Determination of Ferrous Oxidation Kinetics in the Presence of Metals Associated with Printed Circuit Boards to Determine the Potential for Bioleaching of e-Waste

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Abstract

Printed circuit boards (PCBs) contain a wide variety of valuable metals that may be recovered and recycled. Biohydrometallurgical processing techniques are well suited to metal solubilisation and extraction and provide a viable process option for base metal recovery. The presence of base metals, especially copper, in PCB waste is known to compromise extraction of the precious materials; hence, we aim to extract these base metals through acidic biohydrometallurgy prior to extraction of Au, Ag, Pt and Pd, but recognise that these metals also have potential to inhibit the bioprocess. In this study, a mesophilic *Leptospirillum ferriphilum*-dominated culture was adapted to 1 g/L copper (Cu^{2+}) and subsequently exposed to increasing concentrations of prevalent metals associated with PCBs. Metal concentrations representative of PCB composition, at corresponding metal loadings of 0%, 11%, 18% and 27%, were investigated. Metals of interest included Cu^{2+}, Al^{3+}, Ni^{2+} and Zn^{2+}. Copper was found to elicit the most significant inhibitory effect on ferrous iron oxidation. As copper is the most abundant metal in PCB waste, rigorous kinetic studies are required to better understand the impact of Cu^{2+} inhibition on microbial performance and subsequent metal extraction.

Introduction

Printed circuit boards (PCBs) form an integral part of numerous electronic devices and contain 28 - 30% metal. Metals present in PCBs primarily include copper, lead, aluminium, tin and iron alongside other heavy metals such as nickel, zinc and cadmium (Table 1) and precious metals such as gold and silver.

Table 1: Table illustrating content of common base and heavy metals associated with PCBs (adapted from Bizzo et al., 2014; Yoo et al., 2009)

Metal species	**Content (wt%)**
Cu	12.0 – 29.0
Al	1.5 – 7.1
Zn	0.1 – 2.7
Fe	0.1 – 11.5
Ni	0.3 – 5.4
Sn	1.0 – 5.6
Pb	1.3 – 3.9

The ongoing generation of electronic waste (e-waste), resultant from rapid technological innovation, has provided a metal-rich waste stream from which metals may be recovered and recycled. Bioleaching has demonstrated promise as a processing option for the recovery of valuable base metals from e-waste as microbes generate the leach agent (ferric iron) that facilitates solubilisation of the metals embedded within discarded PCBs. However, numerous metals are known to elicit toxic effects on bioleaching microbes via various mechanisms. Inhibition kinetics of iron-oxidising bacteria such as *Leptospirilum ferriphilum* and *Acidithiobacillus ferrooxidans* have been studied extensively within tank and heap leaching contexts and it has been determined that metal ions such as Ni^{2+} and Zn^{2+} induce non-competitive inhibition of ferrous oxidation according to Equation 1 (Nurmi et al., 2009).

$$r_{Fe^{2+}} = -\frac{q_m\, S\, X}{\left(K_s + S + \frac{S^2}{K_i}\right).\left(1 + \frac{I}{K_{iinc}}\right)} \tag{1}$$

where $r_{Fe^{2+}}$ is the oxidation rate, q_m is the maximum specific oxidation rate (mg Fe^{2+}/cell.hr), X is the cell concentration (cell/mL), S is the ferrous iron concentration (mg/L), K_s is the half-saturation constant (mg/L), K_i is the inhibition constant of the self-inhibitory substrate (mg/L), K_{iinc} is the non-competitive inhibition constant and I is the inhibitor concentration (mg/L). However, kinetic information relating to Cu^{2+} and Al^{3+} inhibition is largely lacking.

This body of work aims to quantify the effect of prominent metals associated with PCB waste on microbial oxidation of ferrous iron and subsequently, the generation of the ferric iron lixiviant. Furthermore, this study aims to determine kinetic model parameters for bioleaching oxidation performance when exposed to metals of interest so as to inform reactor configuration and design.

Materials and Methods

Microbial strains, media and growth

The *L. ferriphilum*-dominated mixed mesophilic microbial culture used in this study was maintained in a 100 mL Erlenmeyer flask containing ABS medium (0.15 g/L $(NH4)_2SO_4$, 0.15 g/L Na_2SO_4, 0.05 g/L KCl, 0.5 g/L $MgSO_4.7H_2O$, 0.05 g/L KH_2PO_4 and 0.014 g/L $Ca(NO_3)_2.4H_2O$) at a total working volume of 30 mL. The pH of the medium was adjusted to pH 1.4 with concentrated H_2SO_4 (96%) and was supplemented with 3 g/L ferrous iron (Fe^{2+}), added as $FeSO_4.7H_2O$, and trace element solution (Kolmert and Johnson, 2001). The culture was incubated at 37°C on a platform shaker at 130 rpm. To maintain sustained ferrous iron oxidation activity, the culture was transferred into fresh medium with replenished substrate every three days using a 20% inoculum volume.

Adaptation to copper metal

The mesophilic culture was adapted to copper ions (Cu^{2+}) *via.* serial transfer into ABS medium at a pH of 1.4 with 0.5 g/L Cu^{2+}, added as $CuSO_4.5H_2O$. Following sustained microbial activity, the Cu^{2+} concentration was increased to 1 g/L. The adapted culture was thereafter maintained as outlined above.

Analytical techniques

Ferrous iron utilisation was quantified using the modified spectrophotometric ferric chloride assay on a Thermo Scientific GENESYS 20 spectrophotometer (Govender et al., 2012). Cell numbers were quantified *via*. direct count using a Thoma counting chamber under 1000x magnification using an Olympus CX-40 microscope.

Preliminary experiments

Preliminary batch experiments were conducted in multi-well plates (MWPs), using Greiner Bio-one CELLSTAR® 12 Well Suspension Culture Plates (4 ml volume per well), to investigate the effect of Cu^{2+} on ferrous iron oxidation activity of a mesophilic bioleaching culture. Low copper concentrations were investigated (0 g/L, 0.048 g/L, 0.096 g/L, 0.19 g/L, 0.48 g/L, 0.96 g/L and 1.91 g/L). Each well was charged with nutrient medium, 5 g/L Fe^{2+} as substrate, the designated volume of the Cu^{2+} stock solution and inoculated with 1 x 10^7 cells/mL of the cell culture such that a total working volume of 3 mL was achieved. An AeraSeal™ film was fitted to the MWP and thereafter placed in a humidified container to mitigate evaporative losses. The MWPs were incubated in a shaking incubator at 37°C and agitated at 140 rpm. Over the course of the experiment, 5 μL samples were withdrawn at appropriate time intervals (selected based on the rate of oxidation) for measurement of ferric iron concentrations using the modified ferric chloride colorimetric assay. Cell density was quantified twice daily. These experiments were repeated using a copper-adapted mesophilic bioleaching culture.

Effect of metals on copper-adapted culture

Evaluation of Cu^{2+}, Al^{3+}, Ni^{2+} and Zn^{2+} impact on ferrous iron oxidation

The singular effect of increasing Cu^{2+} concentrations, corresponding to PCB metal loadings of 0%, 11%, 18% and 27%, on microbial Fe^{2+} utilisation and biomass yield was investigated under batch conditions using the copper-adapted culture as inoculum. The Cu^{2+} concentrations investigated were 0 g/L, 4.03 g/L, 6.9 g/L and 10.36 g/L. Following this, the impact of increasing concentrations of a ternary mixture comprising of Al^{3+}, Ni^{2+} and Zn^{2+} at a fixed ratio of 1: 0.76: 0.1, corresponding to the afore-mentioned PCB metal loadings, was investigated. Thereafter a combination of Cu^{2+}, Al^{3+}, Ni^{2+} and Zn^{2+} at the designated PCB metal loadings was assessed. Ferrous iron utilisation was measured at regular time intervals and cell number was enumerated twice daily throughout the time course of the experiment.

Results and Discussion

Preliminary study

Copper is the most abundant metal associated with PCBs and is known to be toxic to bioleaching organisms. The suitability and sensitivity of selected microbial cultures was therefore investigated by evaluating the impact of low-level Cu^{2+} concentrations on Fe^{2+} oxidation rate and growth.

In the unadapted mesophilic culture (Figure 1a and Figure 2), it was found that Fe^{2+} utilisation was impeded when the bioleaching culture was exposed to Cu^{2+} concentrations above 0.096 g/L, with complete microbial arrest (no growth or oxidation activity) noted at Cu^{2+} concentrations greater than 0.48 g/L. With these increasing Cu^{2+} concentrations, an associated increase in the lag period was noted preceding the onset of Fe^{2+} oxidation. Conversely, exposure of the copper-adapted culture to low-level Cu^{2+} concentrations had negligible impact on the initiation of Fe^{2+}oxidation with the rate of oxidation conserved (Figure 1b and Figure 2).

In accordance with Das et al. (1997), in which *At. ferroxidans* was adapted to Cu^{2+}, enhanced Cu^{2+} tolerance with little impact on microbial oxidation activity was observed. The 1 g/L Cu^{2+} adapted culture in this study was functionally optimal at Cu^{2+} concentrations exceeding the adaption concentration (1.0 g/L reaching 1.92 g/L Cu^{2+}). The improvement in the tolerance to Cu^{2+} is likely due to the induction of copper resistant mechanisms. Iron-oxidising acidophiles such as *At. ferrooxidans* are capable of adapting to elevated Cu^{2+} concentrations as the genome contains a suite of genes that are upregulated following Cu^{2+} exposure (Navarro et al., 2013). Several *cop* genes encode for P-Type ATPases, which mediate Cu^{2+} import and export with additional genes involved in copper homeostasis, such as *cusCBA* and *cusF* (Dopson and Holmes, 2014; Navarro et al., 2013). Copper-resistance mechanisms have also been identified in *L. ferriphilum* bacterial strains, namely the P-Type ATPase transporter (Mi et al., 2011), which may account for the similarly observed Cu^{2+} tolerance.

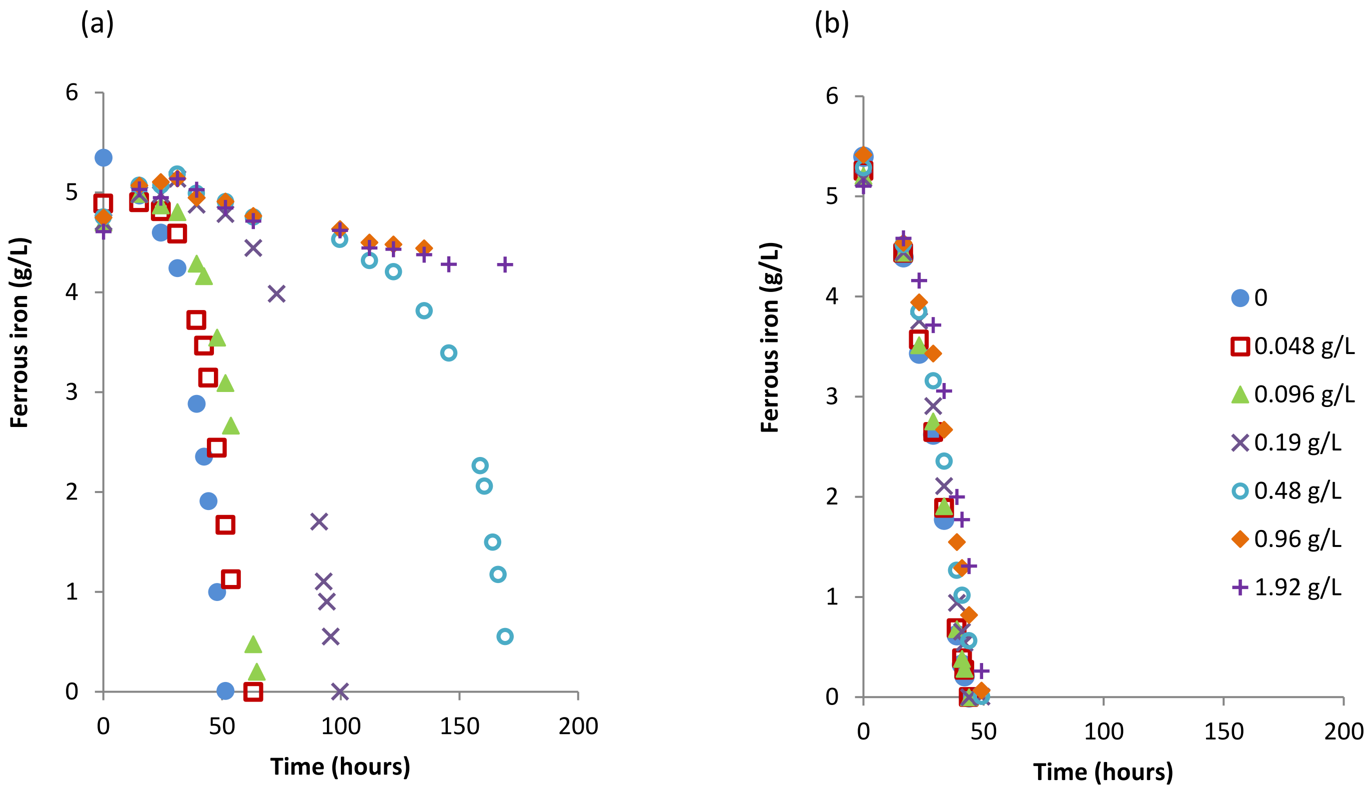

Figure 1: Ferrous iron utilisation profiles for an unadapted and copper-adapted mesophilic bioleaching culture exposed to low-level concentrations of Cu^{2+} at 37°C

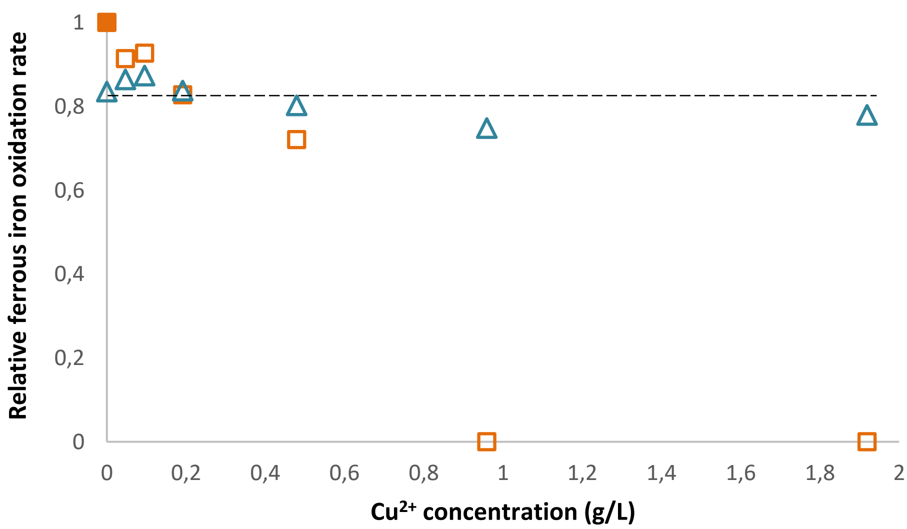

Figure 2: Relative ferrous iron oxidation rates $[(mgL^{-1}\ hr^{-1})_X/(mg\ L^{-1}\ hr^{-1})_R]$ across increasing Cu^{2+} concentrations (g/L) for unadapted (□) and copper-adapted (Δ) mesophilic bioleaching cultures, where 'X' refers to rate of test case and 'R' refers to reference rate (rate of unadapted culture, unexposed to Cu^{2+})

Impact of metals on copper-adapted bioleaching culture

Following successful adaptation to Cu^{2+}, the *L. ferriphilum* dominated culture was exposed to Cu^{2+} concentrations associated with 0%, 11%, 18% and 27% PCB metal loadings to probe the suitability of this culture in metal recovery applications from e-waste. The adapted culture was also exposed to a ternary mixture of Al^{3+}, Zn^{2+} and Ni^{2+} corresponding to the designated PCB metal loadings to evaluate whether these metals elicit an inhibitory effect. Following this, the impact of concurrent exposure to all the metals was investigated. This was to simulate a prospective PCB leachate environment wherein the performance of the microorganisms could be evaluated in the presence of an array of metal cations.

Exposure of the adapted culture to elevated Cu^{2+} concentrations resulted in an increased lag time preceding the initiation of Fe^{2+} oxidation. The onset of microbial activity was further delayed with increasing Cu^{2+} (Figure 3a). However, the average oxidation rates were found to remain largely conserved across the applied metal loadings, except at 27% (10.36 g/L Cu^{2+}) where the Fe^{2+}oxidation rate decreased by *ca*. 20% (Table 2). Exposure to the ternary combination of Al^{3+}, Zn^{2+} and Ni^{2+} was found to elicit no effect on culture activity across all metal loadings investigated suggesting that the metal concentrations were below inhibitory limits (Figure 3b and Table 2). Simultaneous exposure to all metals was found to mimic the response observed for elevated Cu^{2+} concentrations (Figure 4). However, the decrease in the average Fe^{2+}oxidation rate at a 27% metal loading was approximately double that observed in the pure Cu^{2+} cation exposure test (Table 2). The rapid decline in the rate at this loading potentially indicates that although a ternary mixture of Al^{3+}, Zn^{2+} and Ni^{2+} does not inhibit the culture, the addition of Cu^{2+} may metabolically overburden the system and therefore enhance the inhibitory effect.

The behaviour observed at the investigated PCB metal loadings indicate that Cu^{2+} is the limiting cation within the system. The results further suggest that the effect of the ternary combination of Al^{3+}, Zn^{2+} and Ni^{2+} have little effect on the oxidation efficiency of the adapted culture and may not be inhibitory at the concentrations investigated. Copper-adapted strains of *At. ferrooxidans* have been found to be tolerant to systems containing Fe^{3+} and Zn^{2+} in addition to Cu^{2+}, however adaptation to Fe^{3+} iron does not result in reciprocal tolerance of other metals (Das et al., 1997). In addition to this, kinetic studies on *L. ferriphilum* exposed to Zn^{2+} and Ni^{2+} have illustrated the organism's insensitivity to these metals with inhibition constants of 49.1 g/L and 62.7 g/L for Zn^{2+} and Ni^{2+} respectively (Nurmi et al., 2009). Alternatively, tolerance to Al^{3+}, Zn^{2+} and Ni^{2+} may be a function of the mechanism associated with adaptation to Cu^{2+}.

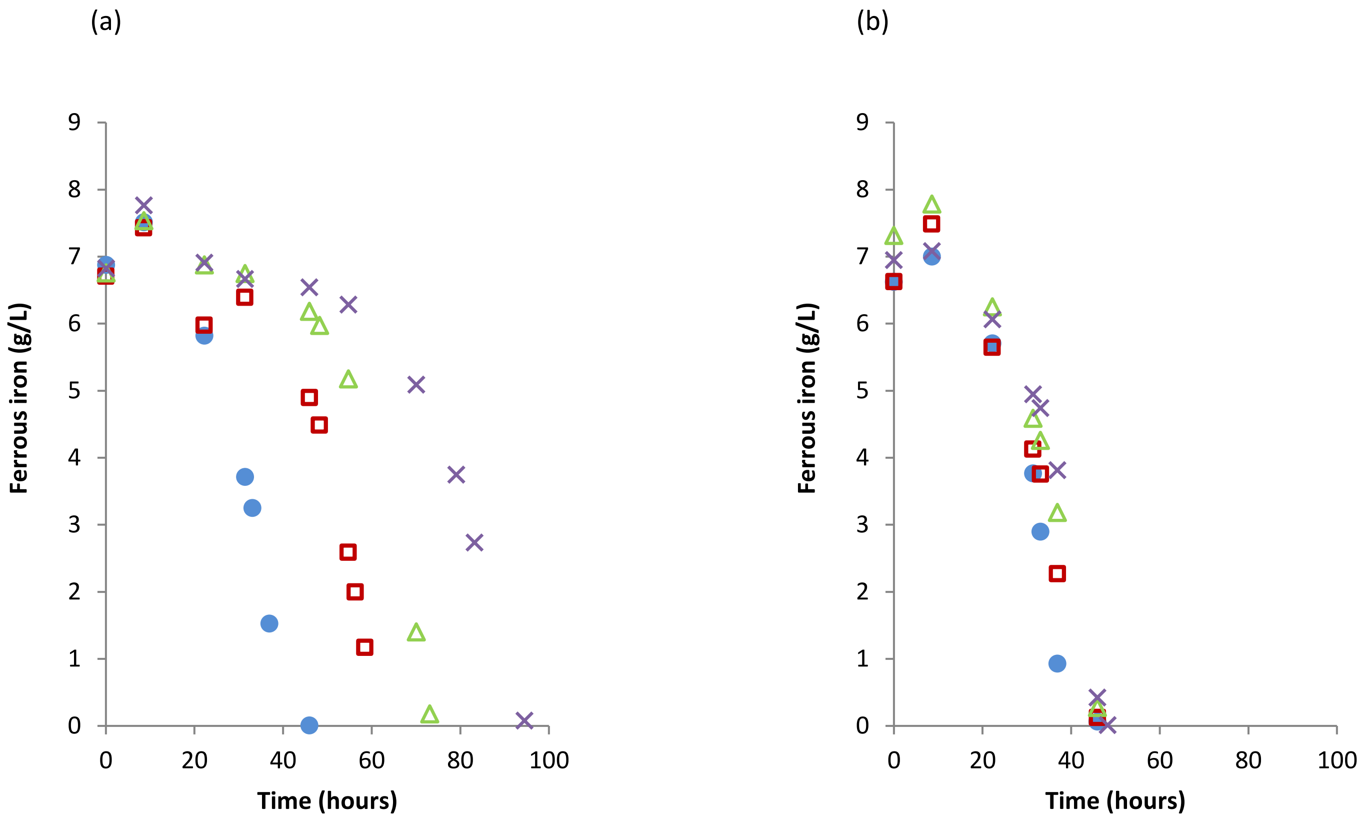

Figure 3: (a) Copper-adapted mesophilic culture exposed to increasing concentrations of Cu^{2+} (● – 0 g/L), (□ – 4.03 g/L), (**Δ** – 6.9 g/L), (**x** – 10.36 g/L) (b) Copper-adapted mesophilic culture exposed to increasing concentrations of mixed metals excluding Cu^{2+} (● – no metals), (□ – 0.49 g/L Al^{3+}, 0.37 g/L Ni^{2+}, 0.05 g/L Zn^{2+}), (**Δ** – 0.85 g/L Al^{3+}, 0.64 g/L Ni^{2+}, 0.088 g/L Zn^{2+}), (**x** – 1.3 g/L Al^{3+}, 0.96 g/L Ni^{2+}, 0.13 g/L Zn^{2+})

Table 2: Average ferrous iron oxidation rates of a copper-adapted mesophilic culture exposed to increasing concentrations of Cu^{2+}, increasing concentrations of a ternary combination of Al^{3+}, Zn^{2+} and $Ni^{2+}$and increasing concentrations of Cu^{2+}, Al^{3+}, Zn^{2+} and Ni^{2+} in proportional concentrations associated with designated PCB metal loadings.

PCB metal loading (wt%)	Average Fe^{2+} oxidation rate ($mg.L^{-1}h^{-1}$)		
	Cu^{2+}	Al^{3+}, Zn^{2+}, Ni^{2+}	All metals
0%	260.9	306.8	240.6
11%	283.2	278.2	258.3
18%	260.7	312.3	234.0
27%	207.5	312.3	133.8

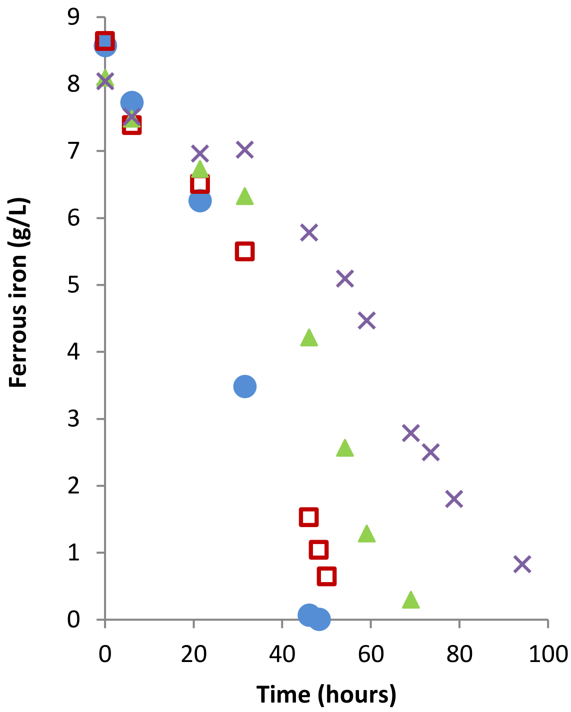

Figure 4: Copper-adapted mesophilic culture exposed to increasing concentrations of Cu^{2+}, Al^{3+}, Ni^{2+} and Zn^{2+} corresponding to increasing metal loadings associated with PCB composition (● – no metals), (□ – 4.03 g/L Cu^{2+}, 0.49 g/L Al^{3+}, 0.37 g/L Ni^{2+}, 0.05 g/L Zn^{2+}), (Δ – 6.9 g/L Cu^{2+}, 0.85 g/L Al^{3+}, 0.64 g/L Ni^{2+}, 0.088 g/L Zn^{2+}), (x – 10.36 g/L Cu^{2+}, 1.3 g/L Al^{3+}, 0.96 g/L Ni^{2+}, 0.13 g/L Zn^{2+})

Conclusions

The results of this study indicate that adaptation to Cu^{2+} is imperative for efficient metal recovery from e-waste. Adaptation to 1 g/L Cu^{2+} was found to mitigate microbial inhibition completely at Cu^{2+} concentrations up to 1.92 g/L. However, exposure to Cu^{2+} concentrations associated with 11%, 18% and 27% PCB metal loadings was found to correspond with increasing lag time linked to the onset of ferrous iron oxidation as well as a reduction in ferrous iron oxidation rate at 27% metal loading. The ternary combination of Al^{3+}, Zn^{2+} and Ni^{2+} was found to impact microbial activity negligibly at the metal loadings investigated. However, evaluation of exposure to Al^{3+}, Zn^{2+} and Ni^{2+} coupled with Cu^{2+} was found to be comparable to the results obtained for the investigation of Cu^{2+} in isolation, therein highlighting its inhibitory significance.

As adaptation to 1 g/L Cu^{2+} was found to improve the culture's tolerance significantly, it is postulated that adaptation to a higher Cu^{2+} concentration may significantly improve microbial tolerance and mitigate the inhibition observed at the metal loadings investigated. Further, these findings indicate that the microbial kinetics associated with Cu^{2+} inhibition must be resolved so as to better understand system performance such that it may be modelled accurately within the context of e-waste processing.

References

Bizzo, W.A., Figueiredo, R.A., De Andrade, V.F., 2014. Characterization of printed circuit boards for metal and energy recovery after milling and mechanical separation. Materials (Basel). 7, 4555–4566. https://doi.org/10.3390/ma7064555

Das, A., Modak, J.M., Natarajan, K.A., 1997. Studies on multi-metal ion tolerance of *Thiobacillus ferrooxidans*. Miner. Eng. 10, 743–749. https://doi.org/10.1016/S0892-6875(97)00052-6

Dopson, M., Holmes, D.S., 2014. Metal resistance in acidophilic microorganisms and its significance for biotechnologies. Appl. Microbiol. Biotechnol. 98, 8133–8144. https://doi.org/10.1007/s00253-014-5982-2

Govender, E., Harrison, S.T.L., Bryan, C.G., 2012. Modification of the ferric chloride assay for the spectrophotometric determination of ferric and total iron in acidic solutions containing high concentrations of copper. Miner. Eng. 35, 46–48. https://doi.org/10.1016/j.mineng.2012.05.006

Kolmert, A., Johnson, D.B., 2001. Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. J. Chem. Technol. Biotechnol. 76, 836–843. https://doi.org/10.1002/jctb.453

Mi, S., Song, J., Lin, J., Che, Y., Zheng, H., Lin, J., 2011. Complete genome of Leptospirillum ferriphilum ML-04 provides insight into its physiology and environmental adaptation. J. Microbiol. 49, 890–901. https://doi.org/10.1007/s12275-011-1099-9

Navarro, C.A., von Bernath, D., Jerez, C.A., 2013. Heavy metal resistance strategies of acidophilic bacteria and their acquisition: Importance for biomining and bioremediation. Biol. Res. 46, 363–371. https://doi.org/10.4067/S0716-97602013000400008

Nurmi, P., Özkaya, B., Kaksonen, A.H., Tuovinen, O.H., Puhakka, J.A., 2009. Inhibition kinetics of iron oxidation by Leptospirillum ferriphilum in the presence of ferric, nickel and zinc ions. Hydrometallurgy 97, 137–145. https://doi.org/10.1016/j.hydromet.2009.02.003

Yoo, J.M., Jeong, J., Yoo, K., Lee, J. chun, Kim, W., 2009. Enrichment of the metallic components from waste printed circuit boards by a mechanical separation process using a stamp mill. Waste Manag. 29, 1132–1137. https://doi.org/10.1016/j.wasman.2008.06.035