UC Berkeley

UC Berkeley Previously Published Works

Title

Analysis of microbial communities associated with bioremediation systems for thiocyanate-laden mine water effluents

Permalink

<https://escholarship.org/uc/item/4bx6s1pw>

ISBN

9783035711806

Authors

Huddy, RJ
Kadzinga, F
Rahman, SF
et al.

Publication Date

2017

DOI

10.4028/www.scientific.net/SSP.262.601

Peer reviewed

Analysis of Microbial Communities Associated with Bioremediation Systems for Thiocyanate-Laden Mine Water Effluents

Robert J. Huddy^{1,a}, Fadzai Kadzinga^{1,b}, Sumayah F. Rahman^{2,c},
Rose Kantor^{2,d}, Jillian F. Banfield^{3,4,e} and Susan T.L. Harrison^{1,f*}

¹Centre for Bioprocess Engineering Research, Department of Chemical Engineering,
University of Cape Town, South Africa

²Department of Plant and Microbial Biology, University of California, Berkeley, USA

³Department of Earth and Planetary Sciences, University of California,
Berkeley, USA

⁴Department of Environmental Science, Policy, and Management, University of California,
Berkeley, USA

^arobert.huddy@uct.ac.za, ^bKDZFAD001@myuct.ac.za, ^crahman.sumayah@gmail.com,
^drkantor@berkeley.edu, ^ejbanfield@berkeley.edu and ^fsue.harrison@uct.ac.za

Keywords: Thiocyanate, Bioremediation, Genome-resolved metagenomics, Bioprocess

Abstract. During the processing of refractory gold ores, cyanide (CN⁻) and residual sulphur species react to form an effluent stream containing thiocyanate (SCN⁻) and residual CN⁻. The release of SCN⁻ and CN⁻ containing effluent water to the environment is prohibited, necessitating effective treatment prior to discharge and/or reuse of contaminated plant water. Biologically mediated effluent remediation processes have been developed for commercial use, to remediate SCN⁻ containing effluents, with the aim of enabling recycling of process water and improving the quality of effluent water prior to disposal. Bioremediation processes to treat these effluents rely on a complex consortium of microorganisms to metabolise the SCN⁻ resulting in the production of ammonium that is in turn removed by conversion to nitrite and subsequent denitrification. Increasingly, genomic methods are being used to investigate processes in wastewater treatment to identify key microbial species and, thereby, inform the rationale design and operation of these bioremediation systems. The microbial ecology of laboratory-based SCN⁻ degrading bioprocesses have been investigated, using genome resolved metagenomics, to provide detailed information on the community composition and metabolic profile of abundant microbial community members. Three *Thiobacillus* strains and an *Afipia* strain capable of SCN⁻ degradation were shown to be highly abundant within a reactor system enabling biofilm formation. In contrast the inclusion of suspended mineral tailings within an alternative reactor system, led to the selection of an active planktonic microbial community with reduced microbial diversity containing only a single SCN⁻ degrading *Thiobacillus* sp. This information is being used to inform further rational development of SCN⁻ degradation processes for treatment of contaminated industrial wastewater effluents.

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 and the significant health and environmental risk that they pose; the discarding of untreated effluent waters, containing residual CN⁻, metal cyanide complexes and SCN⁻, is prohibited. The Activated Sludge Tailing Effluent Remediation (ASTERTM) process (Outotec, Finland) is an industrial bioremediation process that was developed to treat SCN⁻ and CN⁻ contaminated wastewater comprehensively prior to disposal and/or re-use of plant water within BIOX[®] circuits. ASTERTM technology is currently employed at several commercial mining operations worldwide and effectively reduces the CN⁻ and SCN⁻ concentrations to <1 mg/L. This biological SCN⁻ destruction process relies on a complex consortium of microorganisms that are present in suspended flocs or attached biofilm [2]. These microorganisms

aerobically co-operatively metabolise SCN^- by means of either the carbonyl sulfide (COS) or cyanate (CNO^-) pathways, respectively, to produce carbon dioxide, ammonia and sulfide [3, 4]. The formation of biofilm and flocs within the ASTER™ bioreactors is expected to assist with efficient biomass retention and / or recycle, thereby enhancing SCN^- and CN^- destruction.

The microbial ecology of laboratory-scale SCN^- bioremediation processes, based upon the industrial ASTER™ process, has been investigated using 16S rRNA gene surveys [2, 5] and genome-resolved metagenomics [6-8]. The use of these culture-independent techniques facilitates access to the microbial community composition and functional potential of key microbial species within these mixed bioremediation systems. The primary aim of this investigation is to compare genome-resolved metagenomic investigations of the complex mixed microbial communities associated with laboratory-based SCN^- bioremediation systems operated under differing physicochemical conditions [6-8].

Experimental Set Up

The experimental stirred tank bioreactors were inoculated with homogenized biofilm and planktonic samples from a long-running continuous stock culture. The stock culture was maintained in a 1 L continuous glass stirred tank reactor connected to a 2 L clarifier unit. The stock reactor was continuously fed with reactor media [150 mg/L molasses, 27 mg/L phosphate and 1,200 mg/L SCN^- (pH 7.00 ± 0.02)] at a hydraulic residence time of 12 h. The experimental reactor system was operated continuously with incremental increases in the feed SCN^- concentration, as described by Kantor et al. [6, 8]. Samples of biofilm and planktonic cells were removed aseptically from the stock reactor, at regular intervals, in order to understand whether these represented distinct communities. The pH was measured prior to batch SCN^- analysis by high performance liquid chromatography (HPLC). A comparative reactor system was acclimatized to and then operated in the presence of 5.5% (w/v) solids, in the form of mineral tailings, as described by van Zyl et al. [2] ahead of implementation at a site where complete tailings removal was not possible. This SCN^- degrading culture was maintained as a “draw-and- fill” system with 10% volume removal and replacement by fresh feed solution containing 450 mg/L SCN^- per week, as described by Rahman et al. [7]. Two samples of planktonic cells were aseptically removed from the solids reactor, separated by an interval of 25 days for community analysis. DNA was extracted from planktonic and biofilm samples using a NucleoSpin® soil genomic DNA extraction kit (Machery-Nagel, Germany) with the inclusion of a repeated extraction step, per manufacturer’s instructions. Paired end library preparation and sequencing were performed with Illumina HiSeq-2500 run at the rapid mode at the Joint Genome Institute (Walnut Creek, CA) to yield 250 bp paired-end reads. The metagenomic sequences were processed, assembled and annotated, before being assigned to putative bins on the basis of shared characteristics as described by Kantor et al. [6, 8] and Rahman et al. [7]. 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 [6-8]. Of particular interest were genes and pathways potentially involved in breakdown of SCN^- and their degradation products.

Results and Discussion

The performance of the SCN^- reactor treating solution was monitored over approximately 220 days of continuous operation with incremental increases in the SCN^- loading rate (Fig. 1). This SCN^- degrading bioreactor was observed to accumulate biofilm on the internal components, including reactor walls, baffles and impeller. Planktonic and biofilm samples from within the experimental SCN^- reactor were removed over the course of the experimental period for metagenomic analysis of the microbial community within these phases (Fig. 2). Planktonic and biofilm samples harvested at a feed concentration of 500 mg/L SCN^- yielded 53 and 60 near complete assembled genomes, respectively [6]. Four bacterial strains with the capacity for SCN^-

degradation, including three *Thiobacillus* strains and a strain of *Afiplia*, were present within both the planktonic and biofilm communities over the course of the study (Fig. 2). These microorganisms all contained one of the two known types of SCN^- hydrolases necessary for SCN^- degradation [6].

Fig. 1. The SCN^- loading (—) and residual SCN^- concentration (•) within a 1 L CSTR operated at ambient temperature and treating an SCN^- -containing solution. After a period of gradual increase in the dilution rate, the reactor was operated continuously at a hydraulic residence time of 12 hours (from day 68 to 220). Biomass sampling point at a feed concentration of 500 mg/L is indicated by the star.

Fig. 2. Rank abundance and key metabolic potential of dominant organisms within the biofilm (A) and planktonic (B) communities of the continuously operated SCN^- reactor system at a 12 hr residence time and thiocyanate loading of 500 mg/L, and the planktonic phase within the solids SCN^- reactor system (C; Sample 1; 5.5% m/v solids and 450 mg/L thiocyanate). For each reactor, genome bins are ordered from highest to lowest coverage (the average read depth over all scaffolds in the bin).

In contrast, the presence of the suspended particles prevented floc and biofilm formation [5], thereby contributing to reduced biomass loading and microbial diversity [7]. This represented an interesting comparison for the metagenomic analysis of a purely planktonic SCN⁻ degrading microbial community (Fig. 2). Metagenomic sequence analysis resolved 34 near complete genomes from sample 1 taken from the reactor treating the SCN-containing particle suspension [7]. Several of the microorganisms present within the system treating the solids suspension have genes for denitrification and sulfur oxidation, however, only one *Thiobacillus* sp. has the potential for SCN⁻ degradation [5, 7]. Van Zyl et al. [5] attributed this to the absence of a mature biofilm and the associated microenvironments. Despite the presence of solids in suspension, and the associated decline in the quantity and variety of SCN⁻ degrading species (Fig. 2) [5-7], high thiocyanate degradation rates were still achieved [5].

Concluding Remarks

This research continues to demonstrate the importance of characterizing complex mixed microbial communities using metagenomics. The perceived importance of the biofilm, as a reservoir of microbial diversity, has focused our on-going research on the drivers that lead to the formation of a stable biofilm mediated microbial community. This information has informed our on-going attempts at further process optimization, including the use of low-cost biomass supports within alternative reactor configurations, towards a robust and efficient industrial SCN⁻ degrading bioprocess.

Acknowledgements

Funding for this study has been provided by the NRF of South Africa through the SARChI Chair in Bioprocess Engineering (UID 64778) held by Prof. Harrison. Dr. Huddy is funded through a DST/NRF Research Career Advancement Fellowship (UID 91465).

References

- [1] C. van Buuren, N. Makhotla, and J.W. Olivier, Proceedings of the ALTA 2011 Nickel-Cobalt-Copper, Uranium and Gold Conference.
- [2] R.J. Huddy, A.W. van Zyl, R.P. van Hille, S.T.L. Harrison, Characterisation of the complex microbial community associated with the ASTERTM thiocyanate biodegradation system, *Miner. Eng.* 76 (2015) 65–71.
- [3] M.P. Watts, J.W. Moreau, New insights into the genetic and metabolic diversity of thiocyanate-degrading microbial consortia, *Appl. Microbiol. Biotechnol.* 100 (2016) 1101–1108.
- [4] D.P. Kelly, S.C. Baker, The organosulfur cycle: aerobic and anaerobic processes leading to the turnover of C₁-sulfur compounds, *FEMS Microbiol. Rev.* 87 (1990) 241-246.
- [5] A.W. van Zyl, R.J. Huddy, S.T.L. Harrison, R.P. van Hille, Evaluation of the ASTERTM process in the presence of suspended solids, *Miner. Eng.* 76 (2015) 72–80.
- [6] R.S. Kantor, R.J. Huddy, R. Iyer, B.C. Thomas, C.T. Brown, K. Anantharaman, S. Tringe, R.L. Hettich, S.T. L. Harrison, J.F. Banfield, Genome-Resolved Meta-Omics Ties Microbial Dynamics to Process Performance in Biotechnology for Thiocyanate Degradation, *Environ. Sci. Technol.* 51 (2017) 2944–2953.
- [7] S.F. Rahman, R.S. Kantor, R.J. Huddy, B.C. Thomas, A.W. van Zyl, S.T.L. Harrison, J.F. Banfield, Genome-resolved metagenomics of a bioremediation system for degradation of thiocyanate in mine water containing suspended solid tailings, *Microbiol. Open.* 2017 1–9.
- [8] R.S. Kantor, A.W. van Zyl, R.P. van Hille, B.C. Thomas, S.T. L. Harrison, J.F. Banfield, Bioreactor microbial ecosystems for thiocyanate and cyanide degradation unravelled with genome-resolved metagenomics, *Environ. Microbiol.* 17 (2015) 4929-4941.