur compounds, such as thiosulphate ($S_2O_3^{2-}$) and tetrathionate ($S_4O_6^{2-}$), are formed but converted to sulphate immediately due to their instability under acidic conditions by a series of enzymes and electron transport chain reactions (Eqs. (4)-(8)) (Naseri and Mousavi, 2024). The formation of these intermediates reflects the presence of multiple interconnected metabolic pathways, which allow *A. thiooxidans* to adapt to diverse

environmental conditions and use a wide range of sulphur compounds as energy sources. (Ibáñez et al., 2023).

$$S + \frac{3}{2}O_2 + H_2O \rightarrow H_2SO_4 \qquad \Delta G° = -507 \text{ kJ/mol} \qquad (3)$$

$$4S_2O_3^{2-} + O_2 + 4H^+ \rightarrow 2S_4O_6^{2-} + 2H_2O \qquad \Delta G° = -106\ kJ/mol \qquad (4)$$

$$S_4O_6^{2-} + H_2O \rightarrow S_2O_3^{2-} + S\ + SO_4^{2-} + 2H^+ \qquad \Delta G° = -178\ kJ/mol \qquad (5)$$

$$S_2O_3^{2-} + 0.5O_2 \rightarrow S + SO_4^{2-} \qquad \Delta G° = -750\ kJ/mol \qquad (6)$$

$$S_2O_3^{2-} + 2H^+ \rightarrow S + H_2SO_3 \qquad \Delta G° = 48\ kJ/mol \qquad (7)$$

$$2SO_3^{2-} + O_2 \rightarrow 2SO_4^{2-} \qquad \Delta G° = -516\ kJ/mol \qquad (8)$$

Figs. 2(a) and (b) show the production of biogenic sulphate ions and the rate of sulphur oxidation to sulphate ions at different sulphur concentrations (5–20 g/L), using an inoculation size of 2 % v/v. The results indicate that although a higher initial sulphur concentration increased sulphate ion production, the overall oxidation efficiency did not increase proportionally. At an initial concentration of 5 g/L, sulphur oxidation reached 63% after 10 days of incubation, yielding 9 g/L of sulphate. When the concentration was increased to 10 g/L, both sulphur oxidation efficiency and sulphate production increased significantly, with nearly 77% of the sulphur oxidised to sulphate (23 g/L). This trend suggests that 10 g/L provides the best balance between substrate availability and bacterial activity. However, further increasing the sulphur concentration beyond this point inhibited oxidation, as evidenced by the decrease in the oxidation efficiency to about 36% (18 g/L sulphate) at 20 g/L. This suggests that excess sulphur hindered bacterial metabolism, likely due to substrate inhibition, oxygen limitation, or both (Chen and Lin, 2001). In all samples, oxidation remained stable after 14 days. A similar trend was reported by Naseri and Mousavi (2024), who found that increasing the sulphur concentration to 20 g/L decreased sulphur oxidation and that sulphur oxidation remained constant after 10 days. Fig. 2(c) shows the pH variation at a sulphur concentration of 5-20 g/L over time. The pH drop over time demonstrated the bacterial activity and the acid production. The lowest pH was observed at a sulphur concentration of 15 g/L, with a pH of 0.68 after 14 days. The highest sulphate concentration (20 g/L) inhibited both bacterial growth and sulphate production. Fig. 2(d) illustrates the cell density (cells/mL) of *A. thiooxidans* at different elemental sulphur concentrations (5, 10, 15, and 20 g/L) over a period of 16 days. Cell density increased with time for all concentrations. During the initial 2–4 days, the bacteria were in a lag phase with minimal increases in cell density. From day 4 to day 11, the cell population

increased sharply, validating the logarithmic growth phase. The bacterial cell was slightly higher at 15 g/L sulphur between 7 and 11 days, owing to a sufficient energy source for bacterial growth. The maximum cell density of 1×10^8 cells/mL was obtained at an initial sulphur concentration of 10 g/L after 15 days of incubation. This growth pattern closely matched the maximum sulphate yield observed at the same concentration, confirming that microbial growth and sulphur oxidation are closely correlated under these conditions.

3.2 Production of biogenic thiosulphate

In sulphur-oxidising microorganisms such as *A. thiooxidans,* thiosulphate is the key intermediate in the metabolic cycle of sulphur to sulphate conversion, undergoing rapid oxidation to tetrathionate or sulphite, then to sulphate. In the periplasm of *A.thiooxidans*, there is a sulphur oxidising enzyme system (Sox) that oxidises thiosulphate, sulphide, sulphite, and elemental sulphur to sulphate by SoxXA, SoxB, and $Sox(CD)_2$ enzymes. Also, there is a thiosulphate oxidation pathway (tetrathionate intermediate thiosulphate oxidation (S_4I)) that consists of two enzymes: thiosulphate quinol oxidoreductase (TQO or DoxDA), which oxidises thiosulphate to tetrathionate and tetrathionate hydrolase (TetH or TTH), which hydrolyses tetrathionate to thiosulphate. Additionally, thiosulphate can be oxidised to tetrathionate by Thiosulphate Dehydrogenase (TSD) enzyme without the need for any cofactors (Yin et al., 2019). The pH of the periplasmic and cytoplasmic areas typically ranges from 2 to 3 and from 6 to 7, respectively. In *A. thiooxidans*, the generated thiosulphate in the periplasmic area quickly converts to the acid-stable tetrathionate. Studies on thiosulphate-grown *A. thiooxidans* have shown that the optimal pH for thiosulphate and tetrathionate oxidation is approximately 5.0 (Masau et al., 2001). In another study, thiosulphate oxidation with an optimum pH of 3 –5.5 was reported for *A. thiooxidans* (Kamimura et al., 2005). So, maintaining pH above 5 during the growth phase of bacteria can significantly reduce thiosulphate conversion by the sulphur oxygenase reductase (SOR) enzyme, which oxidises cytoplasmic elemental sulphur to thiosulphate, sulphite, and sulphide (Wang et al., 2019). In addition to pH adjustment, adding inhibitors can minimise the activity of thiosulphate-oxidising enzymes and other sulphur compounds. Eqs. (4)-(7) and (9)-(11) list reactions that can be inhibited by certain inhibitors. Thiosulphate production was investigated at different pH levels above 4, and the results showed no detectable thiosulphate formation within the pH range of 4–6 (data not shown). Consequently, thiosulphate production and the inhibitory effect of DDC and NaF at different concentrations were examined at pH 6. DDC directly inhibits by binding to and removing non-heme iron, which is essential for the activity of soluble components and redox enzymes in the

pathway. Removal of Fe disrupts iron-dependent electron carriers within enzymes such as the Sox system and the Heterodisulfide Reductase (Hdr)-like complex, blocking electron transfer from sulphur to the quinone pool and disrupting adenosine triphosphate (ATP) generation and sulphur oxidation activity. NaF inhibits the sulphur oxidation enzyme indirectly by interfering with phosphate metabolism and ATP-dependent reactions required for coupling sulphur oxidation to biosynthetic processes. Although sulphur oxidation is not directly affected, the inhibition of phosphate-ester metabolism prevents effective utilisation of the generated energy and reduces the overall functional output of the system (Iwatsuka et al., 1962; Iwatsuka and Mori, 1960). As illustrated in Fig. 3(a) and (c), the highest thiosulphate concentration without inhibitors was 219 mg/L. In all samples, thiosulphate levels were high during the first 3 days. Still, they then declined due to the high degradation rate and thiosulphate's instability over time, and concentrations remained below 100 mg/L with only small fluctuations. The inhibitor curves were quite similar to those without inhibitors, showing an insignificant effect of the inhibitors. As shown, at high inhibitor concentrations (10^{-2} M), the thiosulphate concentration was lower, not exceeding 112 mg/L and falling below 50 mg/L after a week. This can happen due to the toxic effect of high concentrations of NaF and DDC on bacterial function and metabolism (Pourhossein and Mousavi, 2023). The limited inhibitory effect of DDC and NaF on thiosulphate oxidation can be explained by the presence of multiple, parallel sulphur oxidation pathways in *A. thiooxidans*. Thiosulphate can be oxidised via the S4I pathway, the Sox system, and TSD-mediated oxidation, allowing for metabolic flexibility. Previous research indicates that *Acidithiobacillus* species can regulate these pathways in response to environmental conditions. This means that inhibiting one pathway does not block overall thiosulphate oxidation, as the metabolic flux can be redirected to alternative routes. This redundancy and adaptability in the sulphur oxidation pathway explain the weak inhibitory effect observed in the experiment (Camacho et al., 2020; Wang et al., 2019). Research on the inhibitory effects of NaF in *Acidithiobacillus* species indicates that fluoride interferes with bacterial growth and sulphur or iron oxidation only under particular conditions. For example, Ma et al. (2016) reported that fluoride inhibition in *A. ferrooxidans* is highly dependent on pH, energy source, and growth phase, resulting in significant inhibition under acidic conditions (~pH of 2). In another study, fluoride tolerance was assessed in pure and mixed cultures of various acidophilic bacteria, and *A. thiooxidans* demonstrated the highest fluoride tolerance (0.5 mM F^-), whereas *S. thermosulfidooxidans* was completely inhibited (Fritze and Hedrich, 2026). It means that *A. thiooxidans* was able to grow and maintain metabolic activity, even at high fluoride concentrations, with limited inhibitory effect. Fig. 3(b) and (d) illustrate the effect

of inhibitors on bacterial cell density (cells/mL) after pH adjustment. During the logarithmic growth phase, raising the pH did not significantly affect growth or cell concentration because the bacteria continued to consume and oxidise sulphur to compounds, such as thiosulphate (Masau et al., 2001). Cell density was slightly higher in the samples without the inhibitor, suggesting that the inhibitor had a weak negative effect on cell number and bacterial survival during the first week. After this period, the cell population increased further in all samples, particularly in the inhibitor-containing samples, as the bacteria adapted to the inhibitors over time.

Kinetic studies in acidophilic sulphur-oxidising bacteria have been interpreted using enzyme-like or Monod/Michaelis–Menten models. For example, resting-cell studies of *A. ferrooxidans* showed that the apparent half-saturation constant *(Km)* values, which define the substrate affinity for thiosulphate oxidation, ranged from approximately 1.2 to 25 mM and were strongly pH dependent, with higher affinity at higher pH and greater maximum oxidation rates at low pH. High substrate concentrations outside this range induced substrate inhibition, highlighting non-ideal kinetics under certain conditions (Eccleston and Kelly, 1978). Further pure culture experiments with *A. thiooxidans* quantified microbial thiosulphate oxidation have reached maximal oxidation rates of ~55 ± 3 mg/L h at optimal temperature (~30 °C) and pH (~4), and demonstrated that environmental factors such as nutrient availability and metal ion concentration significantly influence these rates (Silver, 1981). At the enzyme level, thiosulphate dehydrogenase has been purified from *A. thiooxidans* and reached a K_m value for thiosulphate of ~0.81 mM, which indicates relatively high substrate affinity within the periplasmic oxidation system (Nakamura et al., 2001). Here, the modelling of microbial thiosulphate consumption and intermediate oxidation was fitted to the Michaelis–Menten equation, with explicit dependence on pH, substrate concentration, and oxygen availability, as expressed by Eq. (12).

$$v = V_{max}.\ S_t/(K_m + S_t) \quad (12)$$

Where v is the rate of thiosulphate oxidation (e.g. mg/L h or mol/L s), V_{max} is the maximum oxidation rate and S_t is the thiosulphate concentration. The value of K_m ranges from 1–25 mM at the whole-cell level and ~0.81 mM at the enzyme level (thiosulphate dehydrogenase). The relationship between thiosulphate concentration and its consumption rate was evaluated using a Monod-type kinetic model (Fig. 3(e)). To quantitatively describe this behaviour, nonlinear regression and curve fitting were performed using MATLAB R2022a (MathWorks, Natick, MA, USA), which provided a good fit to the experimental data ($R^2 = 0.957$). V_{max} was estimated

at 74 mg/L h, while K_m was 262 mg/L, corresponding to approximately 2 mM. The obtained K_m value falls within the range reported for whole-cell thiosulphate oxidation by acidophilic sulphur-oxidising bacteria, indicating a moderate substrate affinity. These results confirm that thiosulphate oxidation by *A. thiooxidans* under the tested conditions follows classical Monod kinetics.

$$S_2O_3^{2-} + 3H_2O + O_2 \rightarrow 2H_2SO_3 + 2OH^- \quad (9)$$

$$S_2O_3^{2-} + 1\frac{1}{2}O_2 + H_2O \rightarrow H_2SO_3 + SO_4^{2-} \quad (10)$$

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ \quad (11)$$

For comparison, the inhibitory effects of selected compounds on sulphur and thiosulphate oxidation during the *A. thiooxidans* growth cycle were summarised in Table 2. Carboxylic acids such as acetic acid, formic acid, and propionic acid exhibited strong inhibition at 10 mM. Iwatsuka and Mori (1960) have shown that the inhibitory rate of these acids depends on pH. No inhibition was observed at pH 6, but it reached its highest value for succinic acid and fumaric acid at 10 mM and pH 4, reaching 82% and 100%, respectively. Other compounds, such as potassium cyanide and sodium azide, were more effective at pH levels above 5, resulting in complete inhibition. Sodium diethyldithiocarbamate was effective at pH 6 but only slightly effective at pH 4, likely due to its decomposition under acidic conditions. The phosphorus compounds ATP, Adenosine diphosphate (ADP), adenosine monophosphate (AMP), and pyrophosphate were effective inhibitors of sulphur and thiosulphate oxidation. (Takakuwa, 1975). Several components, including arsenate, hydroxylamine, dipyridyl, and magnesium chloride, showed no inhibitory effect on the oxidation of sulphur or thiosulphate.

3.3 Thiosulphate leaching of gold

3.3.1 Ammonia on bio-thiosulphate leaching of gold

Thiosulphate leaching of gold has been demonstrated to be most effective when catalysed by Cu^{2+} as an oxidant in the presence of ammonia, which performs several key functions. For example, it stabilises the Cu^{2+}–ammonia complexes, minimises the decomposition of thiosulphate into polythionates, maintains the solution pH within an optimal range, and enhances leaching kinetics (Ilkhani Aiouache, 2025). Fig. 4(a) illustrates the effect of ammonia (0.5-1 M) on the leaching rate of gold using biogenic thiosulphate of 120 mg/L, Cu^{2+} 0.04 M from residue PCB powder at a pulp density of 1%. The results demonstrate that increasing the ammonia concentration from 0.5 M to 1 M had a slight effect on gold leaching, with a

maximum gold leaching rate of 9%. The active oxidising species in this system was confirmed to be the tetraammine–cupric complex, $Cu(NH_3)_4^{2+}$, which facilitated leaching of gold as $Au(S_2O_3)_2^{3-}$ complex, Eqs. (13)-(17) (Jeon et al., 2020). Oxygen was essential for maintaining a continuous oxidation process to enhance the process. During bio-thiosulphate leaching, $Cu(NH_3)_4^{2+}$ would be reduced to Cu^+ and stabilised as $Cu(S_2O_3)_3^{5-}$ Eq. (17). Oxygen oxidises Cu^+ to Cu^{2+}, thereby regenerating the active $Cu(NH_3)_4^{2+}$ species again. This regeneration step could increase the efficiency of gold leaching from residue PCB (Dong et al., 2019). At ammonia concentrations below 0.5 M, Cu^{2+} ions react mainly with thiosulphate, reducing the availability of ($Cu(NH_3)_4^{2+}$) to oxidise gold. During this reaction, tetrathionate was formed and gradually disproportionated to trithionate and thiosulphate (Eqs. (14)-(15)). Ammonia concentrations exceeding 1 M reduce the leaching rate due to increased hydroxide ion (OH^-) concentration. Ammonia could weakly interact with gold to form the transient $Au(NH_3)_2^+$ complex, but high ammonia concentrations shift the reaction equilibrium, Eqs. (18)-(19) toward less favourable conditions, promoting side reactions and destabilising the $Au(S_2O_3)_2^{3-}$ complex. These findings align with previous studies (Jeon et al., 2020), which indicate that moderate ammonia levels enhance gold leaching, whereas concentrations above 1 M inhibit the process. Fig. 4(b) shows that the pH varied between 10 and 12 because thiosulphate tends to decompose quickly at pH below 9 due to the low ammonia concentration. This is consistent with other studies reporting that a pH of 10 is favourable for enhancing thiosulphate stability and the formation of stable copper–ammine–thiosulphate complexes. Zhang and Senanayake (2016) also reported that Eh must be maintained between 0.1–0.5 V to form stable $Au(S_2O_3)_2^{3-}$ species, because at potentials below 0.1 V, gold leaching will not occur and copper will precipitate as a copper sulphide, and at potentials above 1 V, $Au(NH_3)_4^{3+}$ species will form (Fig. 4(c)). Therefore, maintaining an ammonia concentration in the range of 0.5–1.0 M ensures balanced complex stability, which controls pH and oxidation efficiency.

$$2Cu + 8NH_3 + 2H_2O + O_2 \rightarrow 2Cu(NH_3)_4^{2+} + 4OH^- \quad (13)$$

$$2Cu(NH_3)_4^{2+} + 8S_2O_3^{2-} \rightarrow 2Cu(S_2O_3)_3^{5-} + 8NH_3 + S_4O_6^{2-} \quad (14)$$

$$4S_4O_6^{2-} + 6OH^- \rightarrow 5S_2O_3^{2-} + 2S_3O_6^{2-} + 3H_2O \quad (15)$$

$$Au + 5S_2O_3^{2-} + Cu(NH_3)_4^{2+} \rightarrow Au(S_2O_3)_2^{3-} + 4NH_3 + Cu(S_2O_3)_3^{5-} \quad (16)$$

$$4Cu(S_2O_3)_3^{5-} + 16NH_3 + 2H_2O + O_2 \rightarrow 4Cu(NH_3)_4^{2+} + 4OH^- + 12S_2O_3^{2-} \quad (17)$$

$$Au + Cu(NH_3)_4^{2+} \rightarrow Au(NH_3)_2^+ + Cu(NH_3)_2^+ \quad (18)$$

$$Au^{+} + 2NH_3 \rightarrow Au(NH_3)_2^{+} \quad (19)$$

$$Au(NH_3)_2^{+} + 2S_2O_3^{2-} \rightarrow Au(S_2O_3)_2^{3-} + 2NH_3 \quad (20)$$

3.3.2 Cu^{2+} on bio-thiosulphate leaching of gold

The effect of Cu^{2+} concentration (0.02–0.05 M) on gold leaching was determined under the conditions of 120 mg/L biogenic thiosulphate, 1 M ammonia, and 1% pulp density (Fig. 5(a)). The leaching efficiency was slightly higher at a Cu^{2+} concentration of 0.02 M, reaching 10%, but then decreased as the copper concentration increased. Similar behaviour has been reported in previous studies, in which high Cu^{2+} concentrations negatively affected gold leaching. The reason is that higher Cu^{2+} concentrations narrowed the stability domains of soluble complexes such as $Cu(NH_3)_4^{2+}$ and $Cu(S_2O_3)_3^{5-}$ and promoted the formation of less soluble species, including CuO, Cu_2O, and Cu_2S, thereby hindering the catalytic role of Cu^{2+} in gold leaching (Sitando et al., 2020; Zhang and Senanayake, 2016). Moreover, higher concentrations of Cu^{2+} can lead to the degradation of thiosulphate into by-products, such as tetrathionate, trithionate, sulphate, and copper sulphide (Eqs. (21)-(25)). Based on the findings of Aylmore and Muir (2001), pH values higher than 11 should be avoided to prevent the formation of less soluble species. The pH variation during leaching, shown in Fig. 5(b), indicates elevated pH levels during the first two days, likely due to the formation of copper oxides. The Eh variation shown in Fig. 5(c) indicates a relatively low oxidation potential for gold oxidation compared with the higher Eh values reported in previous studies (Zhang and Senanayake, 2016). A control experiment using sodium thiosulphate was conducted under the favoured conditions, which generated 120 mg/L sodium thiosulphate at 0.02 M Cu^{2+}, 1 M ammonia, and a pulp density of 1%. The results showed that chemical leaching was slightly higher than bioleaching, which can be attributed to the use of a pure and stable chemical reagent in the control system. Biogenic thiosulphate is produced through complex microbial sulphur-oxidising pathways, which can generate additional intermediate species such as sulphite and tetrathionate. These by-products interfere with the leaching process and reduce its efficiency. Liquid thiosulphate in the biogenic system was less stable than commercially available solid sodium thiosulphate. Even after bacterial removal, the solution pH decreased rapidly, promoting thiosulphate decomposition. These findings are consistent with previous studies by Pourhossein and Mousavi (2022), who reported that although no significant difference was observed between bioleaching and chemical thiosulphate leaching, the chemical leaching rate was slightly higher than the biogenic thiosulphate leaching. Nadi et al., (2025) reported significant differences in the leaching of rare

earth elements praseodymium and neodymium when comparing chemical sulphuric acid with biologically sulphuric acid produced by *A. thiooxidans*. Chemical leaching was more effective, achieving 80% neodymium recovery versus 28% for bioleaching, which likely reflects the combined acidic and enzymatic mechanisms influencing bioleaching efficiency.

$$2Cu^{2+} + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2Cu^{+} \quad (21)$$

$$4S_4O_6^{2-} + 6OH^{-} \rightarrow 5S_2O_3^{2-} + 2S_3O_6^{2-} + 3H_2O \quad (22)$$

$$8Cu^{2+} + 3S_2O_3^{2-} + 6OH^{-} \rightarrow 8Cu^{+} + 2S_3O_6^{2-} + 3H_2O \quad (23)$$

$$2Cu(NH_3)_4^{2+} + 6S_2O_3^{2-} \rightarrow 2Cu(S_2O_3)_2^{3-} + S_4O_6^{2-} + 8NH_3 \quad (24)$$

$$Cu^{2+} + S_2O_3^{2-} + H_2O \rightarrow CuS + SO_4^{2-} + 2H^{+} \quad (25)$$

A. thiooxidans has been the most frequently examined organism, producing between 120 and 500 mg/L thiosulphate depending on conditions and the use of inhibitors. For example, Pourhossein and Mousavi (2022) reported that *A. thiooxidans* generated up to 120 mg/L of thiosulphate after pH adjustment above 6 using 0.1 N NaOH. They also investigated the impact of potassium cyanide and sodium azide on thiosulphate oxidation. They found that adding approximately 3 mg/L of potassium cyanide and sodium azide increased thiosulphate concentrations to 350 and 500 mg/L, respectively, resulting in gold leaching rates of 31% and 56% from PCBs. These inhibitors disrupt key sulphur-oxidising enzymes and the electron transport chain through cytochrome oxidase, blocking the conversion of thiosulphate into sulphate and leading to its accumulation at higher concentrations (Pourhossein and Mousavi, 2023). Although the current study produced a comparable amount of thiosulphate, the inhibitors did not significantly affect thiosulphate oxidation during bacterial growth. This study aimed to evaluate safer, non-toxic inhibitors reported in previous research to support the development of a more sustainable bioprocess. Even though earlier studies indicated that 1 mM DDC and NaF could completely inhibit sulphur-oxidising activity, their effects were negligible under the conditions examined (Iwatsuka et al., 1962; Iwatsuka and Mori, 1960).

Previous studies have investigated the bioleaching of gold from e-waste using various biogenic leaching agents by different bacterial species under a range of operating conditions, generally reporting low leaching rates. For example, Ruan et al., (2014) reported a gold leaching rate of approximately 8% using *C. violaceum*, a commonly used cyanide-producing microorganism, at ambient temperature and a pulp density below 2% after 70 h. Similarly, Sahni et al., (2016) employed the same bacterium for gold leaching from SIM card waste and achieved less than

1% leaching after 7 days. Alternative non-cyanide leaching agents have also shown limited performance. Kudpeng et al., (2020) used triiodide-producing bacteria named *Roseovarius tolerans* and *Roseovarius mucosus*, resulting in a leaching rate of around 1%. Although the leaching rate in the present study remains modest, it is comparable to or higher than that of previously reported bioleaching systems, highlighting the potential of biogenic thiosulphate as an alternative technique. Biogenic thiosulphate is still in its early stages, with only a limited number of studies investigating its effectiveness and further optimisation is required to improve its efficiency.

3.4 Thermodynamic analysis

The Eh-pH diagram was generated using HSC 10 software (Metso Outotec, Finland) to validate the location of the thermodynamic stability region of different species and help understand the experimental laboratory results, since the Eh–pH stability range of a species is strongly influenced by the concentrations of thiosulphate, ammonia, and Cu^{2+}/Cu^{+}. The Eh-pH diagram for Cu-NH_3-$S_2O_3^{2-}$-H_2O system (Fig. 6(a)) indicates that Eh affects the equilibrium boundary between Cu^{+} and Cu^{2+} ions. Cu^{2+} ammonia complex dominates at high Eh and pH, whereas Cu^{+} thiosulphate complex is relevant at low Eh and pH (Aylmore and Muir, 2001). At low potentials and with limited oxidants, thiosulphate decomposes to form copper sulphide precipitates. Under these conditions, gold co-precipitates with sulphides, resulting in a decrease in the leaching rate (Wan and LeVier, 2003). A narrow stability region for Cu^{2+} exists at pH values below 5 and Eh above 0.5 V, whereas copper oxides tend to form at approximately neutral pH when the Eh exceeds 0.02 V. The Eh-pH diagram for the quaternary Au-NH_3-$S_2O_3^{2-}$-H_2O system (Fig. 6(b)) shows that the $Au(S_2O_3)_2^{3-}$ complex is predominant at pH values below 11 and an Eh range of approximately 0.4-1.25 V, which is consistent with the previous reports (Chen et al., 2024; Liu et al., 2018). Under certain conditions, gold may also exist in solution as $Au(NH_3)_2^{+}$ over a relatively wide pH range when the Eh exceeds about 0.5 V (Wan and LeVier, 2003). This observation is consistent with the experimental data presented in Fig. 6(b) and (c), where the system operated within a pH range of 10.5–11.5 and an Eh range of 0–0.15 V. The Eh–pH diagram indicates that Eqs (18) and (19) were the predominant in the chemical mechanism of the bioleaching process, which explains the low gold leaching rate. Therefore, to effectively leach gold from the powder, the Eh should be increased to values above 0.4 V. The Eh-pH diagram for the S-H_2O system (Fig. 6(c)) is shown to demonstrate the various stable and metastable sulphur-oxygen species. Looking from a thermodynamic perspective, allyl hydrosulphide (HS^{-}) and dithionate ($S_2O_6^{2-}$) are more stable across a wide

480 Eh-pH range than other species. $S_2O_3^{2-}$ is stable within a relatively narrow region of pH values
481 of 4.5–14 and Eh ranging from −0.6 V to 0.02 V. Once the gold thiosulphate complex is formed,
482 it becomes stable over a larger Eh-pH range as observed in Fig. 6(b). Thiosulphate oxidises to
483 $S_4O_6^{2-}$ between pH 0–5, otherwise it oxidises to other sulphur species, such as $S_2O_6^{2-}$. However,
484 the decomposition mechanism of $S_2O_3^{2-}$ is complex and remains unclear (Muir and Aylmore,
485 2004).

486

487 ## 4. Conclusion

488 This study investigated a sustainable method for gold leaching from residual printed circuit
489 boards using bacteria to produce biogenic thiosulphate as an alternative leaching agent. The
490 achieved gold recovery was discussed in terms of limited thiosulphate accumulation, rapid
491 microbial and chemical thiosulphate degradation, and Eh for the formation of a stable gold-
492 thiosulphate complex.

493 During bacterial growth, adjusting the pH to around 6-7 enabled the production of 219 mg/L
494 thiosulphate within 2 days. Using inhibitors to reduce the thiosulphate oxidation showed
495 limited effectiveness, likely due to multiple parallel metabolic pathways in the bacteria.
496 Biogenic thiosulphate gold leaching in the presence of Cu^{2+} (0.02 M) as a catalyst and 1 M
497 ammonia as a stabiliser resulted in 10% leaching using 120 mg/L thiosulphate at 30 °C and a
498 pulp density of 1% after 3 days. The thermodynamic analysis explained the low gold leaching
499 rate. The use of biogenic thiosulphate proved to be a practical alternative to chemical
500 thiosulphate, opening the door for further exploration of other thiosulphate-generating
501 microorganisms for an efficient and sustainable metal recovery process.

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503 ## References

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