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Effect of thiocyanate on BIOX® organisms: Inhibition and adaptation

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ABSTRACT

Stringent environmental legislation and the desire to become zero discharge have motivated mining operations to treat and recycle process water. Cyanidation tailings effluent contains elevated concentrations of cyanide and thiocyanate (SCN^-), precluding recycling to the BIOX® process without prior treatment to reduce SCN^- to below 1 mg/l. The current study investigated the effect of SCN^- on individual microbial species. Iron oxidation by *Leptospirillum ferriphilum* was not affected by SCN^- concentrations below 0.5 mg/l, with concentration dependent inhibition observed between 0.75 and 1.25 mg/l and complete inhibition of iron oxidation above 1.25 mg/l. Sulphur oxidation by *Acidithiobacillus caldus* showed a similar trend, with limited inhibition below 1.25 mg/l and almost complete inhibition above 1.25 mg/l. Repeated sub-culturing at low concentrations induced adaptation, with adapted cultures currently growing at SCN^- concentrations of 7 mg/l. The phenomenon of inhibition at low concentration, with subsequent adaptation was repeated in stirred tank reactors, leaching a pyrite/arsenopyrite concentrate.

1. Introduction

The mining industry has been responsible for significant economic development in many parts of the world, but the associated environmental cost has been substantial in many cases and the effects are predicted to persist for centuries. The United States Environmental Protection Agency reported that the headwaters of more than 40% of the watersheds in the Western USA have been contaminated by mining and the estimated remediation costs of the almost 500,000 abandoned mines in that country could exceed $35 billion (USEPA, 2000). Incidents associated with the gold mining industry range from relatively contained spills of cyanide impacted effluent to the catastrophic failure of tailings impoundments. In recent years the mining industry has become increasingly focused on sustainability, with the mantra of zero discharge being aspired to in many cases.

Gold exists primarily as free-milling or refractory ores. In free-milling ores the gold is readily available and approximately 95% can be recovered by direct cyanidation, following milling to 80% passing 75 μm (Marsden and House, 2006). Cyanide reacts with elemental gold to form a soluble gold-cyano complex, which can be recovered by adsorption to activated carbon (de Andrade Lima and Hoduin, 2005).

$$2Au + O_2 + 4CN^- + 2H_2O \rightarrow 2[Au(CN)_2]^- + 2OH^- + H_2O_2$$

Refractory ores, where the gold is associated with sulphide minerals are not generally amenable to direct cyanidation, with low recoveries and high cyanide consumption. They require pretreatment to degrade the sulphide mineral. This is typically achieved by pressure oxidation or bioleaching. Bioleaching is a viable alternative to pressure oxidation, with the BIOX® technology applied most widely. There are currently eight active BIOX® operations (van Niekerk, 2009). During bioleaching, ferric iron, the active leaching agent is regenerated via the action of iron-oxidising bacteria and archaea. Sulphur-oxidising microorganisms are responsible for converting the sulphide component of the mineral to sulphuric acid, which helps to maintain the acidic conditions (Rawlings et al., 2003). Sulphide minerals are classified as either acid soluble or acid insoluble, based on the nature of their oxidation (Schippers and Sand, 1999). Acid soluble minerals, such as arsenopyrite, are oxidised via a polysulphide mechanism. The chemical bonds between sulphur and metal are degraded by proton attack such that hydrogen sulphide is released (H_2S). The presence of ferric iron allows for the simultaneous oxidation of the sulphur moiety, resulting in a series of rearrangements and oxidation steps via polysulphides to elemental sulphur (Rawlings, 2007). Acid insoluble minerals, such as pyrite, are degraded via the thiosulphate pathway. Electron transfer proceeds by single transfer steps, with six successive electron transfers resulting in the release of thiosulphate (Rohwerder et al., 2003). The majority of these partially oxidised sulphur intermediates are oxidised to sulphate, by sulphur-oxidising organisms, but some proceed to the cyanidation step where they react readily with cyanide to form thiocyanate (SCN^-).

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This increases cyanide consumption and results in tailings effluent with SCN^- concentrations of up to 3000 mg/l (Soto et al., 1995; Stott et al., 2001).

Thiocyanate is toxic to the environment and specifically to the iron and sulphur-oxidising microbial community. Bioleaching organisms are inhibited at low concentrations (±1 mg/l) of both CN^- and SCN^- (Olson et al., 2006 and van Aswegen et al., 2007; Adams, 2013). The mechanism of thiocyanate inhibition is not well understood and it has been proposed that it is related to acidification of the cytoplasm. Acidophilic bacteria often have an inside-positive membrane potential in order to maintain a neutral cytosol (Alexander et al., 1987). This inside-positive membrane potential leaves the bacteria susceptible to intracellular accumulation of toxins. The magnitude and polarity of the membrane potential is dependent on the pH, with greater toxicity experienced at lower pH. It has been suggested that acidification of the cytoplasm by anions, such as thiocyanate, causes inhibition of the respiratory chain, specifically the cytochromes involved in the terminal O_2-reducing reaction. Iron oxidisers are particularly susceptible to this phenomenon, given that the respiratory chain constituents involved are located periplasmically or on the outer surface of the membrane (Alexander et al., 1987).

The treatment of cyanidation tailings has focused primarily on the free-cyanide component, with most processes targeting the chemical oxidation of cyanide to the less toxic cyanate ion (OCN^-), with little impact on the thiocyanate. The activated sludge tailings remediation (ASTER™) process is a biological option that specifically targets thiocyanate destruction. It employs a complex consortium of microorganisms (Huddy et al., submitted for publication) in an aerated reactor system (van Buuren et al., 2011). ASTER™ has been implemented at commercial scale at the Consort mines in South Africa and the Suzdal mine in Kazakhstan. The process is designed to reduce thiocyanate concentrations to below 1 mg/l and this has been achieved at laboratory (van Zyl, 2014), pilot and commercial scale (van Buuren et al., 2011), although short-term periods of instability, where effluent SCN^- concentrations exceed 1 mg/l, have been observed. The success of ASTER™ has created the potential to recycle treated tailings effluent back to the bioleaching circuit and this is being considered for operations that utilise both the BIOX® and ASTER™ processes.

The effect of low concentrations of thiocyanate on bioleaching performance has been documented, but there is a lack of systematic studies on the effect of thiocyanate on individual species in the BIOX® community. In addition, if ASTER™ treated effluent is to be recycled to the bioleaching circuit, the ability of the community to tolerate SCN^- concentrations above 1 mg/l for short periods needs to be assessed. The current research addresses these issues, as well as the potential of the microbial species to adapt to thiocyanate.

2. Materials and methods

2.1. Microbial cultures

The pure cultures used in this study were isolated from a mixed BIOX® culture obtained from the Fairview mine in Mpumalanga, South Africa.

Leptospirillum ferriphilum was maintained on a modified DSMZ 882 medium, comprised of a basal salt medium (BSM), supplemented with ferrous and ferric iron (to 5 g/l of each as sulphate salts) and trace element solution (1 ml/l). The BSM was comprised of $(NH_4)_2SO_4$ (1.32 g/l), $MgCl_2{\cdot}6H_2O$ (0.53 g/l), KH_2PO_4 (0.27 g/l) and $CaCl_2{\cdot}2H_2O$ (1.42 g/l). The pH of the BSM was adjusted to pH 1.3 using concentrated sulphuric acid (98%) prior to autoclaving. The ferrous iron stock solution ($FeSO_4{\cdot}7H_2O$) was prepared and adjusted to pH 1.3 using concentrated H_2SO_4. The ferric iron stock solution was prepared, using ferric sulphate hydrate and adjusted to pH 1.3 as described above. The ferrous and ferric iron stock solutions were filter sterilised and added aseptically to the medium. The trace element solution consisted of $MnCl_2{\cdot}H_2O$ (62 mg/l), $ZnCl_2$ (68 mg/l), $CoCl_2{\cdot}6H_2O$ (64 mg/l), H_3BO_3 (31 mg/l), Na_2MoO_4 (10 mg/l) and $CuCl_2{\cdot}H_2O$ (67 mg/l). The culture was maintained in a shaking incubator at 45 °C and sub-cultured weekly.

Acidithiobacillus caldus was maintained on a modified DSMZ 150a medium, consisting of 0 K basal salt medium, trace element solution and elemental sulphur as a substrate. The 0 K medium was comprised of $(NH_4)_2SO_4$ (3 g/l), $K_2HPO_4{\cdot}3H_2O$ (0.5 g/l), $MgSO_4{\cdot}7H_2O$ (0.5 g/l), KCL (0.1 g/l) and $Ca(NO_3)_2$ (0.01 g/l). The pH of the 0 K medium was adjusted to pH 2.5 using concentrated H_2SO_4 and autoclaved at 121 °C for 20 min. Thereafter, the filter-sterilised (0.45 μm) trace element solution (1 ml/l) and sterilised (100 °C oven) sulphur (5 g/l) were aseptically added to the medium. Trace element solution was prepared with $FeCl_3{\cdot}6H_2O$ (1.1 g/l), $CuSO_4{\cdot}5H_2O$ (50 mg/l), H_3BO_3 (200 mg/l), $MnSO_4{\cdot}H_2O$ (200 mg/l), $Na_2MoO_4{\cdot}2H_2O$ (80 mg/l), $CoCl_2{\cdot}6H_2O$ (60 mg/l) and $ZnSO_4{\cdot}7H_2O$ (90 mg/l). The culture was maintained in a shaking incubator at 45 °C and sub-cultured weekly.

2.2. Reactor systems

The tests in microwell plates (MWPs) were performed in Greiner Bio-one CELLSTAR® 12 Well Suspension Culture Plates. Each well had a diameter of 23 mm and a depth of 18 mm, and a working volume of 3 ml. The microwell plates have been used previously to study biooxidation of ferrous iron, giving similar results to traditional shake flask studies (Meissner et al., 2014). During the experiments, the MWPs were incubated in a humidified container, to minimise evaporative volume losses, in a Labcon 5082U shaking incubator, set at 170 rpm and 45 °C. A water-filled beaker was kept in the incubator for the duration of the experiments and maintained at a minimum volume of 1 l to ensure a humid environment and further minimise evaporative losses.

The simulated tank leach was performed in two glass, stirred tank reactors with a working volume of 1 l. Each reactor had a height of 220 mm (liquid volume height 118 mm), a diameter of 104 mm and was fitted with four vertical baffles (10 mm width). The contents of the reactor were agitated using a Heidolph overhead stirrer, fitted with a four-bladed marine impeller (58 mm diameter), at an impeller speed of 500 rpm. Air was provided through an L-shaped sparge pipe at a rate of 4.25 l/min. Reactor temperature was maintained at 45 °C by circulating heated water through the jacket surrounding the reactor.

2.3. Analytical techniques

The pH in the multiwell plates was measured using a Eutech CyberScan pH2100 micro-probe, while the pH of the reactor samples was monitored using a Metrohm 716 pH probe. The redox potential, relative to Ag/AgCl, of all samples was measured using a Metrohm 827 pH Lab redox metre.

Ferrous and total iron concentrations were measured colorimetrically using the 1–10 phenanthroline method (Komadel and Stucki, 1988). Ferric iron was determined from the difference. Sulphate ion concentration was determined by high performance liquid chromatography (HPLC) using a Waters IC-Pak AHR (Anion High Resolution) column and a conductivity detector. The system was run isocratically using a sodium borate–gluconate mobile phase at a flow rate of 1 ml/min. Sample injection volumes of 50 μl were used. To quantify the ion concentration, standard solutions (50, 100, 150 and 200 mg/l) of sulphate were prepared from a sulphate sodium salt. The reduced sulphur content of the head grade and leached mineral concentrate was determined using