Recycling bioremediated cyanidation tailings wastewater within the biooxidation circuit for gold recovery: impact on process performance and water management

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Abstract Small scale batch experiments were conducted to assess potential to recycle bioremediated cyanidation tailings water (ASTER™ water) within the biooxidation circuit and reduce nutrient demand of biooxidation operations. Cultures grown in 0K medium or ASTER™ water with and without nutrient supplementation were compared. The microbial community was challenged with thiocyanate (SCN). ASTER™ water cannot provide sufficient nutrients to sustain growth and metabolic activity of the culture. Supplemented ASTER™ water promoted growth and activity comparable to cultures grown in 0 K media. Elevated thiocyanate concentrations (5 mg/L) altered community structure, resulting in loss of iron-oxidising bacterium L. ferriphilum, affecting process performance.

Key words Wastewater recycling, thiocyanate, iron oxidation, sulphur oxidation, mixed biooxidation community

Introduction

Biohydrometallurgical processing schemes are highly dependent on water quality for optimum function, therefore water recycling within these operations has been limited (Stott et al. 2001). The current trend towards integrated, sustainable mining practices within commercial operations and toward 'zero-waste' systems has prompted re-evaluation of plant-wide water management (Mudd 2007; Mudd 2008). Biohydrometallurgical process routes for gold recovery from sulphidic concentrates have typically not recycled cyanidation wastewater due to historical process upsets and the apparent sensitivity of the biooxidation organisms to low levels of thiocyanate (SCN^-) (Adams 2013). The 'rule-of-thumb' exposure limit has been proposed as 1 mg/L SCN^- (van Aswegen et al. 2007); however, data from rigorous study is not yet presented. Cyanidation bioremediation processes such as the Activated Sludge Tailings Effluent Remediation (ASTER™) process have been successful in reducing high levels of SCN^- (± 1500 mg/L) to concentrations of 0.025 and 0.1 mg/L (van Buuren 2014). This suggests potential to close the water cycle within the biooxidation circuit. SCN^- is metabolised by various organisms via reactions (1) to (3) to generate ammonium, sulphate and bicarbonate (Makhotla et al. 2010). These ions, specifically NH_4^+, may contribute to the nutrient requirements of the biooxidation culture that predominantly depend on nitrogen, phosphate and potassium supplemented in the form of sulphate salts (van Aswegen et al. 2007).

$$SCN^- + H_2O \rightarrow HCNO + HS^- \quad (1)$$

$$HCNO + 2H_2O \rightarrow NH_4^+ + HCO_3^- \quad (2)$$

$$SH^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \quad (3)$$

Biooxidation cultures are typically dominated by three species: *Acidithiobacillus caldus, Leptospirillum ferriphilum* and *Acidiplasma cupricumulans*. These microorganisms provide leach agents to facilitate the degradation of the mineral concentrate encapsulating the gold metal, making it available for recovery via cyanidation. In this research, the feasibility of recycling ASTER™ treated cyanidation wastewater within the biooxidation circuit is explored through quantifying resilience of the mixed microbial community used in biooxidation process to SCN^-.

Materials and methods

Microbial culture and growth medium: A mixed moderately thermophilic biooxidation culture, originating from commercial gold biooxidation leaching tanks, was maintained in an aerated 1 L stirred reactor at 45°C and pH 1.3. The culture was maintained at a 15 % w/v solids loading of arsenopyrite/pyrite mineral concentrate in 0 K medium. The reactor was operated in 'draw-and-fill' mode at a residence time of 5 days. The bacterially-dominated culture was comprised of *At. caldus* (42.4%), *L. ferriphilum* (25.8%), other bacteria (29.4%) and *Ac. cupricumulans* (2.4%).

Experimental design: Batch experiments were conducted in multiwell plates (MWPs) using Greiner Bio-one CELLSTAR® 12 Well Suspension Culture Plates (4 ml volume per well). One MWP was used to investigate the effect of thiocyanate (SCN^-) on cell growth, and ferrous and sulphur oxidation activity under SCN^- loadings of 0 mg/L, 0.1 mg/L, 1 mg/L and 5 mg/L using 0 K nutrient medium. The second MWP was set up to investigate the potential for utilizing ASTER™ water as a nutrient solution in place of 0 K medium. ASTER™ water was obtained from a 1 L stirred tank reactor run at similar conditions to that of the commercial ASTER™ operations (Kantor et al. 2015). The continuous ASTER™ reactor was fed 750 mg/L SCN^-, supplemented with molasses and phosphate to give a residence time of 12 hours and an effluent SCN^- concentration of 0.4 mg/L. The ASTER™ water was clarified through a 0.22 μm filter to remove cells associated with SCN^- degradation before use. SCN^- concentrations of 0.4 mg/L, 1 mg/L and 5 mg/L were evaluated. Biooxidation rates in 0 K supplemented ASTER™ water at a SCN^- loading of 1 mg/L were also read.

Each well was charged with the specified nutrient matrix (0 K medium, ASTER™ water or 0 K supplemented ASTER™ water), 5 g/L each of ferrous and ferric iron, 0.5 % (w/v) tyndalised elemental sulphur and the designated volume of SCN^- stock solution (20 mg/L). The wells were inoculated with biooxidation culture at 1 x 10^7 cells/ml. A total working volume of 3 ml at starting pH 1.7 was used. To mitigate evaporative losses, each MWP was fitted with an AeraSeal™ film and placed in a humidified container. The MWPs were incubated in a shaking incubator at 45°C and 140 rpm. During the experiment, 20 μl samples

were withdrawn at regular intervals to analyze ferrous iron concentrations using the 1-10 phenanthroline colorimetric assay. Sulphur oxidation was quantified at the start and end of experiment by ion chromatography. Cell numbers were measured twice daily under phase contrast microscopy and 1000x magnification.

The completion of the experiment was marked by depletion of ferrous iron in the experiments in 0 K medium supplemented with 0, 0.1 and 1 mg/L SCN^- and also the 0 K supplemented ASTER process water with 1 mg/L SCN^- loading. For final cell numbers in excess of 10^8 cells/ml (SCN^- supplemented 0 K medium studies and 0 K and 1 mg/L SCN^- supplemented ASTER™ water study), a 2 ml aliquot from each well was centrifuged at 14, 000 × g for 5 min to pellet cells. For the remaining samples (<10^8 cells/ml), the triplicate wells were combined and centrifuged as described to ensure a sufficient number of cells were harvested for DNA extraction. Cell pellets were washed twice with 10 mM citrate buffer (9.88 mM citric acid anhydrous, 0.22 mM sodium citrate dihydrate, pH 2) to remove soluble iron and neutralised by washing twice with TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8), centrifuging at 14, 000 x g for 5 min between washes. Pellets were stored at -20°C until DNA was extracted using the High Pure PCR Template Preparation Kit (Roche) with a modified extraction protocol. Cells were re-suspended in 200 µl tissue lysis buffer with the inclusion of 50 µg lysozyme and 2 µg RNaseA and incubated at 37°C for 30 min. Following incubation, proteinase K was added as per manufacturer's description and the protocol described for the isolation of DNA from bacteria and yeast followed from this point. DNA extracted was quantified using a Nanodrop® ND2000 spectrophotometer and 10 ng/µl working concentrations prepared for quantitative real-time polymerase chain reaction (qPCR). The qPCR analysis was performed as described using the UniBact, *L. ferriphilum*, *At. caldus*, UniArch and *Ac. cupricumulans* specific primer sets.

Results and discussion

The first set of experiments were designed to assess microbial performance and growth when exposed to a range of SCN^- concentrations characteristically associated with ASTER™ water. A SCN^- concentration of 0.1 mg/L was investigated as it is the typical concentration of residual SCN^- reported in commercial ASTER™ wastewaters (van Buuren 2014). In addition, a SCN^- concentration of 1 mg/L was investigated as it has been suggested as the maximum tolerable concentration for biooxidation organisms (van Aswegen et al. 2007). Furthermore, the impact of potential perturbations in the post-cyanidation bioremediation circuit was evaluated by monitoring oxidation performance when exposed to 5 mg/L SCN^-.

Table 1 illustrates that the maximum specific growth rate (μ_{max}), lag time and maximum cell numbers achieved in the microbial culture in 0 K medium was unaffected by the presence of increasing concentrations of SCN^- up to 1 mg/L. Potentially the μ_{max} was marginally increased at 5 mg/L. Most notably, at 5 mg/L SCN^- the lag time was markedly increased (~2 fold) relative to the cultures with lower SCN^- loadings and the maximum cell concentration achieved at termination of the experiment of the culture exposed to 5 mg/L SCN^- was less than half that of the control culture exposed to 0 mg/L SCN^-. The reduced cell concentration may have been responsible for the negligible ferrous oxidation activity seen at 5 mg/L SCN

in Table 2. The total sulphate generation was unaffected by the presence of SCN^-, even at 5 mg/L, in the 0 K medium test (Table 2).

In the second set of experiments, the potential of ASTER™ water to provide nutrients and its use as a recycled water source for biooxidation was investigated. No cell growth or ferrous oxidation was observed in cultures grown in ASTER™ water only, irrespective of SCN^- concentration (Tables 1 and 2). However, sulphur oxidation activity was detected within the ASTER™ water cultures measured as an increase in sulphate concentration. Interestingly, the culture grown in 0 K supplemented ASTER™ water exhibited a maximum cell concentration circa. 20% higher than that of the culture grown in 0 K medium at the same SCN^- concentration. This may be attributed to the increased supply of nitrogen, phosphate and potassium from the 0 K nutrient solution. The maximum specific growth rate, lag time for microbial growth, average ferrous iron oxidation rate and net sulphate generated are comparable to that of the corresponding 0 K medium culture at 1 mg/L SCN^- (Table 2). Moreover, the 0 K supplemented ASTER™ water culture exhibits a noticeable delay in ferrous utilisation (Figure 1), thereby suggesting that the ASTER™ water may contain constituents that elicit an inhibitory effect.

***Table 1**: Comparison of the maximum cell growth rate, cell growth lag and maximum cell concentration of the mixed biooxidation culture when exposed to SCN- concentrations of interest with 0 K medium as nutrient solution, with ASTER™ water as nutrient medium and with 0 K supplemented ASTER™ water as nutrient matrix*

Medium matrix	SCN^- concentration (mg/L)	μ_{max} (h^{-1})	Lag (h)	Max. cell concentration (10^7 cells/ml)
0 K medium	0	0.073	12.5	36.28
	0.1	0.068	7.5	31.78
	1	0.078	15.5	38.25
	5	0.098	33.1	14.34
ASTER™ water	0.4	0	-	1.56
	1	0	-	1.13
	5	0	-	0.84
ASTER™ water with 0 K medium	1	0.076	9.18	47.25

These findings are supported by the study of Stott et al (2001) who observed that when nutrient solution was prepared with biologically treated cyanidation tailings wastewater with a residual SCN^- concentration less than 1 mg/L, the ferrous iron oxidation of the mixed biooxidation culture was not impeded. They concluded that the by-products of SCN^- degradation present within the bioremediated wastewater do not negatively impact the microbial activity.

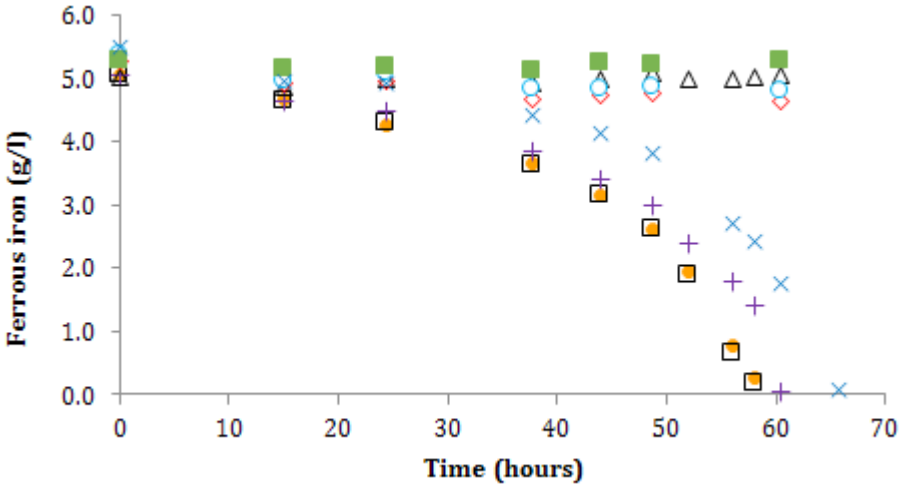

***Figure 1**: Ferrous iron utilisation plot for mixed biooxidation culture exposed to various SCN^- concentrations in the presence of 0 K nutrient solution, Bioremediated cyanidation wastewater and nutrient supplemented bioremediated cyanidation wastewater. Mixed culture in 0 K medium with 0 mg/L SCN^- (●), 0.1 mg/L SCN^- (□), 1 mg/L SCN^- (+) and 5 mg/L SCN^- (Δ). Mixed culture in bioremediated cyanidation wastewater at 0.4 mg/L SCN^- (◊), 1 mg/L SCN^- (○) and 5 mg/L SCN^- (*). Mixed culture in nutrient supplemented bioremediated cyanidation wastewater at 1 mg/L SCN^- (x)*

***Table 2**: Comparison of average ferrous iron oxidation rates and net sulphate generation of a mixed biooxidation culture subjected to increasing concentrations of SCN- with variation of the nutrient solution. Averaged data represents the mean of triplicate measurements with associated standard deviation*

Medium matrix	SCN^- concentration (mg/L)	Average Fe^{2+} oxidation rate (g/L.h)	SO_4^{2-} generated (g/L)
0 K medium	0	0.23 ± 0.012	5.69
	0.1	0.26 ± 0.017	5.43
	1	0.25 ± 0.009	5.80
	5	0	5.75
ASTER™ water	0.4	0	2.30
	1	0	2.57
	5	0	2.89
ASTER™ water with 0 K medium	1	0.22 ± 0.006	5.13

Speciation of the microbial community present in the MWPs following the completion of the experiment indicated the presence of varying proportions of *L. ferriphilum*, *At. caldus* and the archaeon *Ac. cupricumulans* (Figure 2). Interestingly, no proportion of 'other bac-

teria' was detected in these samples. This may suggest that the growth of the 'other bacteria' present in the inoculum may not be favoured under the conditions tested in this experiment. It is unlikely that the absence of the 'other bacteria' is a result of lethal sensitivity to SCN^- as they are also absent in the 0 mg/L SCN^- culture. The 16S rRNA copy numbers detected for *Ac. cupricumulans* accounted for the total archaeal 16S rRNA gene copies detected using the UniArch primer set, suggesting that it is the dominant archaeon present within the community.

The biooxidation culture exposed to SCN^- concentrations of 0.1 mg/L and 1 mg/L in 0 K medium exhibited sustained growth and biooxidation activity similar to that of the control (0 mg/L SCN^-). From Figure 2 it may be inferred that *L. ferriphilum* and *At. caldus* were responsible for the iron and sulphur oxidation activity observed at 0, 0.1 and 1 mg/L SCN^- loadings respectively. At 5 mg/L SCN^- however, *L. ferriphilum* is largely absent (<0.5%) thus demonstrating the sensitivity of *L. ferriphilum* to SCN^- at concentrations above 1 mg/L. *At. caldus* is present as the dominant bacterium within the microbial community when exposed to 5 mg/L SCN^- therefore suggesting that *At. caldus* has a greater tolerance to SCN^- than *L. ferriphilum*. This is further supported by the sulphate generation and iron oxidation rates reported in Table 2. No ferrous iron oxidation was observed at 5 mg/L SCN^- however sulphur oxidation activity was detected.

Microbial growth rates and oxidation activity achieved in the 0 K supplemented ASTER™ water culture relative to the negligible microbial growth and activity in the ASTER™ water culture at 1 mg/L SCN^- (Table 1) suggests that the ASTER™ water alone is nutrient deficient and cannot support the nutrient requirement of biooxidation organisms. It may be noted from Figure 2 that the cultures grown in ASTER™ water generally reflect higher proportions of the archaeon species *Ac. cupricumulans* with the culture exposed to 5 mg/L SCN^- in ASTER™ water reflecting archaeal dominance. The ASTER™ water may have carbon containing organics originating from the metabolic waste products and lysed cellular content of the ASTER™ organisms. Moreover, the effluent water may also retain trace amounts of molasses, a component included as carbon source for the ASTER™ process. These organics may support the growth of the heterotroph *Ac. cupricumulans*. Although *Ac. cupricumulans* can oxidise iron, its ferric production rate has been reported at less than 0.03 h^{-1} at a pH higher than 1.5 (Hawkes et al. 2006). This is less than half the rate of 0.07 h^{-1} achieved by *L. ferriphilum*, under the same conditions (Plumb et al. 2008). Therefore, iron oxidation by this organism was not detected during the short duration of this experiment. The total cell numbers remained low in the ASTER™ water samples and therefore, although an increase in the proportion of *Ac. cupricumulans* was detected within the 5 mg/L culture, it only represented 5.8 x 10^6 cells/ml. This, however, represented a 10 fold increase in the *Ac. cupricumulans* cell numbers compared to that present at the start of the experiment (2.4 x 10^5 cells/ml) based on the inoculated cell concentration of 1 x 10^7 cells/ml. This suggests that ASTER™ water contains beneficial constituents that support *Ac. cupricumulans* cell growth.

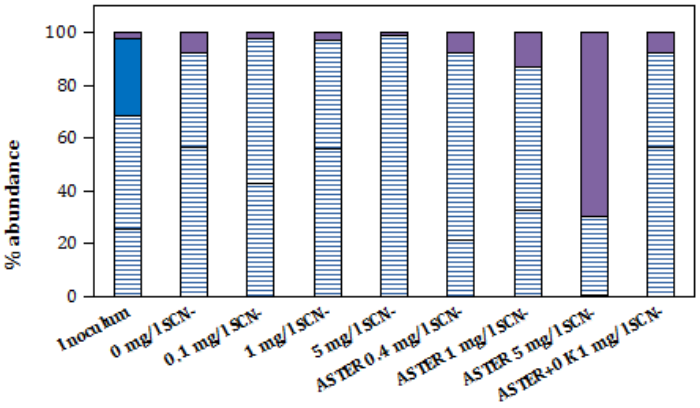

***Figure 2**: Graphical representation of the percentage species abundance of a biooxidation culture exposed to various SCN- concentrations in the presence of 0 K medium, ASTER water or 0 K supplemented ASTER water. Bars indicate the average abundance of L. ferriphilum (▭), At. caldus (▭), other bacteria (■) and Ac. cupricumulans (■). Error bars indicate standard error for samples where three biological replicates were available due to high cell numbers.*

Conclusions

The current study suggests the presence of inhibitors in the ASTER™ water, as the microbial iron utilisation in nutrient supplemented ASTER™ water was delayed relative to the control experiment grown in 0 K medium at the corresponding SCN^- concentration of 1 mg/L. This study further details the shift in the microbial ecology as a function of SCN^- concentration within a biooxidation culture, linked to both the iron oxidation and sulphate generation and thus providing comprehensive insight into the possibility of recycling ASTER™ water within the biooxidation circuit.

Furthermore, it was found that ASTER™ water does not contain a sufficient nutrient profile to sustain microbial growth and activity without supplementation. Nutrient supplemented ASTER™ water, however, was shown to reflect adequate and similar cell growth, ferrous iron oxidation and sulphur oxidation, despite the presence of SCN^- at a concentration of 1 mg/L. A further elevated concentration of SCN^- (5 mg/L) was shown to affect the biooxidation performance and community structure significantly. The microbial cultures exposed to 5 mg/L SCN^- were found to have a negligible proportion of *L. ferriphilum* within the population, consequently resulting in no iron oxidation and reduced overall cell numbers. These results illustrate that ASTER™ water may be recycled successfully within biooxidation circuits, provided the water is appropriately supplemented with nutrients to support microbial growth and that SCN^- concentration does not exceed tolerable limits. Recycling of treated cyanidation wastewater has potential to reduce the water demand associated with the biooxidation operations and to improve water management. Further work is required to assess the feasibility of recycling ASTER™ water through quantifying the impact of prolonged SCN^- exposure on process efficiency, microbial community and its resilience.

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