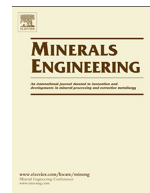
Minerals Engineering 76 (2015) 72–80

Contents lists available at ScienceDirect

Minerals Engineering

journal homepage: www.elsevier.com/locate/mineng



Evaluation of the ASTER™ process in the presence of suspended solids

Andries W. van Zyl, Robert Huddy, Susan T.L. Harrison, Robert P. van Hille *

Centre for Bioprocess Engineering Research, Department of Chemical Engineering, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

ARTICLE INFO

Article history:
Received 11 September 2014
Revised 5 November 2014
Accepted 6 November 2014
Available online 9 December 2014

Keywords:
Thiocyanate destruction
BIOX® process
Biomass retention
Microbial ecology

ABSTRACT

The ability to recycle and reuse process water is a major contributing factor toward increased sustainability in the mining industry. However, the presence of toxic compounds has prevented this in most bioleaching operations. The ASTER™ process has been used for the bioremediation of cyanide (CN) and thiocyanate (SCN^-) containing effluents at demonstration and commercial scale, increasing the potential for recycling of the treated effluent. The process relies on a complex consortium of microorganisms and laboratory tests have shown that the biomass retention, in suspended flocs or attached biofilm, significantly improved SCN^- degradation rates. The current research evaluated the process performance in the presence of suspended solids (up to 5.5% m/v) ahead of implementation at a site where complete tailings removal is not possible. Experiments were performed in four 1 l CSTRs (with three primary reactors in parallel at an 8 h residence time, feeding one secondary reactor at a 2.7 h residence time). Stable operation at the design specifications (5.5% solids, 100 mg/l SCN^- feed, effluent SCN^- <1 mg/l) was achieved within 50 days, including a period of adaptation. The pH had the most significant effect on performance, with significant inhibition below pH 6. The presence of gypsum and anhydrite phases in the fresh tailings was most likely responsible for the observed decrease in pH. A maximum SCN^- degradation rate of >57 mg/l/h was achieved, despite no obvious floc formation. Microbial ecology studies (16S rRNA clone library) revealed reduced diversity relative to reactors operated without suspended solids.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Cyanide is extensively used in various industries, including gold extraction, steel works, for drug manufacturing, pesticide and plastics manufacturing, electroplating and photo development. The most recent figures presented by the International Cyanide Management Code for the Gold Mining Industry (www.cyanidecode.org) indicate that 6% of the 1.1 million tons of cyanide produced each year is used for the production of cyanide reagents for gold processing.

Gold ores can be classified as free milling or refractory. In the case of free milling gold ores, the milled ore is exposed to dilute cyanide solutions, in the presence of oxygen, resulting in the formation of gold-cyanide complexes (Gönen et al., 2004). Refractory gold ores are resistant to recovery by the conventional cyanidation and carbon adsorption processes. The majority of refractory ores contain gold that is associated with or occluded by sulphide minerals, typically pyrite (FeS_2) or arsenopyrite (FeAsS), although a range of base metal sulphides may also be present. As a consequence, froth flotation of the milled ore is normally used to produce a high grade concentrate. The processing options for the sulphide mineral concentrate represent the greatest challenge from a technical perspective and the choice of processing option has a significant impact on the commercial viability.

Historically, roasting of the concentrate was used to remove sulphur and arsenic, after which the calcine was leached. The process had relatively low capital and operating costs, but produced significant amounts of sulphur dioxide gas. The adaptation of the technology to meet more stringent environmental regulations has meant that roasting is no longer attractive from a capital and operating cost perspective. Pressure oxidation (POX) has become a more attractive option, resulting in very high gold recovery, while the cost of neutralising the calcrete is low. The capital costs remain high, particularly as POX operations generally require an on-site oxygen plant.

Technology based on the biological oxidation of sulphide minerals has emerged as a viable alternative, with the BIOX® process leading the way in terms of process development and optimisation. There are currently eight active BIOX® operations, with two more in the development phase (van Niekerk, 2009). Biological oxidation relies on the action of specific iron and sulphur oxidising microorganisms to regenerate ferric iron, the chemical leach agent, and oxidise reduced sulphur compounds to sulphuric acid. The dissolution

* Corresponding author. Tel.: +27 21 6502513; fax: +27 21 6505501.
E-mail address: rob.vanhille@uct.ac.za (R.P. van Hille).

http://dx.doi.org/10.1016/j.mineng.2014.11.007
0892-6875/© 2014 Elsevier Ltd. All rights reserved.

Nomenclature

D	impeller diameter (m)
d_p	particle size (m)
g	acceleration of gravity (m^2/s)
N_{js}	critical impeller speed (rpm)
X	solids mass fraction
S	dimensionless geometrical factor

Greek symbols

ρ	density (kg/m^3)
ρ_L	liquid density (kg/m^3)
ν	kinematic velocity (m^2/s)

of the mineral is primarily a redox reaction, with ferric iron acting as the electron acceptor. Sulphide minerals can be divided into two broad groups on the basis of their leaching characteristics. Acid insoluble minerals, including pyrite, are solubilised by ferric iron. The process requires six single-electron removal steps and results in the release of a thiosulphate intermediate. The thiosulphate can be further oxidised to sulphuric acid by sulphur oxidising microorganisms, or undergo chemical rearrangement to sulphur (S_8) and sulphite. The second group of sulphide minerals are termed acid soluble and include arsenopyrite. These are solubilised by the combined action of ferric iron and protons, releasing hydrogen sulphide, which spontaneously rearranges to polysulphide. The polysulphide may be chemically oxidised, by ferric iron, to sulphur or biologically oxidised to sulphuric acid (Schippers and Sand, 1999).

The sulphide oxidation efficiency of a typical BIOX® operation is greater than 95%, indicating that a certain amount of reduced sulphur passes into the cyanidation reactor. Laboratory tests have shown that sulphide minerals are unlikely to react with cyanide, while the reaction of elemental sulphur and cyanide is slow. However, the two most reactive forms of sulphur are polysulphide and thiosulphate (Dzombak et al., 2005). These react rapidly with cyanide to form thiocyanate (SCN^-). Despite passing through the counter current decantation (CCD) circuit, it is likely that some reduced sulphur intermediates, particularly thiosulphate, would be retained in the interstitial liquid and enter the carbon in leach (CIL) circuit. Thiocyanate concentrations of up to 1500 mg/l have been recorded in coke wastewater (Luthy and Bruce, 1979; Oulego et al., 2014) and up to 3000 mg/l in cyanidation tailings effluent from gold operations (Soto et al., 1995; Stott et al., 2001; van Buuren, *pers. comm.*).

Thiocyanate is particularly toxic to bioleaching organisms, with significant inhibition of iron and sulphur oxidation observed at concentrations greater than 1 mg/l (Suzuki et al., 1999; Olson et al., 2006). This precludes the recycling of untreated process water upfront of the leaching reactors and negatively impacts the overall water balance of the plant.

The treatment of waste from cyanidation processes ranges from natural destruction of simple-cyanide solutions, which is a slow process free of human intervention, to complex processes treating slurries which require specialised infrastructure and technical knowledge. Treatment technologies can be classified into three broad groups: (1) physical processes, (2) chemical processes or (3) biological processes (Akcil, 2003; Baxter and Cummings, 2006). The majority of chemical processes target the oxidation of free cyanide to the less harmful cyanate (OCN^-), and achieve little thiocyanate destruction.

Cyanide (HCN and CN^-) and thiocyanate are biodegradable under both aerobic and anaerobic conditions and can be used as the sole nitrogen source by a range of microorganisms. A number of metabolic pathways have been proposed for this. These are reviewed in Dzombak et al. (2005). Cyanide degrading microorganisms have been employed in a number of biological processes, utilising both suspended culture and attached biomass systems, for the treatment of cyanide containing effluents (Whitlock, 1987; Whitlock and Smith, 1989; Kuyucak and Akcil, 2013).

A collaborative project between Gold Fields and BHP Billiton was initiated in the mid 1990's to develop a process to treat CN and SCN^- containing water from BIOX® operations. The resulting ASTER™ process is an example of an activated sludge type biological treatment system. The initial development work was conducted at laboratory scale in an aerated glass reactor (4 l). Fresh feed was pumped in continuously and the effluent cascaded into a clarifier, where the solid sludge was settled. The sludge could be recycled back to the reactor or purged. Compressed air was injected into the reactor to provide sufficient oxygen for the oxidation reactions and to ensure sludge suspension. Specific thiocyanate degradation rates of 14–17 mg/l h per g dry biomass were achieved at feed concentrations between 200 and 550 mg/l SCN^- (van Buuren et al., 2011). Process development continued through increasingly large laboratory (80 l), pilot (2 and 6 m^3) and demonstration scale (25 m^3) systems. The first commercial scale system was commissioned at the Consort Mine in Mpumalanga, South Africa and was not coupled to a BIOX® plant. The ASTER™ plant at Consort Mine was designed to treat 320 m^3/d at a feed SCN^- concentration of 120 mg/l and free cyanide at 10–30 mg/l (van Buuren et al., 2011). It has operated successfully since September 2010. A second, larger plant was commissioned in 2013 to treat effluent from the Suzdal BIOX® plant in Kazakhstan. The plant is currently treating 800 m^3/d at a feed SCN^- concentration of 3500 mg/l (van Buuren, *pers. comm.*).

Based on the relevance of the ASTER™ process for the remediation of a wide range of effluent streams, the Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town has been conducting both applied and fundamental research on the ASTER™ process since 2010 to interrogate the bounds of the operating window, to establish factors influencing process performance and robustness, to determine key requirements for reactor design and to provide fundamental insight into the complex mixed microbial community implicated in thiocyanate and cyanide degradation. The research has focused on determining the effective operating window (van Zyl et al., 2011; van Zyl et al., submitted for publication), characterising the microbial community (Huddy et al., 2015) and optimising the thiocyanate degradation rate. Degradation rates in excess of 125 mg/l h have been achieved, although high rates have been dependent on efficient biomass retention, either through recycling of suspended flocs or the colonisation of the reactor surface by a thick biofilm (van Zyl, 2014).

The work described in this paper investigates the potential to operate the ASTER™ in the presence of suspended solids. To date, complete removal of the tailings from the liquid effluent stream has been achieved prior to application of the ASTER™ process. However, a number of advantages result from processing in the presence of tailings, including the remediation of the tailings stream. This study was motivated by the proposed exploitation of a refractory gold deposit in the Philippines, using the BIOX® technology. The topography of proposed site constrained the

overall footprint of the plant, such that complete removal of the tailings from the cyanidation effluent is not possible. It is estimated that the feed to the ASTER™ plant will contain between 4% and 6% (m/v) suspended solids. The aim of the research was to determine the impact of these residual solids on the ASTER™ community and its associated performance in terms of thiocyanate degradation, using the reactor configuration of the proposed ASTER™ plant.

2. Materials and methods

2.1. Mineral solids

The solid material was provided by Gold Fields in two batches. It was generated by SGS during small scale leaching and gold recovery tests as part of the bankable feasibility process for the development of the Runruno operation in the Philippines. The solids represented the expected size and composition of tailings to be produced during full scale operation. The characteristics of the solids were consistent for both batches, being fine grained, with a D_{50} of 6.122 μm (D_{10} of 0.939 μm and D_{90} of 38.026 μm). The measured density was 2.677 g/ml.

2.2. The ASTER™ culture

The mixed microbial consortium used to inoculate the reactors was derived from the stock ASTER™ culture (Huddy et al., 2015). A sample from the stock reactor was removed and pre-adapted to suspended solids (5.5% m/v) in a series of 500 ml Erlenmeyer flasks, agitated at 160 rpm on an orbital shaker. The growth medium contained SCN^- (100 mg/l, as KSCN), molasses (150 mg/l) and phosphate (27 mg/l, as KH_2PO_4).

2.3. Reactor system

The work was conducted using four continuously stirred tank reactors, each with an operating volume of 1 l. Each reactor had a height of 220 mm (liquid volume height of 118 mm), a diameter of 104 mm and was fitted with four vertical baffles (10 mm width). Three of the reactors were operated as primary reactors, in parallel, with the effluent from each combined to pass into a single secondary reactor, to remain consistent with the proposed plant design. Each reactor was agitated using a four bladed marine impeller (58 mm diameter) connected to a variable speed overhead stirrer. The impeller was set at 36.7 mm from the base of the reactor. Air was supplied through an L-shaped sparger (tip length 40 mm) at a rate of 840 ml/min. Feed solution, containing the solids, thiocyanate, and basal medium of molasses (150 mg/l) and phosphate (27 mg/l, as KH_2PO_4), was prepared in a separate 10 l container. The pH of the feed was initially adjusted to pH 7.0 using sodium hydroxide. The feed in the container was agitated (marine impeller at 700 rpm) vigorously, to ensure complete solids suspension and feed was pumped to the primary reactors at the desired rate using a variable speed peristaltic pump. The reactors were sampled daily to determine pH, redox potential and residual thiocyanate concentration, the latter by HPLC (van Zyl et al., 2011). In addition, samples were taken periodically to quantify solids suspension efficiency, estimate cell concentration and determine residual ammonia by HPLC.

2.4. Experimental programme

The reactors for the experimental work were configured to replicate the proposed design of the Runruno ASTER™ plant and the experimental parameters selected to evaluate whether the system could meet the design specifications (reduction of SCN^- from a feed concentration of 100 mg/l to below 1 mg/l in the effluent in the presence of suspended solids at 5.5%, with a hydraulic residence time in the primary reactors of 8 h). A number of the interventions described below were in response to system performance, so the programme is described chronologically.

Each of the primary reactors was inoculated with 200 ml of the adapted culture and 800 ml of basal medium. The initial thiocyanate concentration was controlled at 100 mg/l. The reactors were initially operated in the presence of 5.5% solids, with a residence time in the primary reactors of 8 h, resulting in a residence time in the secondary reactor of 2.7 h. The impeller speed in all reactors was set to 270 rpm. Temperature was not specifically controlled, but the pilot plant resided in an air conditioned laboratory (22 ± 2 °C).

However, performance under these conditions was poor, with only limited SCN^- degradation. From day 7, the solids were removed from the feed. Once complete degradation of the SCN^- was achieved in the primary reactors (day 26), solids were gradually introduced over a 23 day period, at increments equivalent to 1–1.5% total loading, until the desired loading of 5.5% was reached (day 49).

The system was operated under the desired specifications (5.5% solids, 8 h residence time in primary reactors, residual SCN^- below 1 mg/l in effluent from the secondary reactor) between day 49 and day 77 (84 residence times), although some accumulation of solids at the bottom of the reactors was observed. This was confirmed experimentally by increasing the stirrer speed (to 500 and 700 rpm) for short periods and measuring solids suspension. The impeller speed was increased to 400 rpm on day 75 and further to 500 rpm on day 77. The latter coincided with an increase in feed SCN^- concentration to 125 mg/l. On day 81, following a period of poor performance, the operating conditions were restored to previous levels (100 mg/l, 270 rpm), but performance remained erratic.

From day 102 the pH of the feed was increased from pH 7 to pH 9 and the residence time in the primary reactors was increased from 8 to 12 h on day 105. The feed SCN^- concentration was incrementally increased from 100 to 300 mg/l (increments of 50 mg/l) between day 107 and 157. On day 170 the residence time in the primary reactors was decreased to 10 h and further to 8 h on day 177. Finally, on day 180, the feed SCN^- concentration was increased to 450 mg/l and the reactors were operated under these conditions until the end of the experiment (day 198).

2.5. Community structure analysis

A dilution series of a 10 ml reactor sample, harvested using a sterile syringe, was prepared from the reactor in sterile basal growth medium (pH 7.0) and spread onto solid reactor medium (reactor growth medium with the addition of 15 g/l bacteriological agar, 50 mg/l SCN^- and 50 μg/ml Cyclohexamide), yeast extract malt extract agar (YEME; 4 g/l yeast extract, 10 g/l malt extract, 4 g/l glucose, 15 g/l bacteriological agar, 50 mg/l SCN^- and 50 μg/ml Cyclohexamide, pH 7.3) and Czapek solution agar (30 g/l sucrose, 2 g/l $NaNO_3$, 1 g/l K_2HPO_4, 0.5 g/l KCl, 0.5 g/l $MgSO_4.7H_2O$, 10 mg/l $FeSO_4.7H_2O$, 15 g/l bacteriological agar, 50 mg/l SCN^- and 50 μg/ml Cyclohexamide, pH 7.3) for the isolation of prokaryotes. Diluted samples were also plated onto Sabourand agar (10 g/l peptone, 40 g/l glucose, 15 g/l bacteriological agar, 50 mg/l SCN^- and 30 μg/ml Chloramphenicol) for the isolation of eukaryotes. The plates were incubated at 22 °C and individual colonies, displaying a unique phenotype, were aseptically transferred onto fresh solid growth media, to confirm culture purity, before being inoculated into liquid growth media (prepared as described above with the omission of bacteriological agar). Cultures were incubated at

22 °C on a rotary shaker, at 200 rpm, for 48 h or until the liquid medium appeared turbid, indicating growth of the organisms. Genomic DNA was extracted from the respective liquid cultures and used as the template for the PCR amplification of 16S and 18S rRNA genes from the individual isolates. The PCR were performed using the universal bacterial, 27F (5′-GAGAGTTTGATCIT GGCTCAG-3′) and 1492R (5′-GTACGGITACCTTGTTACGACTT-3′), and universal 18S rRNA, Uni18S-F (5′-GAAACTGCGAATGGCTC-3′) and Uni18S-R (5′-CACCTACGGAAACCTTGTTA-3′), primers respectively. Cycling conditions for the universal bacterial primers included an initial denaturation step (6 min at 96 °C), followed by 35 cycles of denaturation (15 s at 96 °C), annealing (15 s at 60 °C) and extension (20 s at 72 °C), followed by a final extension (5 min at 72 °C). Cycling conditions for the universal 18S rRNA primers included an initial denaturation step (6 min at 96 °C), followed by 30 cycles of denaturation (10 s at 96 °C), annealing (15 s at 60 °C), and extension (20 s at 72 °C), followed by a final extension (5 min at 72 °C). The PCR for both primer pairs was carried out in 50 μl reaction volumes, containing 2 mM $MgCl_2$, 1 U Kapa*Taq* polymerase (Fermentas), 200 μM dNTPs, 0.5 μM of each primer and approximately 20 ng of total genomic DNA. The PCR products were evaluated following electrophoresis on a 0.8% (w/v) TAE agarose gel. The PCR amplicons were purified using a PCR purification kit (Macher-Nagel) and cloned into the pGEM®-T Easy vector (Promega), according to the manufacturer's instructions.

To assess the culture-independent microbial community, total genomic DNA was extracted from a 150 ml reactor sample using the High Pure PCR Template Preparation Kit (Roche Applied Sciences), according the manufacturer's instructions. The 16S and 18S rRNA genes of the individual isolates contained within the reactor sample were amplified by PCR and cloned into the pGEM®-T Easy vector system, as described above. Recombinant clones were grouped into Operational Taxonomic Units (OTU), based on Amplified Ribosomal DNA Restriction Analysis (ARDRA) restriction patterns generated by single digestions using *Alu*I and *Hae*III (Fermentas).

A single representative of each 16S and 18S rDNA OTU was sequenced using the universal M13F (5′-GTAAAACGACGGCCAGT-3′) and M13R (5′-GCGGATAACAATTTCACACAGG-3′) primers (Macrogen). Homology and similarity searches of DNA consensus sequences were performed using the nucleotide–nucleotide basic local alignment search tool (BLASTN) program (Altschul et al., 1989; Altschul et al., 1997), as provided by the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/). For phylogenetic analysis, reference strains, selected based on lowest *E*-values or highest sequence similarity from BLASTN results, were included. Sequences were downloaded from the GenBank database (NCBI), aligned using CLUSTAL_X, version 2.1 (Larkin et al., 2007) and a portion of the respective 16S and 18S rRNA sequences were manually edited to a common length. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011) and a neighbour-joining (Saitou and Nei, 1987) tree constructed. Bootstrap values were based upon 1000 re-sampled data sets (Felsenstein, 1985) and only bootstrap values greater than 40% are indicated.

3. Results and discussion

3.1. System performance

The initial attempt to operate the system under conditions similar to those anticipated at the operation (100 mg/l SCN^-, 8 hour residence time, 5.5% solids) was unsuccessful, despite the adaptation of the culture to the presence of 5.5% solids in shake flasks. The residual SCN^- concentrations in all reactors, including the secondary, increased rapidly (Fig. 1) with the SCN^- degradation efficiency below 20%. It is well recognised that the solids suspension achieved in shake flasks and well-mixed stirred tank reactors is not the same. In the shake flask, the expanded solids bed provides substantial attrition between particles, whereas in a well mixed stirred tank, particulates are fully suspended from the floor of the vessel and carry a higher momentum. This impacts particle–particle collision events (Scholtz et al., 1997) and may result in increased shear stress associated with the impeller, particularly where cell aggregates or flocs form (Illing and Harrison, 1999). The removal of solids from the feed resulted in a rapid improvement in performance, with the residual SCN^- concentration in the secondary reactor falling below the desired threshold (1 mg/l) by day 16. This confirmed the negative impact of suspended solids on process performance in the reactor system in which adaptation has not been achieved.

The reintroduction of solids, from day 26, was performed gradually. Effective SCN^- degradation, to less than 1 mg/l, was observed in the primary reactors at 2% and 3% solids loading. A further increase to 4% solids resulted in some inconsistency in primary reactor performance, particularly in primary reactor 1, where SCN^- concentrations of up to 20 mg/l were observed. During this period the SCN^- concentration in the effluent from the secondary reactor was also above 1 mg/l (Fig. 1), indicating that very little SCN^- degradation had occurred in the secondary reactor. However, by day 46 the system had stabilised, with residual SCN^- concentrations across all three primary reactors at below 1 mg/l, so the solids loading in the feed vessel was increased to 5.5%. This represented the anticipated operating conditions at the Runruno plant. The laboratory scale system performed well under these conditions, with almost complete SCN^- degradation observed across the primary reactors and on the occasions when some residual SCN^- was detected in a primary reactor, this was completely degraded in the secondary reactor. At this point, proof of concept had been successfully demonstrated.

Visual observation of the primary reactors indicated some accumulation of solids at the bottom of the reactors. Prior to setting up the reactors, a mixing study was conducted to determine empirically the critical impeller speed (N_{js}) required to ensure just off-bottom suspension of all particles. Triplicate samples (2 ml) were taken from three different levels (top, middle and just off bottom) and suspended solids mass calculated. The preliminary study suggested that 270 rpm was sufficient to achieve complete suspension, within the error associated with the gravimetric assay. However, during extended operation (84 residence times at 5.5% solids loading) there was some accumulation of the coarser material. A subsequent calculation of N_{js}, based on the Zwietering equation (Eq. (1)) (Zwietering, 1958), using an S value of 4.65, calculated assuming an exponential dependence of critical impeller speed on the relationship between impeller clearance and tank diameter (Armenante and Nagamine, 1988), yielded a value of 314 rpm, which would account for some accumulation of solids.

$$N_{js} = S\frac{\upsilon^{0.1} d_p^{0.2}\left(\frac{g\Delta\rho}{\rho_L}\right)^{0.45} X^{0.13}}{D^{0.85}} \quad (1)$$

The volume in the reactor was maintained by a level control overflow port near the top of the reactor, so only particles that were suspended to that level would exit the reactor. The increase in impeller speed to 500 rpm resulted in a measured solids concentration of 7.45 ± 0.25% across the three primary reactors within 15 minutes of the change.

The solids used in this study were generated during small-scale metallurgical testing, to assess gold recovery, so a limited mass was provided at the start. As a consequence, the solids had to be recycled by centrifuging the accumulated effluent from the secondary reactor and drying the recovered material (70 °C for

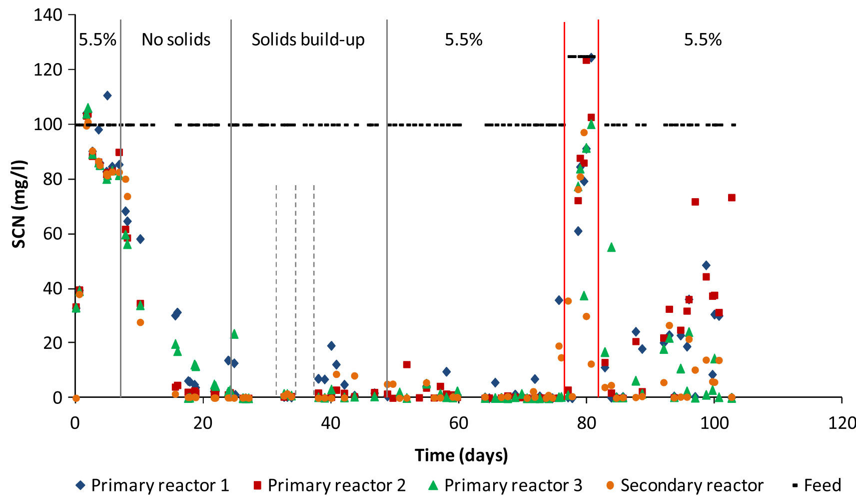


Fig. 1. Summary of performance data across the three primary and one secondary reactor. Data represent residual SCN^- concentrations. Solids build-up began on day 24 and dashed lines indicate increases in solids loading (2%, 3% and 4%). Impeller speed was increased from 270 to 500 rpm between day 77 and 81. The feed adjusted to pH 7 across the time span 0–102 days.

48 h). This was a time consuming and labour intensive process. Midway through the experiment a second batch of tailings (5 kg) was provided, following further metallurgical testing. The particle size distribution and density were similar to the first batch and these solids were introduced into the reactor system from day 77. As a result, no solids recycling was required for approximately 20 days.

The combined effect of the increased agitation rate, increased feed SCN^- concentration and use of fresh solids was a rapid and significant decrease in performance across the system (Fig. 1), with residual SCN^- concentrations increasing to 100 mg/l. Reducing the feed SCN^- concentration back to 100 mg/l and the impeller speed to 270 rpm resulted in some improvement, but overall performance remained inconsistent, with the SCN^- concentration in the effluent from the secondary reactor remaining between 10 and 20 mg/l. This period coincided with a decrease in the measured pH in the reactors, which will be discussed in greater detail later.

The pH of the feed suspension was increased from pH 7 to pH 9 on day 102 and the feed rate was reduced on day 105, resulting in an increase in residence time from 8 to 12 h in the primary reactors. These interventions resulted in a rapid improvement in performance, with SCN^- concentrations falling below 1 mg/l in all reactors (Fig. 2). With stable performance achieved, the system was challenged with increasing SCN^- concentrations. While each incremental increase resulted in a short term increase in residual SCN^- in the primary reactors, all SCN^- passing into the secondary reactor was degraded and the effluent SCN^- concentration remained below 1 mg/l for the duration.

Following stable performance (20 residence times) at a feed SCN^- concentration of 300 mg/l the residence time in the primary reactors was reduced to 10 h (day 170). This had no impact on performance, so the residence time was further reduced, back to the original design specification of 8 h. This resulted in a transient increase in the SCN^- concentration in the primary reactors, but again complete degradation was achieved in the secondary reactor. Finally, on day 180, the feed SCN^- concentration was increased from 300 to 450 mg/l, again with no effect on the overall performance of the system (Fig. 2).

The thiocyanate degradation rate data are represented in Fig. 3. The periods of unstable performance are clearly visible, particularly over the first ten days and between day 77 and 102. During periods of efficient performance, the SCN^- degradation rate in the secondary reactor was low, due to the low residual SCN^- concentrations in the primary reactors. The two points, around day 80,

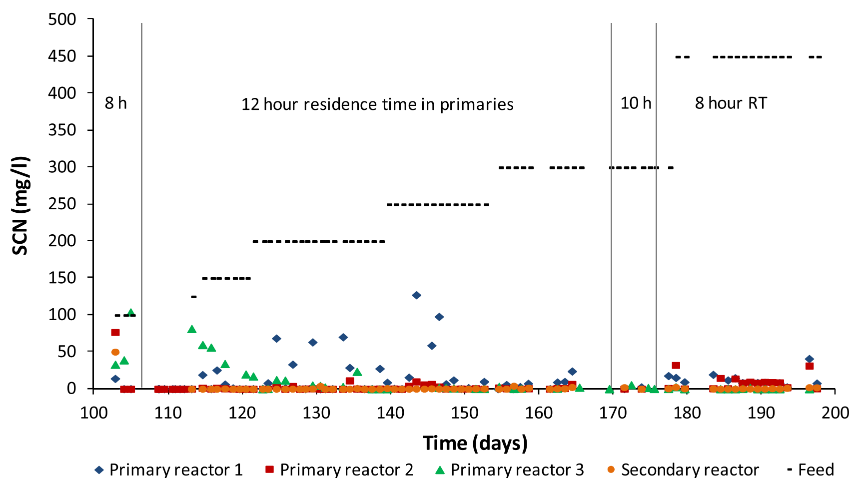


Fig. 2. Summary of performance data across the three primary and one secondary reactor from day 102. Data represents residual SCN^- concentrations. Increases in feed concentration and changes in residence time are indicated. The feed pH was adjusted to pH 9 from day 102 to 198.

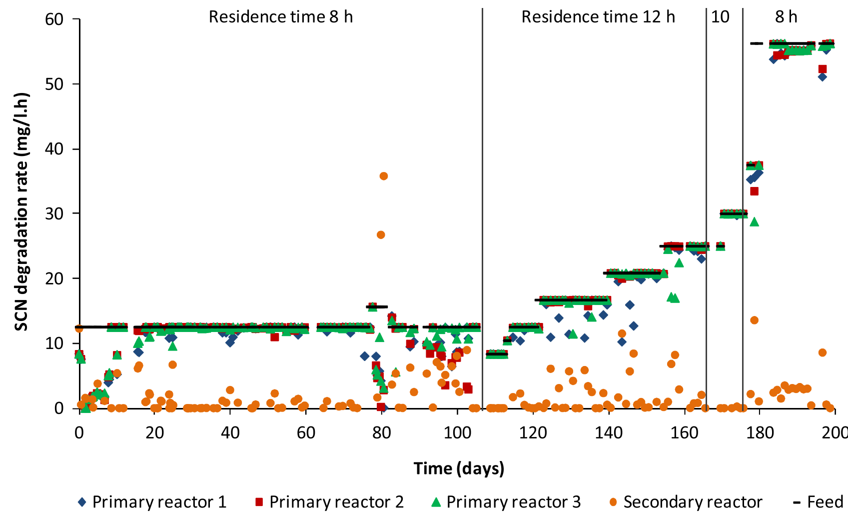


Fig. 3. Calculated SCN^- degradation rates for each of the reactors. The loading rate to the primary reactors is represented by the feed (–), while the loading to the secondary was calculated based on SCN^- concentrations in the primary reactors.

where the rate in the secondary reactor was high were due to the rapid failure of the primary reactors, resulting in a very high loading to the secondary. During the period between day 81 and 102, the degradation rate in the secondary reactor was consistently lower than that of the primary reactors. This is not a consequence of limited loading, as SCN^- was detected in the effluent from the secondary reactor, which suggests inhibition of the microbial culture. This was most likely related to the pH.

3.2. Effect of pH on system performance

The pH during the first days of the study was between pH 5.3 and pH 6.1 (Fig. 4), despite the feed having been adjusted to pH 7. During the period over which the system was operated without solids the pH in the reactors was stable between pH 6.5 and 7 and this continued during the solids build-up phase, with the occasional exception. The decreased pH around day 40 was associated with the increase in solids loading from 3% to 4%, while the decrease around day 55 coincided with the increase from 4% to 5.5%. The most substantial decrease in pH occurred after day 75, when the new batch of solids was introduced to the reactor, with the pH dropping to below pH 5 in many cases. This suggested that, when agitated, the solids react with the reactor medium, releasing acidity or consuming alkalinity, resulting in a decrease in the measured pH. This phenomenon was confirmed in offline tests. In addition to the pH change, agitation of the solids resulted in the leaching of calcium and sulphate. The effect became progressively less significant when recycled solids were used, explaining relatively stable pH up to day 77 and the significant decrease thereafter. Fresh solids were used to perform quantitative X-ray diffraction (XRD) to determine the relative proportion of crystalline phases. The results are summarised in Table 1.

The dominant mineral phases were bytownite, a calcium rich member of the plagioclase solid solution series of feldspar minerals, muscovite, an aluminium and potassium rich phyllosilicate and quartz. These minerals accounted for over 60% of the total composition, but have very low solubility so would not have influenced the solution chemistry. The gypsum and anhydrite (anhydrous $CaSO_4$) have greater solubility and their partial dissolution would account for the increase in calcium and sulphate concentrations. Gypsum (Ehrlich and Wygal, 1977) and anhydrite (Kazempour et al., 2012) have been shown to be strong consumers of alkalinity,

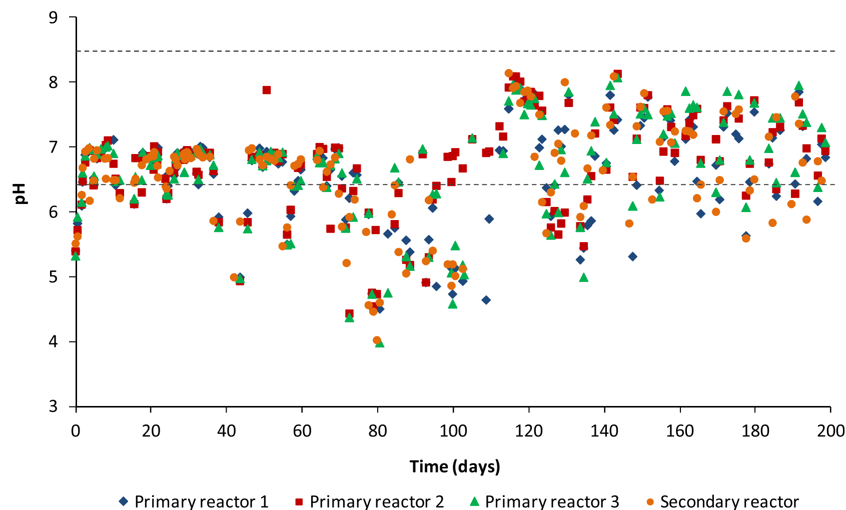


Fig. 4. Summary of pH data for the duration of the experiment. From day 0 to day 102 the pH of the feed solution was initially adjusted to pH 7 after which it was adjusted to pH 9. The dashed lines represent the optimal pH window, determined in a parallel study.

Table 1
Summary of the composition of the fresh tailings material as determined by quantitative X-ray diffraction.

Mineral	Relative (%)	Mineral	Relative (%)	Mineral	Relative (%)
Quartz	10.5	Hematite	0.05	Titanite	5.62
Chlorite	6.0	Magnetite	1.50	Anhydrite	1.0
Muscovite	20.9	Beryl	2.27	Gypsum	5.11
Zircon	0.5	Pentlandite	1.15	Anglesite	1.91
Monazite	2.2	Dolomite	2.78	Jarosite	7.36
Bytownite	31.1	Szomolnokite	0.37		

which would account for the decrease in pH when fresh solids were added. Recycled solids had a limited impact on pH, indicating that the soluble calcium sulphate mineral phases were largely consumed during the initial exposure.

The apparent relationship between pH and performance of the system prompted a parallel investigation to determine the effective operating window, with respect to pH. The study identified an optimum between pH 7 and pH 7.5, with decrease in degradation rate of over 50% below pH 6.5 and above pH 8.5 (van Zyl et al., submitted for publication). This is consistent with the performance data from the reactor system.

3.3. Microbial community structure

The presence of suspended solids had a significant impact on the habit of the microbial community. The stock reactor, in the absence of solids, became colonised with a thick, gelatinous biofilm. The biofilm was densely packed with microbial cells, with several distinct morphologies visible (Huddy et al., 2015). As the biofilm developed the planktonic cell concentration decreased, to around 2×10^6 cells/ml, suggesting the majority of the biomass was confined to the biofilm. The absence of attached biofilm and the relatively higher planktonic cell concentration ($1–3 \times 10^7$ cells/ml) prompted a more detailed investigation of the microbial community in the reactors with solids.

The microbial community was characterised by a combination of culture-dependent and –independent methods. Single PCR amplicons were observed for both 16S and 18S rRNA genes (data not shown), from total DNA extracted from the reactor, indicating the presence of bacteria and eukaryotes, respectively. A total of 16 bacterial and three eukaryotic isolates were cultured. In addition, 48 clones of the bacterial 16S rRNA gene clone library were analysed by Amplified Ribosomal DNA Restriction Analysis (ARDRA) and 30 ARDRA patterns were selected for sequencing. A total of 24 18S rRNA gene library clones were analysed by ARDRA and four were sequenced. The sequenced Operational Taxonomic Units

Fig. 5. Unrooted 16S rRNA gene phylogenetic tree of 16S rRNA library clones, generated with universal bacterial primers, and prokaryotic isolates from the ASTER™ stock reactor and related sequences. The tree was based on the sequence alignment of a common length portion (1,420 nucleotides) and was obtained using the neighbour-joining method. Bootstrap values are based upon 1000 resampled data sets and only values of greater than 40% are indicated. Accession numbers (where available) are shown in brackets. The bar represents 0.05 nucleotide substitutions per nucleotide position. *Methanoculleus bourgensis* was included as an outlier.

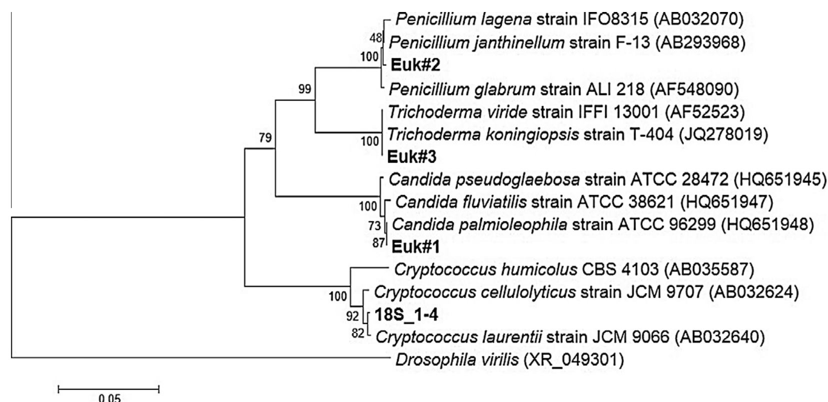


Fig. 6. Unrooted 18S rRNA gene phylogenetic tree of 4 clones, generated with universal 18S rRNA primers from the ASTER™ stock reactor and related sequences. The tree was based on the sequence alignment of a common length portion (1140 nucleotides) and was obtained using the neighbour-joining method. Bootstrap values are based upon 1000 resampled data sets. Accession numbers (where available) are shown in brackets. The bar represents 0.05 nucleotide substitutions per nucleotide position. *Drosphila virilis* was included as an outlier.

(OTUs) formed the basis for the 16S and 18S rRNA phylogenetic analyses of the bacterial and eukaryotic communities within the reactor system.

The analyses indicated approximately 21 different bacterial genera within the 16S rRNA clone library (Fig. 5). Of particular interest are the 16S rRNA phylotypes found to be closely related to *Bosea, Microbacterium* and *Thiobacillus* species. van Buuren and co-workers (2011) implicated *Bosea thiooxidans* and *Microbacterium schleiferi* as dominant ASTER^{TM} consortium members involved in the biodegradation of thiocyanate.

The 18S rRNA gene clone library revealed the presence of four eukaryotes within the solids reactor system (Fig. 6). All four are fungi, with two identified as filamentous fungi, namely Euk#2 and Euk#3, and two yeasts, namely Euk#1 and 18S_1-4. The cultivated yeast isolate, Euk#1, is related to a *Candida* species. *Candida humulis* has previously been shown to be present in the ASTER™ microbial consortium (du Plessis et al., 2001). To date we have not identified a fungal species closely related to *Fusarium oxysporium*, within the solids reactor system. *F. oxysporium* has been previously shown to be capable of cyanide degradation as well as being present in the ASTER™ microbial consortium (du Plessis et al., 2001).

4. Conclusions

The data presented in this paper show that efficient thiocyanate degradation is possible in the presence of suspended solids, at solids concentrations of up to 5.5%. A period of adaptation was required prior to stable performance being achieved. The ease with which the community is able to adapt appears dependent on the properties of the solid material. Adaptation to tailings with a greater mean particle size and density has progressed more slowly. Thiocyanate degradation rates of up to 57 mg/l h were achieved in the primary reactors during the current study and ongoing research suggests these can be improved further.

The primary constraint on performance of the system was found to be pH, with the efficiency of thiocyanate degradation falling sharply when the pH in the reactors fell below pH 6. The optimal pH window for the community was determined to be between pH 6.5 and pH 8.5 in a parallel study. The tailings provided for this study resulted in the consumption of alkalinity in the medium, coupled with the release of calcium and sulphate. This suggests that pH control will be a critical element of the process operation at Runruno.

The presence of suspended solids had a profound effect on the composition of the microbial community, with a significant reduction in diversity relative to reactors operated in the absence of solids. This appears to be related to the absence of biofilm and the anaerobic and microaerobic microenvironments associated with the biofilm. Despite the lower diversity and the lack of biomass retention, high rates of thiocyanate degradation were achieved.

The results show that the ASTER^{TM} process is viable in the presence of suspended solids and that the thiocyanate degradation rates required to treat the anticipated effluent from the Runruno operation should be comfortably achieved. The addition of the ASTER^{TM} process to the overall flow sheet offers the possibility of recycling large volumes of process water that had previously been discharged to the tailings impoundments, significantly improving the overall water balance and sustainability of the operation.

Acknowledgements

The authors thank Dr Nico Fischer and Dr Kirsten Corin for their assistance with the XRD analysis and data interpretation. The authors would like to acknowledge Gold Fields Ltd. and Biomin SA for their financial support, supply of materials and valuable discussions. Sue Harrison and Andries van Zyl acknowledge the financial support from the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology (DST) and National Research Foundation (NRF) in South Africa. Robert Huddy acknowledges the financial support from the South African DST and NRF Innovation Postdoctoral Fellowship.

References

Akcil, A., 2003. Destruction of cyanide in gold mill effluents: biological versus chemical treatments. Biotechnol. Adv. 21, 501–511.
Altschul, S.F., Gish, W., Miller, W., Meyers, E., Lipman, D.J., 1989. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
Armenante, P.M., Nagamine, E.U., 1988. Effect of low off-bottom impeller clearance on the minimum agitation speed for complete suspension of solids in stirred tanks. Chem. Eng. Sci. 53, 1757–1775.
Baxter, J., Cummings, S.P., 2006. The current and future applications of microorganism in the bioremediation of cyanide contamination. Anton. Leeuw. Int. J.G. 90, 1–17.
du Plessis, C.A., Barnard, P., Muhlbauer, R.M., Naldrett, K., 2001. Empirical model for the autotrophic biodegradation of thiocyanate in an activated sludge reactor. Lett. Appl. Microbiol. 32, 103–107.
Ehrlich, R., Wygal, R.J., 1977. Interrelation of crude-oil and rock properties with recovery of oil by caustic water flooding. Soc. Petrol. Eng. J. 17, 263–270.
Dzombak, D.A., Ghosh, R.S., Wong-Chong, G.M., 2005. Cyanide in Water and Soil: Chemistry, Risk, and Management, second ed. CRC Press, USA.
Felsenstein, J., 1985. An approach to using the bootstrap. Evolution 39, 783–791.

Gönen, N., Kabasakal, O.S., Özdil, G., 2004. Recovery of cyanide in gold leach waste solution by volatilization and absorption. J. Hazard. Mater. 113, 231–236.

Huddy, R., van Zyl, A.W., van Hille, R.P., Harrison, S.T.L., 2015. Characterisation of the complex microbial community associated with the ASTER^{TM} thiocyanate biodegradation system. Miner. Eng. 76, 65–71.

Illing, S., Harrison, S.T.L., 1999. The kinetics and mechanism of *Corynebacterium glutamicum* aggregate breakup in bioreactors. Chem. Eng. Sci. 54, 441–454.

Kazempour, M., Sundstrom, E., Alvarado, V., 2012. Geochemical modeling and experimental evaluation of high-pH floods: impact of Water–Rock interactions in sandstone. Fuel 92, 216–230.

Kuyucak, N., Akcil, A., 2013. Cyanide and removal options from effluents in gold mining and metallurgical processes. Miner. Eng. 50–51, 13–29.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Luthy, R.G., Bruce, S.G., 1979. Kinetics of reaction of cyanide and reduced sulfur species in aqueous solution. Environ. Sci. Technol. 11, 1481–1487.

Olson, G.J., Brierley, C.L., Briggs, A.P., Calmet, E., 2006. Biooxidation of thiocyanate-containing refractory gold tailings from Minacalpa, Peru. Hydrometallurgy 81, 159–166.

Oulego, P., Collado, S., Garrido, L., Laca, A., Rendueles, M., Díaz, M., 2014. Wet oxidation of real coke wastewater containing high thiocyanate concentrations. J. Environ. Manage. 132, 16–23.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Scholtz, N.J., Pandit, A.B., Harrison, S.T.L., 1997. Effect of solids suspension on microbial cell disruption. In: Nienow, A.W. (Ed.), Bioreactor & Bioprocess Fluid Dynamics. Wiley.

Schippers, A., Sand, W., 1999. Bacterial leaching of metal sulfide proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Appl. Environ. Microbiol. 65, 319–321.

Soto, H., Nava, F., Leal, J., Jara, J., 1995. Regeneration of cyanide by ozone oxidation of thiocyanate in cyanide tailings. Miner. Eng. 8, 273–281.

Stott, M.B., Franzmann, P.D., Zappia, L.R., Watling, H.R., Quan, L.P., Clark, B.J., Houchin, M.R., Miller, P.C., Williams, T.L., 2001. Thiocyanate removal from saline CIP process water by a rotating biological contactor, with reuse of the water for bioleaching. Hydrometallurgy 62, 93–105.

Suzuki, I., Lee, D., Mackay, B., Harahuc, L., Oh, J.K., 1999. Effect of various ions, pH and osmotic pressure on oxidation of elemental sulphur by *Thiobacillus thiooxidans*. Appl. Environ. Microbiol. 65, 5163–5168.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

van Buuren, C., Makhotla, N., Olivier, J.W., 2011. The ASTER process: technology development through to piloting, demonstration and commercialization. In: Proceedings of the ALTA 2011 Nickel–Cobalt–Copper, Uranium and Gold Conference, Perth 23–28 May 2011.

van Niekerk, J., 2009. Recent advances in BIOX® technology. In: Proceedings of the Hydrometallurgy Conference, The Southern African Institute of Mining and Metallurgy, pp. 167–176.

van Zyl, A.W., 2014. PhD thesis, University of Cape Town, in preparation.

van Zyl, A.W., Harrison, S.T.L., van Hille, R.P., 2011. Biodegradation of thiocyanate by a mixed microbial population. In: Proceedings of the 11th International Mine Water Association Congress, pp. 119–123.

van Zyl, A.W., Harrison, S.T.L., van Hille, R.P., submitted for publication. Defining an operating window for the degradation of thiocyanate by a mixed microbial community.

Whitlock, J., 1987. Performance of the Homestake mining company biological cyanide degradation wastewater treatment plant August 1984-August 1987. In: Proceedings of the Society of Mining Engineers and American Institute of Mining Engineers Conference, Denver, Colorado.

Whitlock, J.L., Smith, G.R., 1989. Operation of Homestake's cyanide biodegradation wastewater system based on multi-variable trend analysis. In: Proceeding of the International Symposium on Biohydrometallurgy, Jackson Hole, Wyoming, pp. 613–619.

Zwietering, T.N., 1958. Suspending solid particles in liquids by agitators. Chem. Eng. Sci. 8, 244–253.