

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

3
4 Abstract

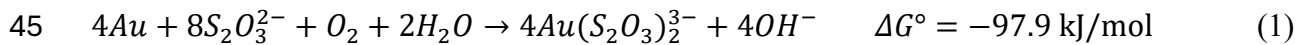
5 This study investigated biogenic thiosulphate as an environmentally friendly alternative to
6 cyanide for gold leaching from waste printed circuit boards. Biogenic thiosulphate, an unstable
7 intermediate formed during sulphur oxidation by *Acidithiobacillus thiooxidans*, was stabilised
8 by pH adjustment and use of inhibitors; the effects of sodium fluoride and sodium
9 diethyldithiocarbamate were evaluated. The inhibitory effects of sodium
10 diethyldithiocarbamate and sodium fluoride at concentrations up to 10^{-2} M on thiosulphate
11 oxidation were not sufficiently strong, which was attributed to differences in metabolic
12 pathways during bacterial growth. The highest thiosulphate concentration, 219 mg/L, was
13 achieved without inhibitors at pH 6. The maximum gold leaching rate reached 10% after 3 days
14 under conditions of 30 °C, 120 mg/L biogenic thiosulphate, 0.02 M Cu^{2+} , 1 M ammonia, and
15 1% pulp density. Ammonia and copper were added as stabiliser and catalyst, respectively.
16 Kinetic and thermodynamic analyses supported these findings and guided the routes for
17 biogenic thiosulphate production as a sustainable leaching agent for gold leaching from
18 electronic waste. Overall, this study demonstrates that biogenic thiosulphate produced by
19 *Acidithiobacillus thiooxidans* is a promising alternative to chemical thiosulphate for
20 sustainable gold recovery from electronic waste, offering a viable route toward efficient and
21 green microbial-based processing.

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

23
24 1. Introduction

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

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



46 In thiosulphate-based leaching, Cu^{2+} ion acts as an oxidising agent that increases the dissolution
47 of gold and thiosulphate consumption at the same time. The consumption of thiosulphate can
48 be controlled by adding ammonia to the solution, forming a cupric ammonia complex that
49 reduces the interaction between Cu^{2+} ions and thiosulphate and increases the interaction
50 between gold and thiosulphate (Ilkhani and Aiouache, 2025). Ammonia also prevents the
51 formation of passivation layers on the gold surface and enhances the leaching kinetics.
52 Camelino et al. (2015) reported a 70% gold leaching rate from waste PCBs using 0.08 to 0.12
53 M ammonium thiosulphate, 0.1 to 0.2 M ammonia, and 15 mM Cu^{2+} ions at pH 10.5 within 2
54 h. In another study, approximately 57% of gold was leached under the optimum conditions of
55 0.1 M ammonium thiosulphate, 40 mM Cu^{2+} , pulp density of 1%, pH 10-10.5, at room
56 temperature and a stirring speed of 250 rpm for 8 h (Tripathi et al., 2012). However, the high
57 consumption of thiosulphate and the formation of by-products such as sulphate, tetrathionate,
58 and trithionate are the main challenges of this process, which can be mitigated by controlling
59 key factors such as temperature, agitation speed, pH, Cu^{2+} ions and ammonia concentration
60 (Ilkhani and Aiouache, 2025). Also, using additives such as ethylenediaminetetraacetic acid
61 (EDTA), humic acid (HA), ethylenediamine (EDA), triethanolamine (TEA), ammonium
62 alcohol polyvinyl phosphate, citric acid, carboxymethyl cellulose (CMC), and amino acids can
63 help reduce thiosulphate consumption by decreasing the direct interaction between thiosulphate
64 and Cu^{2+} ions (Breuer and Jeffrey, 2003). For example, Zhao et al. (2020) reported that adding
65 TEA increased the gold leaching rate in a thiosulphate–copper–ammonia system by 50%. It

66 also reduced thiosulphate consumption by 10%. Thiosulphate has shown strong potential as a
67 leaching agent for gold leaching from waste PCBs. Replacing the commercial thiosulphate with
68 biogenic thiosulphate produced by microorganisms via bioleaching would be a breakthrough,
69 as it would consume fewer toxic substances and less energy for thiosulphate synthesis. This
70 approach would help develop an environmentally friendly and sustainable technology
71 (McNeice et al., 2022a; Pourhossein and Mousavi, 2022). Bioleaching relies on
72 microorganisms' ability to produce leaching agents as metabolites, allowing metals to dissolve
73 from solid waste into the leaching solution. At present, Nevada Gold Mines utilises an
74 industrial thiosulphate leaching process with treatment of sulphur and calcium hydroxide at
75 90 °C under high pressure (550 kPa) (Soleymani et al., 2021). Advancing a biologically based
76 leaching process that operates under mild conditions, such as room temperature and
77 atmospheric pressure, offers a promising and environmentally friendly alternative.
78 Cyanobacteria and proteobacteria have been among the primary microbial groups known to
79 produce thiosulphate. For instance, *Microcoleus chthonoplastes* produced up to 695 mg/L of
80 thiosulphate by utilising light, sulphide, and hydrogen as energy sources, while relying on
81 carbonate as a carbon source at 22 °C and pH 8 (de Wit and van Gemerden, 1987).
82 *Methylophaga sulfidovorans* produced approximately 672 mg/L thiosulphate by utilising
83 methanol or dimethyl sulphide (DMS) as carbon sources at a temperature ranging from 22 to
84 30 °C and alkaline conditions (pH > 7.5) (McNeice et al., 2022b). *Acidithiobacillus thiooxidans*
85 can also produce thiosulphate as an intermediate metabolite. As thiosulphate is unstable under
86 acidic conditions, it is stabilised by adjusting pH and adding inhibitors. Previous studies have
87 reported that inhibitors such as potassium cyanide, sodium azide, 2-Heptyl-4-hydroxy-quinoline
88 sodium fluoride (NaF), sodium diethyldithiocarbamate (DDC), and 2-Heptyl-4-hydroxy-quinoline
89 (CCCP) have the potential to reduce the activity of sulphur, thiosulphate, sulphite, and
90 tetrathionate oxidising enzymes (Iwatsuka et al., 1962; Kamimura et al., 2005; Takakuwa,
91 1975).

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

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

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

115

116 2. Methods and materials

117 2.1 Pre-processing of printed circuit boards

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

126 2.2 Bacterial culture conditions

127 The sulphur-oxidising bacterium *A. thiooxidans* (DSMZ 9463) was cultivated in a medium
128 containing: $(\text{NH}_4)_2\text{SO}_4$ (2 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.25 g/L), K_2HPO_4 (0.1 g/L), KCl (0.1 g/L),

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

134 2.3 Thiosulphate production

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

145 2.4 Bioleaching of gold

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

155 2.5 Analytical method

156 Sulphate ion concentration was determined by adding 100 µL of culture sample to 2 mL of a
157 buffer solution (30 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 5 g/L $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$; 1 g/L KNO_3 ; 20 mL CH_3COOH
158 (99%) in 1 L deionised water, followed by the addition of 0.06 g BaCl_2 and made up to 10 mL.
159 Then, the absorbance of the solution was measured at 420 nm using an Ultraviolet–visible

160 (UV–Vis) spectrometer (Evolution 220, Thermo Fisher Scientific, USA) (Rossum and
161 Villarruz, 1961). Iodine solution (0.05 M) was titrated with samples until the colour changed
162 from brown to pale yellow, then 2-3 mL of starch was added as an indicator, and the colour
163 changed to blue instantly due to the presence of iodine. The titration was then continued until
164 the solution became colourless, indicating the endpoint. pH was monitored and controlled by a
165 pH controller (Bluelab Corporation Limited, New Zealand). The temperature was controlled
166 using a temperature controller (RS PRO/RS Components Ltd, Corby, United Kingdom). An
167 aquarium air pump (Airvolution AV3, Interpet Ltd, United Kingdom) was used to supply
168 oxygen for bacterial growth in the reactor. The Eh was measured with an ORP meter (HI-
169 991003, Hanna Instruments, United Kingdom). The metal content of powder was measured by
170 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent 5100 VDV,
171 Agilent Technologies Inc, Australia) and gold content in the leachate was measured using ICP-
172 MS (Agilent 7700, Santa Clara, CA, USA). The metal leaching rate was calculated by the
173 following Eq. (2):

$$174 \text{ Metal leaching (\%)} = C \times V/M \times m \quad (2)$$

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

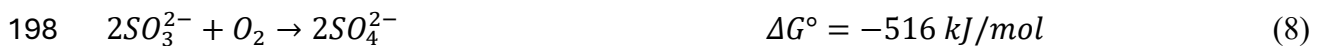
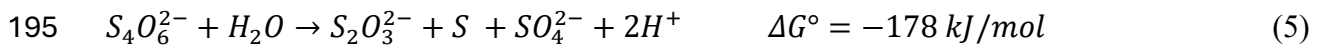
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179 3. Results and discussions

180 3.1 Production of biogenic sulphate

181 Sulphur oxidation mechanism by the bacterium *A. thiooxidans* follows a series of reactions
182 (Eqs. (3)-(8)) via cleavage of elemental sulphur to produce sulphate (SO_4^{2-}) as the final product.
183 In bacterial systems, elemental sulphur is first adsorbed to the cell surface and then transported
184 into the periplasmic space by outer membrane proteins (OMPs). After a series of biooxidation
185 reactions, it is converted into sulphate ions and exported from the cell (Yang et al., 2019).
186 During this process, some intermediate sulphur compounds, such as thiosulphate ($\text{S}_2\text{O}_3^{2-}$) and
187 tetrathionate ($\text{S}_4\text{O}_6^{2-}$), are formed but converted to sulphate immediately due to their instability
188 under acidic conditions by a series of enzymes and electron transport chain reactions (Eqs. (4)-
189 (8)) (Naseri and Mousavi, 2024). The formation of these intermediates reflects the presence of
190 multiple interconnected metabolic pathways, which allow *A. thiooxidans* to adapt to diverse

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



199 Figs. 2(a) and (b) show the production of biogenic sulphate ions and the rate of sulphur
200 oxidation to sulphate ions at different sulphur concentrations (5–20 g/L), using an inoculation
201 size of 2 % v/v. The results indicate that although a higher initial sulphur concentration
202 increased sulphate ion production, the overall oxidation efficiency did not increase
203 proportionally. At an initial concentration of 5 g/L, sulphur oxidation reached 63% after 10
204 days of incubation, yielding 9 g/L of sulphate. When the concentration was increased to 10 g/L,
205 both sulphur oxidation efficiency and sulphate production increased significantly, with nearly
206 77% of the sulphur oxidised to sulphate (23 g/L). This trend suggests that 10 g/L provides the
207 best balance between substrate availability and bacterial activity. However, further increasing
208 the sulphur concentration beyond this point inhibited oxidation, as evidenced by the decrease
209 in the oxidation efficiency to about 36% (18 g/L sulphate) at 20 g/L. This suggests that excess
210 sulphur hindered bacterial metabolism, likely due to substrate inhibition, oxygen limitation, or
211 both (Chen and Lin, 2001). In all samples, oxidation remained stable after 14 days. A similar
212 trend was reported by Naseri and Mousavi (2024), who found that increasing the sulphur
213 concentration to 20 g/L decreased sulphur oxidation and that sulphur oxidation remained
214 constant after 10 days. Fig. 2(c) shows the pH variation at a sulphur concentration of 5-20 g/L
215 over time. The pH drop over time demonstrated the bacterial activity and the acid production.
216 The lowest pH was observed at a sulphur concentration of 15 g/L, with a pH of 0.68 after 14
217 days. The highest sulphate concentration (20 g/L) inhibited both bacterial growth and sulphate
218 production. Fig. 2(d) illustrates the cell density (cells/mL) of *A. thiooxidans* at different
219 elemental sulphur concentrations (5, 10, 15, and 20 g/L) over a period of 16 days. Cell density
220 increased with time for all concentrations. During the initial 2–4 days, the bacteria were in a
221 lag phase with minimal increases in cell density. From day 4 to day 11, the cell population

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

228 3.2 Production of biogenic thiosulphate

229 In sulphur-oxidising microorganisms such as *A. thiooxidans*, thiosulphate is the key
230 intermediate in the metabolic cycle of sulphur to sulphate conversion, undergoing rapid
231 oxidation to tetrathionate or sulphite, then to sulphate. In the periplasm of *A. thiooxidans*, there
232 is a sulphur oxidising enzyme system (Sox) that oxidises thiosulphate, sulphide, sulphite, and
233 elemental sulphur to sulphate by SoxXA, SoxB, and Sox(CD)₂ enzymes. Also, there is a
234 thiosulphate oxidation pathway (tetrathionate intermediate thiosulphate oxidation (S₄I)) that
235 consists of two enzymes: thiosulphate quinol oxidoreductase (TQO or DoxDA), which oxidises
236 thiosulphate to tetrathionate and tetrathionate hydrolase (TetH or TTH), which hydrolyses
237 tetrathionate to thiosulphate. Additionally, thiosulphate can be oxidised to tetrathionate by
238 Thiosulphate Dehydrogenase (TSD) enzyme without the need for any cofactors (Yin et al.,
239 2019). The pH of the periplasmic and cytoplasmic areas typically ranges from 2 to 3 and from
240 6 to 7, respectively. In *A. thiooxidans*, the generated thiosulphate in the periplasmic area quickly
241 converts to the acid-stable tetrathionate. Studies on thiosulphate-grown *A. thiooxidans* have
242 shown that the optimal pH for thiosulphate and tetrathionate oxidation is approximately 5.0
243 (Masau et al., 2001). In another study, thiosulphate oxidation with an optimum pH of 3–5.5
244 was reported for *A. thiooxidans* (Kamimura et al., 2005). So, maintaining pH above 5 during
245 the growth phase of bacteria can significantly reduce thiosulphate conversion by the sulphur
246 oxygenase reductase (SOR) enzyme, which oxidises cytoplasmic elemental sulphur to
247 thiosulphate, sulphite, and sulphide (Wang et al., 2019). In addition to pH adjustment, adding
248 inhibitors can minimise the activity of thiosulphate-oxidising enzymes and other sulphur
249 compounds. Eqs. (4)-(7) and (9)-(11) list reactions that can be inhibited by certain inhibitors.
250 Thiosulphate production was investigated at different pH levels above 4, and the results showed
251 no detectable thiosulphate formation within the pH range of 4–6 (data not shown).
252 Consequently, thiosulphate production and the inhibitory effect of DDC and NaF at different
253 concentrations were examined at pH 6. DDC directly inhibits by binding to and removing non-
254 heme iron, which is essential for the activity of soluble components and redox enzymes in the

255 pathway. Removal of Fe disrupts iron-dependent electron carriers within enzymes such as the
256 Sox system and the Heterodisulfide Reductase (Hdr)-like complex, blocking electron transfer
257 from sulphur to the quinone pool and disrupting adenosine triphosphate (ATP) generation and
258 sulphur oxidation activity. NaF inhibits the sulphur oxidation enzyme indirectly by interfering
259 with phosphate metabolism and ATP-dependent reactions required for coupling sulphur
260 oxidation to biosynthetic processes. Although sulphur oxidation is not directly affected, the
261 inhibition of phosphate-ester metabolism prevents effective utilisation of the generated energy
262 and reduces the overall functional output of the system (Iwatsuka et al., 1962; Iwatsuka and
263 Mori, 1960). As illustrated in Fig. 3(a) and (c), the highest thiosulphate concentration without
264 inhibitors was 219 mg/L. In all samples, thiosulphate levels were high during the first 3 days.
265 Still, they then declined due to the high degradation rate and thiosulphate's instability over
266 time, and concentrations remained below 100 mg/L with only small fluctuations. The inhibitor
267 curves were quite similar to those without inhibitors, showing an insignificant effect of the
268 inhibitors. As shown, at high inhibitor concentrations (10^{-2} M), the thiosulphate concentration
269 was lower, not exceeding 112 mg/L and falling below 50 mg/L after a week. This can happen
270 due to the toxic effect of high concentrations of NaF and DDC on bacterial function and
271 metabolism (Pourhossein and Mousavi, 2023). The limited inhibitory effect of DDC and NaF
272 on thiosulphate oxidation can be explained by the presence of multiple, parallel sulphur
273 oxidation pathways in *A. thiooxidans*. Thiosulphate can be oxidised via the S4I pathway, the
274 Sox system, and TSD-mediated oxidation, allowing for metabolic flexibility. Previous research
275 indicates that *Acidithiobacillus* species can regulate these pathways in response to
276 environmental conditions. This means that inhibiting one pathway does not block overall
277 thiosulphate oxidation, as the metabolic flux can be redirected to alternative routes. This
278 redundancy and adaptability in the sulphur oxidation pathway explain the weak inhibitory
279 effect observed in the experiment (Camacho et al., 2020; Wang et al., 2019). Research on the
280 inhibitory effects of NaF in *Acidithiobacillus* species indicates that fluoride interferes with
281 bacterial growth and sulphur or iron oxidation only under particular conditions. For example,
282 Ma et al. (2016) reported that fluoride inhibition in *A. ferrooxidans* is highly dependent on pH,
283 energy source, and growth phase, resulting in significant inhibition under acidic conditions
284 (~pH of 2). In another study, fluoride tolerance was assessed in pure and mixed cultures of
285 various acidophilic bacteria, and *A. thiooxidans* demonstrated the highest fluoride tolerance
286 (0.5 mM F^-), whereas *S. thermosulfidooxidans* was completely inhibited (Fritze and Hedrich,
287 2026). It means that *A. thiooxidans* was able to grow and maintain metabolic activity, even at
288 high fluoride concentrations, with limited inhibitory effect. Fig. 3(b) and (d) illustrate the effect

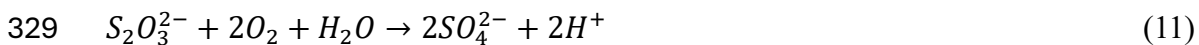
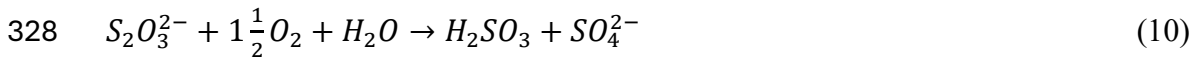
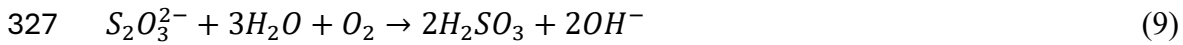
289 of inhibitors on bacterial cell density (cells/mL) after pH adjustment. During the logarithmic
290 growth phase, raising the pH did not significantly affect growth or cell concentration because
291 the bacteria continued to consume and oxidise sulphur to compounds, such as thiosulphate
292 (Masau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

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

$$314 \quad v = V_{max} \cdot S_t / (K_m + S_t) \quad (12)$$

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

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



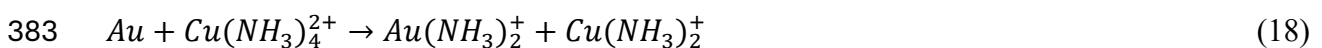
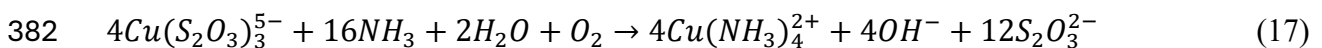
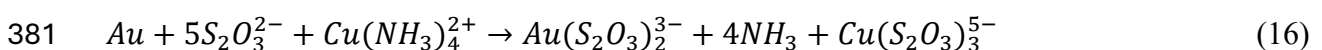
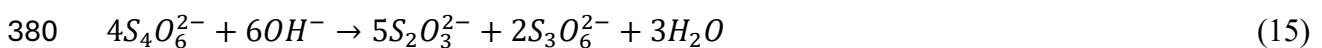
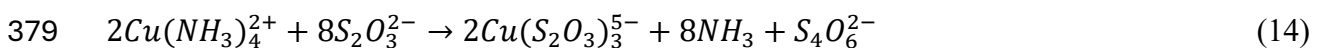
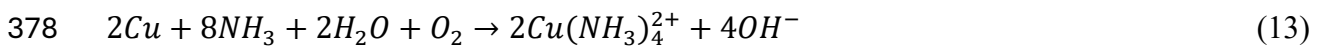
330 For comparison, the inhibitory effects of selected compounds on sulphur and thiosulphate
331 oxidation during the *A. thiooxidans* growth cycle were summarised in Table 2. Carboxylic acids
332 such as acetic acid, formic acid, and propionic acid exhibited strong inhibition at 10 mM.
333 Iwatsuka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

343 3.3 Thiosulphate leaching of gold

344 3.3.1 Ammonia on bio-thiosulphate leaching of gold

345 Thiosulphate leaching of gold has been demonstrated to be most effective when catalysed by
346 Cu^{2+} as an oxidant in the presence of ammonia, which performs several key functions. For
347 example, it stabilises the Cu^{2+} -ammonia complexes, minimises the decomposition of
348 thiosulphate into polythionates, maintains the solution pH within an optimal range, and
349 enhances leaching kinetics (Ilkhani Aiouache, 2025). Fig. 4(a) illustrates the effect of ammonia
350 (0.5-1 M) on the leaching rate of gold using biogenic thiosulphate of 120 mg/L, Cu^{2+} 0.04 M
351 from residue PCB powder at a pulp density of 1%. The results demonstrate that increasing the
352 ammonia concentration from 0.5 M to 1 M had a slight effect on gold leaching, with a

353 maximum gold leaching rate of 9%. The active oxidising species in this system was confirmed
 354 to be the tetraammine–cupric complex, $\text{Cu}(\text{NH}_3)_4^{2+}$, which facilitated leaching of gold as
 355 $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ complex, Eqs. (13)-(17) (Jeon et al., 2020). Oxygen was essential for maintaining
 356 a continuous oxidation process to enhance the process. During bio-thiosulphate leaching,
 357 $\text{Cu}(\text{NH}_3)_4^{2+}$ would be reduced to Cu^+ and stabilised as $\text{Cu}(\text{S}_2\text{O}_3)_3^{5-}$ Eq. (17). Oxygen oxidises
 358 Cu^+ to Cu^{2+} , thereby regenerating the active $\text{Cu}(\text{NH}_3)_4^{2+}$ species again. This regeneration step
 359 could increase the efficiency of gold leaching from residue PCB (Dong et al., 2019). At
 360 ammonia concentrations below 0.5 M, Cu^{2+} ions react mainly with thiosulphate, reducing the
 361 availability of $(\text{Cu}(\text{NH}_3)_4^{2+})$ to oxidise gold. During this reaction, tetrathionate was formed
 362 and gradually disproportionated to trithionate and thiosulphate (Eqs. (14)-(15)). Ammonia
 363 concentrations exceeding 1 M reduce the leaching rate due to increased hydroxide ion (OH^-)
 364 concentration. Ammonia could weakly interact with gold to form the transient $\text{Au}(\text{NH}_3)_2^+$
 365 complex, but high ammonia concentrations shift the reaction equilibrium, Eqs. (18)-(19)
 366 toward less favourable conditions, promoting side reactions and destabilising the $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$
 367 complex. These findings align with previous studies (Jeon et al., 2020), which indicate that
 368 moderate ammonia levels enhance gold leaching, whereas concentrations above 1 M inhibit
 369 the process. Fig. 4(b) shows that the pH varied between 10 and 12 because thiosulphate tends
 370 to decompose quickly at pH below 9 due to the low ammonia concentration. This is consistent
 371 with other studies reporting that a pH of 10 is favourable for enhancing thiosulphate stability
 372 and the formation of stable copper–ammine–thiosulphate complexes. Zhang and Senanayake
 373 (2016) also reported that Eh must be maintained between 0.1–0.5 V to form stable $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$
 374 species, because at potentials below 0.1 V, gold leaching will not occur and copper will
 375 precipitate as a copper sulphide, and at potentials above 1 V, $\text{Au}(\text{NH}_3)_4^{3+}$ species will form (Fig.
 376 4(c)). Therefore, maintaining an ammonia concentration in the range of 0.5–1.0 M ensures
 377 balanced complex stability, which controls pH and oxidation efficiency.

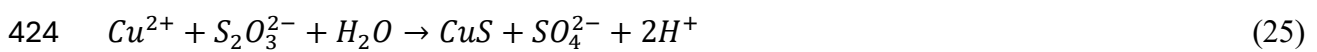
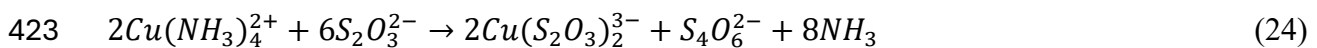
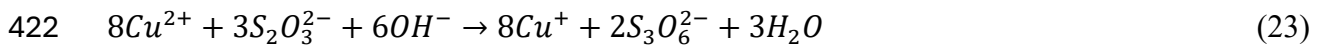
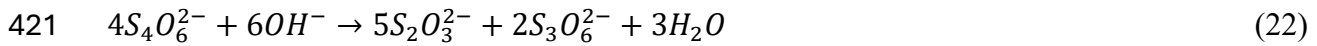




386 3.3.2 Cu^{2+} on bio-thiosulphate leaching of gold

387 The effect of Cu^{2+} concentration (0.02–0.05 M) on gold leaching was determined under the
388 conditions of 120 mg/L biogenic thiosulphate, 1 M ammonia, and 1% pulp density (Fig. 5(a)).
389 The leaching efficiency was slightly higher at a Cu^{2+} concentration of 0.02 M, reaching 10%,
390 but then decreased as the copper concentration increased. Similar behaviour has been reported
391 in previous studies, in which high Cu^{2+} concentrations negatively affected gold leaching. The
392 reason is that higher Cu^{2+} concentrations narrowed the stability domains of soluble complexes
393 such as $Cu(NH_3)_4^{2+}$ and $Cu(S_2O_3)_3^{5-}$ and promoted the formation of less soluble species,
394 including CuO , Cu_2O , and Cu_2S , thereby hindering the catalytic role of Cu^{2+} in gold leaching
395 (Sitando et al., 2020; Zhang and Senanayake, 2016). Moreover, higher concentrations of Cu^{2+}
396 can lead to the degradation of thiosulphate into by-products, such as tetrathionate, trithionate,
397 sulphate, and copper sulphide (Eqs. (21)-(25)). Based on the findings of Aylmore and Muir
398 (2001), pH values higher than 11 should be avoided to prevent the formation of less soluble
399 species. The pH variation during leaching, shown in Fig. 5(b), indicates elevated pH levels
400 during the first two days, likely due to the formation of copper oxides. The Eh variation shown
401 in Fig. 5(c) indicates a relatively low oxidation potential for gold oxidation compared with the
402 higher Eh values reported in previous studies (Zhang and Senanayake, 2016). A control
403 experiment using sodium thiosulphate was conducted under the favoured conditions, which
404 generated 120 mg/L sodium thiosulphate at 0.02 M Cu^{2+} , 1 M ammonia, and a pulp density of
405 1%. The results showed that chemical leaching was slightly higher than bioleaching, which can
406 be attributed to the use of a pure and stable chemical reagent in the control system. Biogenic
407 thiosulphate is produced through complex microbial sulphur-oxidising pathways, which can
408 generate additional intermediate species such as sulphite and tetrathionate. These by-products
409 interfere with the leaching process and reduce its efficiency. Liquid thiosulphate in the biogenic
410 system was less stable than commercially available solid sodium thiosulphate. Even after
411 bacterial removal, the solution pH decreased rapidly, promoting thiosulphate decomposition.
412 These findings are consistent with previous studies by Pourhossein and Mousavi (2022), who
413 reported that although no significant difference was observed between bioleaching and
414 chemical thiosulphate leaching, the chemical leaching rate was slightly higher than the biogenic
415 thiosulphate leaching. Nadi et al., (2025) reported significant differences in the leaching of rare

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



425 *A. thiooxidans* has been the most frequently examined organism, producing between 120 and
 426 500 mg/L thiosulphate depending on conditions and the use of inhibitors. For example,
 427 Pourhossein and Mousavi (2022) reported that *A. thiooxidans* generated up to 120 mg/L of
 428 thiosulphate after pH adjustment above 6 using 0.1 N NaOH. They also investigated the impact
 429 of potassium cyanide and sodium azide on thiosulphate oxidation. They found that adding
 430 approximately 3 mg/L of potassium cyanide and sodium azide increased thiosulphate
 431 concentrations to 350 and 500 mg/L, respectively, resulting in gold leaching rates of 31% and
 432 56% from PCBs. These inhibitors disrupt key sulphur-oxidising enzymes and the electron
 433 transport chain through cytochrome oxidase, blocking the conversion of thiosulphate into
 434 sulphate and leading to its accumulation at higher concentrations (Pourhossein and Mousavi,
 435 2023). Although the current study produced a comparable amount of thiosulphate, the
 436 inhibitors did not significantly affect thiosulphate oxidation during bacterial growth. This study
 437 aimed to evaluate safer, non-toxic inhibitors reported in previous research to support the
 438 development of a more sustainable bioprocess. Even though earlier studies indicated that 1 mM
 439 DDC and NaF could completely inhibit sulphur-oxidising activity, their effects were negligible
 440 under the conditions examined (Iwatsuka et al., 1962; Iwatsuka and Mori, 1960).

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

447 1% leaching after 7 days. Alternative non-cyanide leaching agents have also shown limited
448 performance. Kudpeng et al., (2020) used triiodide-producing bacteria named *Roseovarius*
449 *tolerans* and *Roseovarius mucosus*, resulting in a leaching rate of around 1%. Although the
450 leaching rate in the present study remains modest, it is comparable to or higher than that of
451 previously reported bioleaching systems, highlighting the potential of biogenic thiosulphate as
452 an alternative technique. Biogenic thiosulphate is still in its early stages, with only a limited
453 number of studies investigating its effectiveness and further optimisation is required to improve
454 its efficiency.

455 3.4 Thermodynamic analysis

456 The Eh-pH diagram was generated using HSC 10 software (Metso Outotec, Finland) to
457 validate the location of the thermodynamic stability region of different species and help
458 understand the experimental laboratory results, since the Eh-pH stability range of a species is
459 strongly influenced by the concentrations of thiosulphate, ammonia, and $\text{Cu}^{2+}/\text{Cu}^+$. The Eh-pH
460 diagram for Cu-NH₃-S₂O₃²⁻-H₂O system (Fig. 6(a)) indicates that Eh affects the equilibrium
461 boundary between Cu^+ and Cu^{2+} ions. Cu^{2+} ammonia complex dominates at high Eh and pH,
462 whereas Cu^+ thiosulphate complex is relevant at low Eh and pH (Aylmore and Muir, 2001). At
463 low potentials and with limited oxidants, thiosulphate decomposes to form copper sulphide
464 precipitates. Under these conditions, gold co-precipitates with sulphides, resulting in a decrease
465 in the leaching rate (Wan and LeVier, 2003). A narrow stability region for Cu^{2+} exists at pH
466 values below 5 and Eh above 0.5 V, whereas copper oxides tend to form at approximately
467 neutral pH when the Eh exceeds 0.02 V. The Eh-pH diagram for the quaternary Au-NH₃-S₂O₃²⁻-
468 -H₂O system (Fig. 6(b)) shows that the $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ complex is predominant at pH values
469 below 11 and an Eh range of approximately 0.4-1.25 V, which is consistent with the previous
470 reports (Chen et al., 2024; Liu et al., 2018). Under certain conditions, gold may also exist in
471 solution as $\text{Au}(\text{NH}_3)_2^+$ over a relatively wide pH range when the Eh exceeds about 0.5 V (Wan
472 and LeVier, 2003). This observation is consistent with the experimental data presented in Fig.
473 6(b) and (c), where the system operated within a pH range of 10.5–11.5 and an Eh range of 0–
474 0.15 V. The Eh-pH diagram indicates that Eqs (18) and (19) were the predominant in the
475 chemical mechanism of the bioleaching process, which explains the low gold leaching rate.
476 Therefore, to effectively leach gold from the powder, the Eh should be increased to values
477 above 0.4 V. The Eh-pH diagram for the S-H₂O system (Fig. 6(c)) is shown to demonstrate the
478 various stable and metastable sulphur-oxygen species. Looking from a thermodynamic
479 perspective, allyl hydrosulphide (HS^-) and dithionate ($\text{S}_2\text{O}_6^{2-}$) 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.

502

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