Analysis of the microbial community associated with a bioprocess system for bioremediation of thiocyanate- and cyanide-laden mine water effluents

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Abstract. Gold extraction by cyanidation from refractory gold ores results in the formation of thiocyanate- and cyanide-contaminated wastewater effluents that must be treated before recycle or discard. Activated sludge processes, such as ASTER™, can be used for biodegradation of these effluent streams. The destruction of these compounds is catalyzed by a mixed microbial culture, however, very little is known about the community composition and metabolic potential of the thiocyanate- and cyanide-degrading microorganisms within the community. Here we describe our on-going attempts to better understand the key microorganisms, within the ASTER™ bioprocess, that contribute to the destruction of thiocyanate and cyanide, and how this knowledge relates to further process optimisation.

Introduction

The reaction between cyanide (CN^-) and residual sulfur species, during the processing of refractory gold ores, results in a tailings effluent stream containing thiocyanate (SCN^-; >300 mg/L) and residual CN^- (>20 mg/L) [1]. Due to their toxicity, the presence of residual CN^-, metal cyanide complexes and SCN^- the release of effluent water to the environment is prohibited. The Activated Sludge Tailing Effluent Remediation (ASTER™) process is an example of a biologically-catalysed process that was developed to remediate CN^- and SCN^- containing effluents (Fig. 1). The ASTER™ process was developed, as a collaborative project between Gold Fields and BHP Billiton, in the mid 1990's, to treat process water from BIOX® operations [1]. ASTER™ aims to facilitate comprehensive destruction of SCN^- and CN^- contaminated wastewater prior to environmental discard and ultimately enable the recycling of process water within the plant to improve the water balance and improve effluent water quality for environmental disposal. ASTER™ technology is currently employed at a number of commercial mining operations worldwide and effectively reduces the CN^- and SCN^- concentrations to <1 mg/L [2].

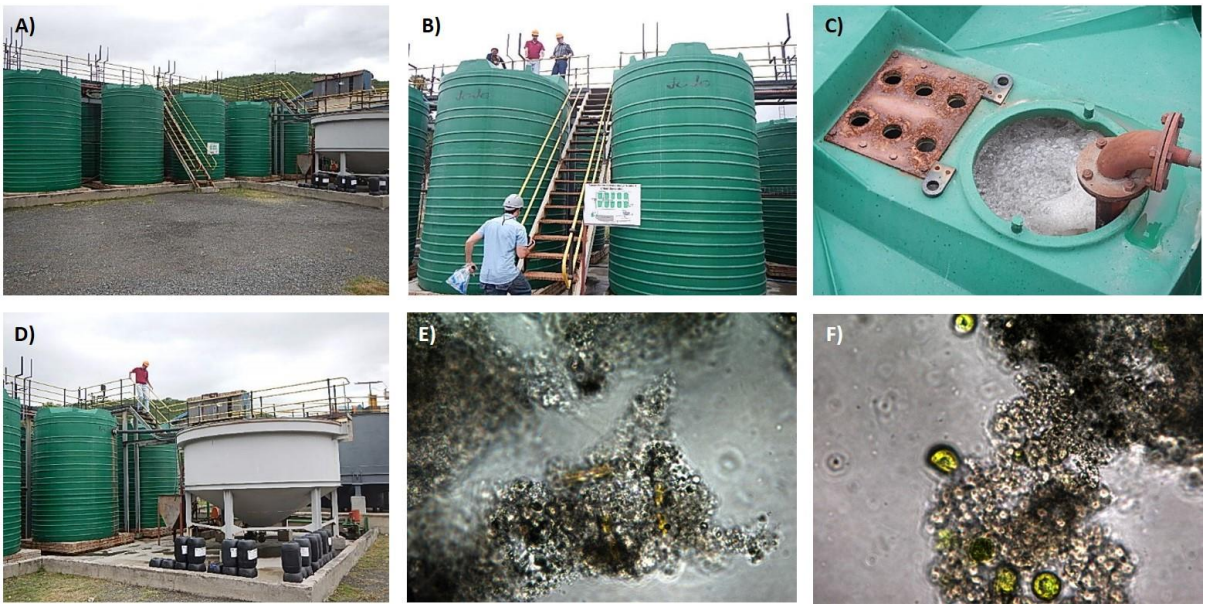

Figure 1. The Consort ASTER™ Plant, South Africa (A-D) showing the primary and secondary reactors. Light microscope images (Magnification x100) of suspended floc's, densely packed with a variety of microorganisms, from the Consort ASTER™ Plant.

The ASTER™ process relies on a complex consortium of microorganisms, in suspended flocs or attached biofilm, to metabolise SCN^- (Equations 1-3) and CN^- (Equations 4 and 5), yielding sulfate and ammonium.. The formation of biofilm and flocs within the system assist with the efficient retention of the biological catalysts for SCN^- and CN^- destruction. Furthermore, the microbial communities associated with biofilm and flocs represent a reservoir of microbial diversity for the continuous operation of this system. This leads to a robust commercial bioprocess capable of treating dynamic wastewater streams.

$$SCN^- + 3H_2O + 2O_2 \rightarrow CNO^- + HS^- \quad \text{Equation 1}$$
$$HS^- + 2O^2 \rightarrow SO_4^{2-} + H^+ \quad \text{Equation 2}$$
$$CNO^- + 3H^+ + HCO_3^- \rightarrow NH_4^+ + 2CO_2 \quad \text{Equation 3}$$
$$HCN + 2H_2O \rightarrow HCOOH + NH_3 \quad \text{Equation 4}$$
$$HCN + H_2O \rightarrow HCONH_2 \quad \text{Equation 5}$$

Previous research has linked enhanced SCN^- destruction rates to the process operating conditions and increased biomass loading [3]. The microbial ecology of the ASTER™ process has been investigated, using 16S rRNA gene surveys and metagenomics, and revealed to be far more complex than previously reported [1,4,5,6]. Collectively these investigations provide detailed information on the diversity and composition of the community and of the abundant microbial community members.

Experimental Setup

Microbial culture. The microbial consortium was collected from the ASTER™ plant, Barberton Mines, Consort Plant, South Africa. The stock culture was maintained in a 1 L continuous glass stirred tank reactor connected to a 2 L glass clarifier unit. The stock reactor

was continuously fed with reactor media [0.15 g/L molasses, 0.010 g/L P provided as a phosphate salt. and 1,200 mg/L SCN^- (pH 7.00 ± 0.02)]. Samples were removed aseptically from the stock reactor, at regular intervals. The pH was measured prior to batch SCN^- analysis by high performance liquid chromatography (HPLC).

Ecology and functional annotation of the microbial community. The stock reactor was sampled for 16S rRNA genes [4] and metagenomic surveys [5] as a means of monitoring the microbial community associated with the laboratory stock culture. Total genomic DNA was extracted from sludge harvested from the stock reactor [4,5]. The 16S rRNA genes were PCR amplified using universal bacterial 16S rRNA primers, before being sequenced [4]. In addition, the genomic DNA was subjected to Illumina® library preparation and sequencing, using an insert-size of 500 bp and read length of 100 bp [5]. The metagenomic sequences were processed, assembled and annotated, before being assigned to putative bins on the basis of shared characteristics.

Metabolic analysis. Metabolic pathways were predicted, from sequences within each genome, in order to identify metabolic pathways of interest and the potential role(s) of the microorganisms that contain these pathways in this system [5]. Of particular interest were genes and pathways potentially involved in breakdown of SCN^-, CN^- and their degradation products, including sulfur, carbon, and nitrogen compounds.

Results

The SCN^- reactor degradation performance prior to sampling was stable over an extended period of continuous operation (Figure 1). Biomass, in the form of a thick biofilm attached to the reactor walls, baffles and impeller, was observed to begin accumulating when the SCN^- loading reached 100 mg/L.h (data not shown).

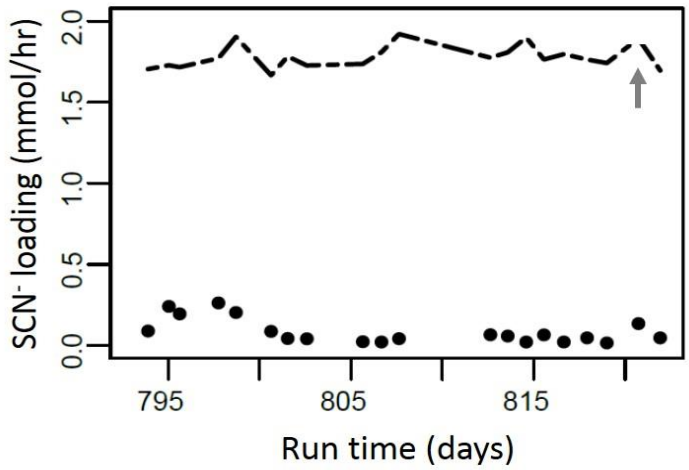

Figure 2. Reactor performance, of the SCN^- degrading stock culture, over an extended period of time. SCN^- loading into the reactor was achieved by SCN^- addition to the reactor feed. Indicated are the rates of SCN- being fed into the system (—) and the residual SCN^- present in the effluent (●). Sampling of biomass from the reactor, for metagenomic sequencing, was performed where indicated (→).

Overall, based on the metagenomic approach employed in this study, the microbial community in the SCN- reactor was far more complex than previously reported. Rank abundance analysis, based on assembled and binned metagenomic data, demonstrated the dominance of *Thiobacillus* spp. (Figure 3). The survival and proliferation of multiple *Thiobacillus* spp. under conditions of high SCN^- loading, indicates tolerance to and/or the capacity to use SCN^-. In particular, the two highest abundance *Thiobacillus* spp. genomes

present in this system contain the genes and pathways potentially involved in breakdown of SCN^- and its degradation products.

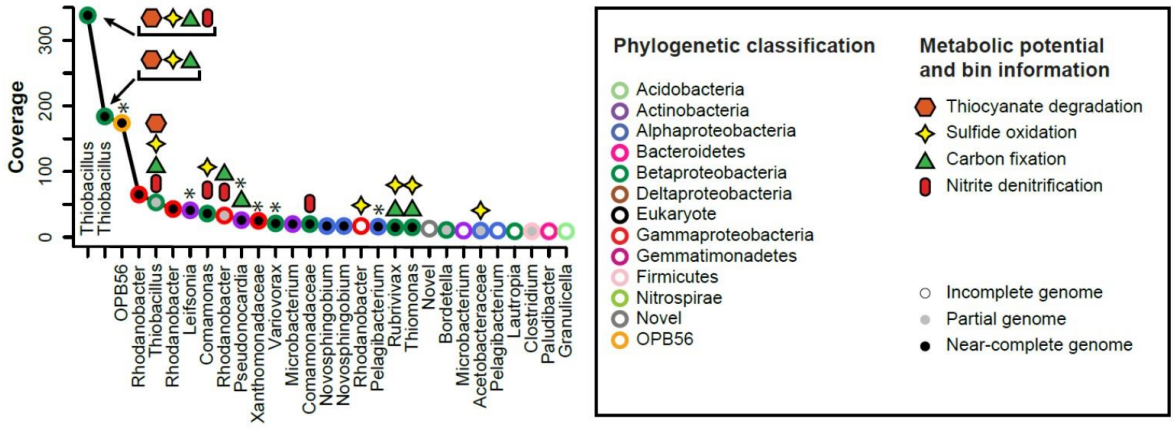

Figure 3. Rank abundance and metabolic potential for organisms in the reactor treating SCN^-. The genome bins are ordered from highest to lowest coverage as a proxy for abundance within the system. The outer circle colour indicates phylogeny, and circle fill indicates genome completeness measured by presence or absence of 51 conserved single-copy marker genes. Symbols represent metabolic potential in organisms based on the presence of all associated functional genes in a given bin.

Conclusions

This cross-disciplinary research suggests that due to the apparent dominance of autotrophic SCN^- degraders within the SCN-degrading system. Furthermore, our on-going research is focused on the drivers leading to the formation of the microbial biofilm, and the role that these sessile microbial communities play, within the current reactor configurations, to ensure efficient SCN^- degradation kinetics. The spatial distribution of key microorganisms within the ASTER™-associated biofilm is also currently being assessed. Ultimately, this information will be chelated and applied to inform further optimisation of the bioprocess for efficient treatment of contaminated mining wastewater effluents, including rational reactor design and a detailed understanding of the response of the microbial community to process perturbations.

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