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Characterisation of the complex microbial community associated with the ASTER™ thiocyanate biodegradation system

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ABSTRACT

The ASTER™ process is used to bioremediate cyanide- (CN^-) and thiocyanate- (SCN^-) containing waste water. This aerobic process is able to reduce the CN^- and SCN^- concentrations to below 1 mg/L efficiently in a continuous system, facilitating reuse of process water or safe discharge. Such remediation systems, which completely eliminate risk associated with the pollutants, are essential for sustainable mineral processing and the long term minimisation of environmental burden through both pollutant destruction and exploiting opportunities for nutrient recycle. Process robustness of these bioremediation options can be enhanced by good understanding of the microbial community involved in the process. To date, the microbial consortia associated with the ASTER™ bioprocess have been poorly characterised using isolation approaches only. As a result, the relative abundance and diversity of the community has been significantly under-represented. In this study, both planktonic and biofilm-associated biomass have been observed. Microscopy has revealed the diversity of these communities, including bacteria, motile eukaryotes, filamentous fungi and algae, with the biofilm densely packed with microorganisms. The results of the molecular characterisation study reported here, using a clone library approach, demonstrate that the microbial community associated with the ASTER™ bioprocess system is far more complex than previously suggested, with over 30 bacterial species identified thus far. On-going investigations focus on identification of key microbial community members associated with SCN^- biodegradation and other critical metabolic functions, as well as the expected dynamic response of this complex microbial community to shifts in the operating window of the process.

1. Introduction

During cyanide-processing of refractory gold ores or concentrates, free cyanide (CN^-) reacts with metals, reduced sulphur species, including sulphide and/or thiosulphate, to form thiocyanate (SCN^-) as well as weak- and strong acid dissociable cyanide complexes (CN_{wad} and CN_{sad}) (Stott et al., 2001). The presence of cyanide compounds, including SCN^-, in effluent streams represent a significant environmental and ecological risk, as SCN^- is non-hydrolysable and non-volatile. Depending on the efficiency of the preceding sulphide oxidation steps, including roasting, pressure or biological oxidation, effluent streams may inadvertently produce significant SCN^- levels (20–5000 mg/L; http://www.biomin.co.za/aster/history.html). The SCN^-, and residual free CN^-, are deported with the tailings as components of the effluent fraction and contribute to making many of these processes technically challenging and economically unfavourable (van Buuren et al., 2011). In addition to its potentially hazardous effects upon release into the aquatic environment, SCN^- is highly toxic to iron and sulphide-oxidising microorganisms within stirred tank bio-oxidation plants. These features result in the inability to re-use the water or residual nutrients associated with the bio-oxidation process. In addition, tailings cannot be considered for further downstream uses, as proposed by Harrison et al. (2013) without prior decontamination. This has necessitated the development of specific treatment technologies for efficient remediation of SCN^- contaminated waste streams prior to discard or reuse. Such remediation processes have potential to enhance sustainable mineral processing and reduce environmental burden through the complete removal of risk associated with pollutants, through their degradation. In addition, they enable resource recycling or re-use, in terms of water, nutrients and particulate materials, in accordance with an industrial ecology approach.

Biological treatment, such as that demonstrated by the Homestake Mining Company in the 1990s, based on the inherent metabolic capability of microorganisms within a mixed community has been shown to biodegrade CN^-, SCN^- as well as weak and

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strong acid dissociable cyanide complexes effectively, in a cost-effective and reliable manner (du Plessis et al., 2001; Ebbs, 2004). During biological treatment, microorganisms metabolise thiocyanate and other cyanide species under aerobic conditions to produce carbon dioxide, ammonia, sulphate and, in the case of metal complexes, free metals which are either adsorbed to the biofilm-containing sludge or precipitated. The successful and stable operation of a biological treatment process in the face of variable feed streams largely depends on the intrinsic metabolic capability of the mixed microbial community to degrade the targeted contaminant and their robustness under the operating conditions of the process. The Activated Sludge Tailings Effluent Remediation (ASTER™) process, developed in South Africa in the late 1990s by researchers of the then Billiton Process Research Laboratories and engineers from Gold Fields Limited, uses biodegradation to remove SCN^- from process and waste water streams following cyanide leaching. The demonstration scale ASTER™ plant at the Consort Mine, Barberton, South Africa, is comprised of a series of aerated reactors and a clarifier unit (Fig. 1) that contain a mixed microbial community (van Buuren et al., 2011). On a lab-scale, the ASTER™ microbial community was shown to degrade SCN^- in feeds containing 550 mg/L to levels below 1 mg/L (du Plessis et al., 2001). In-depth investigations of the operating conditions of the ASTER™ process have been conducted in order to inform the commercial operations (van Zyl, in prep.), demonstrating the ability to process effluent streams with incoming SCN^- concentrations as high as 1000 mg/L with an 24 h residence time. However, a detailed assessment of the composition of the ASTER™ microbial consortium has not yet been conducted, despite the nature and composition of the active microbial community being a fundamental aspect of process performance. Based upon a cultivation dependent approach, the community is documented to contain approximately ten active members, comprising both prokaryotes and eukaryotes (Table 1). However, it is generally accepted that less than 1% of environmental microorganisms are culturable under standard laboratory conditions (Amann et al., 1995). Hence, the cultivation and isolation of microorganisms for the characterisation of the ASTER™ community only provides limited insight into the composition of the consortium and its key role players. Metagenomic sequencing followed by the assembly of well-curated genomes has been shown to provide insights into the relative abundance as well as metabolic potential of species and strains within a particular system (Sharon et al., 2013). From a process perspective this may lead to the identification of critical species and an understanding of their role in operational robustness and the community metabolism.

In the present work a culture-independent approach is employed as a first step to better the current description of the microbial consortium associated with the ASTER™ bioprocess, including those microorganisms that may not be readily culturable, in order to obtain a more complete assessment of the process-associated microbial community. A more defined appreciation of the community composition is needed to inform further improvements in the efficiency and operating conditions of the industrial process, as well as design for robustness. These factors will enhance the application of such bioremediation processes in sustainable mineral processing. Furthermore, our results emphasise the importance of employing culture-independent microbial ecology, in conjunction with culture-based studies and process optimisation, to understand the community composition of a complex industrial mixed microbial community, such as the ASTER™ consortium.

2. Materials and methods

2.1. Microbial consortium and culture conditions

The active microbial consortium used in this study was collected from the ASTER™ plant at the Barberton Mines, Consort Plant, South Africa. The inoculum was maintained in a 1 L continuous glass stirred tank reactor (CSTR) connected to a 2 L glass clarifier unit, described in detail by van Zyl et al. (in prep.). The stock reactor was charged with and continuously fed reactor media [0.15 g/L molasses, 0.027 g/L PO_{4-} and 1200 mg/L SCN^- (pH 7.00 ± 0.02)]. Following a batch setup phase, the hydraulic retention time was maintained at 12 h with a sludge recycle rate of 900 mg/h. Aeration was set to 900 mL/min and the system was operated at ambient temperature (23–25 °C). Mixing was achieved using an overhead stirrer, fitted with a pitched-blade impeller, set to 270 rpm. Throughout the duration of the experiment the residual SCN^- concentration in the stock reactor was below 0.5 mg/L.

2.2. Sampling and SCN^- analyses

Samples from repeat experiments were removed aseptically from the stock reactor, at regular intervals, using a syringe and filtered through a 0.45 μm membrane filter before the pH was measured, using a Cyberscan 2500 micro pH meter, and samples stored at 4 °C. SCN^- analysis was performed on batches of stored samples by high performance liquid chromatography (HPLC). The SCN^- analysis was performed as described by Tamosiunas et al. (2006), employing the Thermo Scientific HPLC spectra system with a UV detector. The stationary phase consisted of a reversed phase C_{18} Discovery HS column fitted with a Supelguard pre-column (5 μm, 250 mm × 4.6 mm). The mobile phase consisted of 40% (v/v) acetonitrile (Merck) in deionised water, with 2 mM

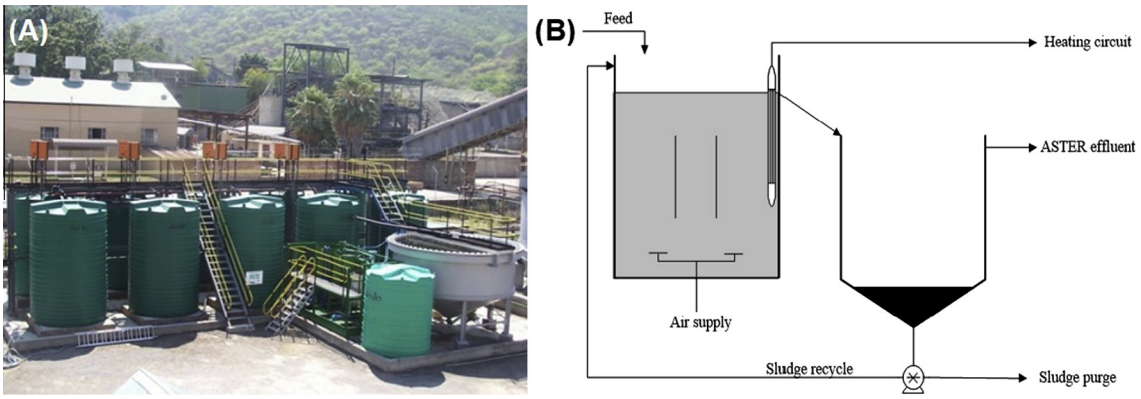

Fig. 1. The Consort ASTER Plant, Barberton, South Africa (A) (BIOMIN) and a schematic diagram of an ASTER unit operation (B) (van Buuren et al., 2011). Sludge, when produced, is recycled to the primary ASTER™ bioreactors in parallel while the secondary reactors, in series, receive primary reactor overflow.