Microbial-mediated Ni and Co recovery from mine tailings

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Abstract

This study shows the microbe-metal interactions that allow the fractionation of Ni and Co in sulphate reducing bioreactors. Ni and Co precipitation experiments with sulfate reducing bacteria (SRB) or with biogenic sulfide effluent were carried out. Lower NiS precipitation occurred in the presence of SRB as compared to the experiments with biogenic sulphide, even when both Ni and Co were added. This and the identified proteins expressed by SRB shows that Ni is complexed by extracellular proteins generated by SRB, which could allow selective recovery of Ni and Co from tailing streams in Australia.

Keywords: sulphate reducing bacteria, Nickel, Cobalt, recovery, proteins

Introduction

Mine tailings and waste streams are known as "the largest environmental liability of the mining industry" left over due to the historical mining activities in Australia (Thurtell et al. 2018). Some of these tailings came from the mining of Ni sulphidic ores (Nehdi and Tariq 2007; Sima et al. 2011), where Co is mostly present (Crundwell et al. 2011). Due to the low recovery efficiencies (70–85% Ni, 20–50% Co), tailing lagoons from this mine activity represents a potential pollutant threat, but also an opportunity to recover these metals for economic revenue (Simonnot et al. 2018).

Up to date, conventional technologies for the treatment of metal-containing waste streams are based on the addition of chemicals for precipitation, thus creating high amounts of a secondary waste with no option of reprocessing. Selective recovery of Ni and Co in waste streams for reprocessing is a challenge due to their similar chemical behaviour. Therefore, the addition of reagents is a common practice for concentrated streams that allows selective separation through precipitation, ion exchange and solvent extraction (Flett 2004). These options are not economic nor environmentally supportable.

Sulphide precipitation, on the other hand, is an excellent chemical to remove metals from waste streams because it allows the formation of highly insoluble salts, even at ppm and ppb concentrations, that can be directly reprocessed (Villa-Gomez and Lens 2017). In this sense, sulfide produced by biological sulphate reduction is a step forward, because it eliminates the costs associated with the acquisition of sulphide reagents, as it uses a pollutant already present in tailings (sulphate) and finally, it allows the production of sulphide on-site, thus avoiding transportation of a hazardous chemical (Villa-Gomez and Lens 2017). The process relies on the activity of sulphate reducing bacteria (SRB) that reduces sulphate (SO_4^{2-}) to sulphide (S^{2-}), which can be used to recover metals as sulphidic precipitates (Sánchez-Andrea et al. 2016; Villa-Gomez and Lens 2017). So far, biological sulphate reduction has been assessed at full-scale to treat acid mine drainage in passive systems and to recover metals such as copper, and zinc from wastewaters coming from metal associated processes in active systems

(bioreactors) (Sánchez-Andrea et al. 2016). While showing very successful results, this technology needs to be further developed to allow separation of metals such as Ni and Co for their recovery.

In bioreactors, the interactions between metals and the microbial environment can affect metal precipitation. This can be due by substances associated to microorganisms can enhance aggregation/settling or metal complexation (Hennebel et al. 2015). All these could open alternatives for metals recovery and separation. It has been demonstrated that SRB generate extracellular proteins that complex metals such as zinc and Ni, chromium, and molybdenum in natural environments (Beech and Cheung 1995; Fortin et al. 1994; Guibaud et al. 2005; Moreau et al. 2007a; Moreau et al. 2007b). This is a protection mechanism against metals and occurs by an alteration of their protein expression profiles (Gillan 2016; Moreau et al. 2007b; Schneider and Riedel 2010). While these defence mechanisms are reported, no one has looked at how these mechanisms complex metals and if there is a difference in complexation depending on the metal. Therefore, the aim of this study was to determine the metal-microbe interactions in sulphate reducing bioreactors as a way to foresee opportunities for selective metal recovery of Ni and Co. The affinity proteins produced by SRB due to the presence of Ni and Co were identified through metagenomics, proteomics and metaproteomics techniques were applied.

Methods

A fist set of batch experiments with Co and Ni and centrifuged biogenic sulphide effluent (170 mg sulphide/L) from a sulphate reducing bioreactor were carried out to assess the differences in the characteristics of the Ni and Co sulphide precipitates. 100 or 500 ppm of Ni or Co as chlorides were added into 160ml of biogenic sulphide that contained 25 mM of sulphide. To determine which compound(s) can contribute to the difference of the metal precipitates formed in biogenic sulphide, acetate (0.4 g/L sodium acetate) and phosphate (1.86 g/L KH_2PO_4 and 1.1 g/L K_2HPO_4), mainly present in the VFA-S was added individually into chemically produced sulphide. Each experiment was done in triplicate in serum bottles of 250 mL. Samples were collected after 20 minutes for particle size distribution (PSD) and 4 days for scanning electron microscopy (SEM) analysis.

A second set of experiments were carried out with SRB biomass from a Continuous Stirred-Tank Reactor (CSTR). The experiments were done in triplicate using serum bottles (120 mL) with a working volume of 100 mL at 120 rpm and 30 °C. In each system, biomass (0.2g VSS/L), nutrients (Alexander J. B. Zehnder 1980), carbon source (sodium lactate), sulphate (sodium sulphate) and metals ($NiCl_2$ and/or $CoCl_2 \cdot 6H_2O$) were added. Sulphide concentration was measured every day, while COD, sulphate, pH and metals were measured at the end of the experiments. The following metals concentrations were evaluated: 1) 0, 10, 50, 100, 200 and 500 mg Ni^{2+}/L; (2) 0, 10, 50, 100, 200 and 500 mg Co^{2+}/L; (3) a mixture of 100 mg Ni^{2+}/L with 10, 50 and 100 mg Co^{2+}/L.

Ni and Co concentrations were measured with Perkin Elmer AAnalyst 400 Atomic Absorption Spectrometry (AAS) in an air-acetylene flame. Dissolved sulfide was determined spectrophotometrically by the colorimetric method described by Cord-Ruwisch (Cord-Ruwisch 1985) using a UV-VIS-NIR spectrophotometer, while sulfate was quantified using Dionex™ ICS-1100 Ion Chromatography System (IC) equipped with a Dionex AS-DV Autosampler. Visual characterization and semi-quantitative analysis of the Ni and Co sulfides produced in VFA-S were carried out using JEOL JSM 6610 Scanning Electron Microscope coupled with Energy-dispersive X-ray spectroscopy (SEM-EDS). PSS Nicomp Accusizer 780 AD was used to analyze PSD of Ni and Co sulfides. 0.5 ml of the samples was diluted to 80ml of Milli-Q water to ensure minimal particles were present in the water.

Samples of the liquid phase from the batch experiments were taken for protein identification using a Q-Exactive HF-X available through Proteomics Australia after trypsin digestion. The analyses of microbial community structure will be carried out using the Illumina platform at the Australian Centre for Ecogenomics at UQ, this information will be used as data library on the protein analysis.

Results and discussion

Metal precipitation experiments with biogenic sulphide showed that Ni precipitates were larger than Co precipitates (Figure 1). Acetate and phosphate present in the bioreactor were mainly responsible for the presence of larger Ni precipitates (7.80±0.52 µm and 12.28±0.75 µm of mean size), while these compounds did not affected Co precipitates (3.70±0.27 µm and 6.18±0.79 µm of mean size). Both compounds have been previously reported to affect particle size of metals (Esposito et al. 2006; Villa-Gomez et al. 2012). By contrast, Co solids could showed aggregation in the matrix (Figure 1), thus demonstrating a different interaction with the substances present on the biogenic sulphide.

The experiments with SRB biomass showed that an increase in Co addition, increase the sulphide production by SRB up to 277.8 mg/L (200 mg Co/L) due to Co sulphide precipitation (Figure 2), while at 500 mg/L, a significant decrease (129.8 mg/L) was observed, suggesting that the inhibitory concentration threshold was surpassed. By contrast, sulphide produced in Ni system decreased to 20.8 mg/L at 100 mg/L Ni supplementation, before increasing to 97.7 mg/L at 500 mg/L addition (Figure 2).

Co precipitation was high (90-95%) at 10-200 mg Co/L added in the experiments with SRB biomass (Figure 3a), while low removal (42%) at 500 mg Co/L was observed, probably due to the lack of sulphide for precipitation (Figure 2). Ni removal was lower than 45% at the different Ni supplementation concentrations (Figure 3a), and even non-removal was detected at 100 mg Ni/L

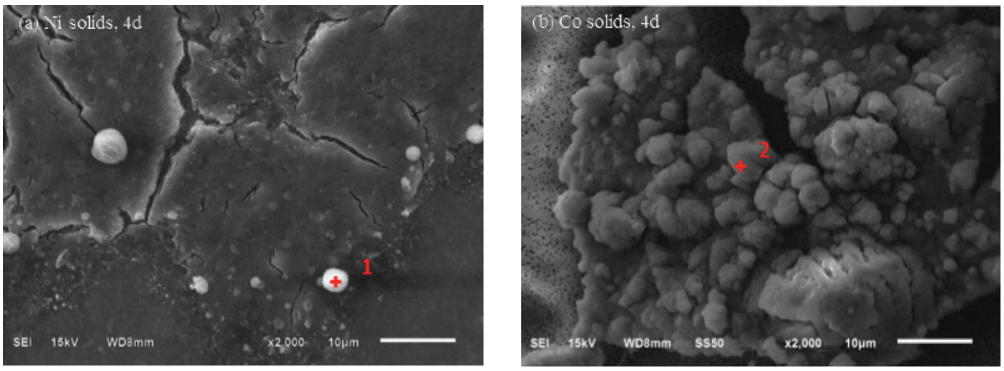

Figure 1 Secondary electron images and EDS of Ni and Co precipitates formed with biogenic sulphide.

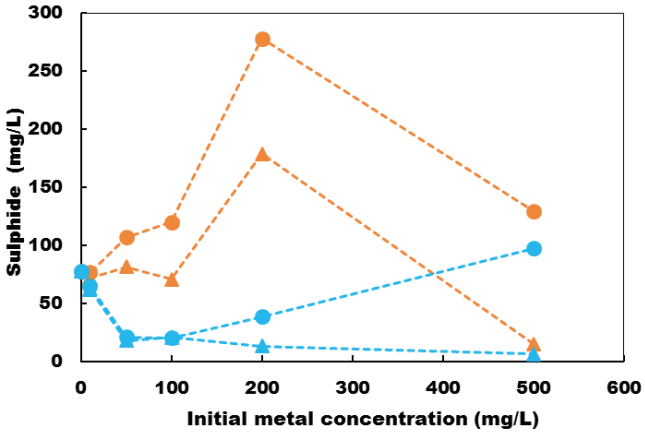

Figure 2 Remaining sulphide (Δ) and total sulphide (o) production (=remaining sulphide + sulphide in metal sulphide precipitates) in the experiments carried out with SRB biomass with Co addition (blue) and Ni addition (orange).

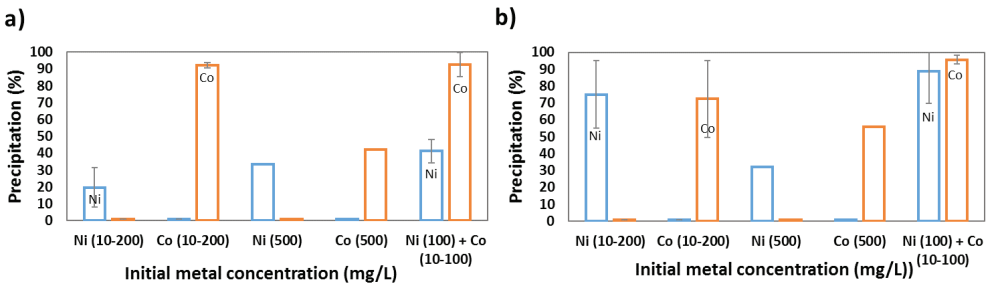

Figure 3 Ni (blue) and Co (orange) precipitation results in the experiments with a) SRB biomass and c) biogenic sulphide effluent.

(data not shown) despite the available sulphide for precipitation (Figure 2). Similar behaviour (low Ni precipitation and high Co precipitation) occurred when both metals were added together (Figure 2). These results suggest that in the presence of Ni, SRB generates extracellular proteins that selectively complex Ni, while Co does not generate this response. Unlike the experiments with SRB biomass, the experiments carried out with only biogenic effluent displayed 75% of Ni removal and 72% of Co removal for Ni and Co addition at 10-200 mg/L (Figure 3b). The decreased Ni and Co removal at 500 mg/L supplementation was a result of a lack of enough sulphide for metal precipitation (Figure 2). In these experiments, also both Ni and Co equally precipitated regardless being added together (Figure 3). Overall comparison among the experiments with SRB biomass and with only sulphide effluent allows to confirm that complexation of Ni is occurring in the system, that could be due to extracellular proteins generated by SRB in response to Ni stress. This has been previously observed by Fortin et al. (1994) on the SRB *Desulfotomaculum* sp., where in the presence of Ni with or without Fe, Ni remained mostly soluble at the cell surface, when cells were subjected to 100 mg/L of both metals, while with Fe only, large amounts of FeS (70% of the Fe) precipitated at the bacterial cell surface and extracellularly.

Microbial and protein analysis

This is the first study that shows the proteins involved in the complexation of Ni in sulphate reducing bioreactors. The high-throughput techniques used in this study have been scarcely used to understand the effect of metals on protein and gene expression in engineered systems. Previous work has identified proteins in the complexation of metals in pure cultures and with non-specific protein identifiers (Fortin et al. 1994; Lenz et al. 2011; Moreau et al. 2007b). Such information is highly relevant to develop a *technology that maintains Ni complexation in continuous sulphate reducing bioreactors and thus, separates Ni from Co.* In total, over 200 proteins were isolated from the experiments with SRB biomass, but this study only considered the first 10 identified proteins as they had the maximum number of peptides to bind metals. Some of these proteins were exclusively encountered on Ni or Co experiments (Table 1), while others were shared in both experiments as well as in the control experiments with no metals added (data not shown). The high affinity proteins associated with Ni precipitation were involved in transfer of electrons (Rubredoxin-oxygen oxidoreductase), iron uptake (Bacterioferritin) and outer membrane. *Desulfovibrio desulfurincans ND132* and *Desulfomicrobium baculatum* DSM 4028 were the main responsible for these proteins. The proteins encountered exclusively in the SRB biomass experiments with Co, were related to ATP, oxidoreductase, periplasmic and binding proteins. The same SRB organism was found to be responsible for the expression of these proteins but also *Pseudomones* and *Marinobacterium*. [NiFe] hydrogenases were also detected in the experiments, which obviously harbor Ni (Ogata et al. 2016) and are widely predominant in SRB species with versatile respiratory electron transport chain system such as *Desulfovibrio* (Zhuang et al. 2015). However, they were not

Table 1 Dominant proteins and the associated organisms encountered in the SRB biomass experiments.

	Characteristic protein	Accession ID	Gene Organism Name
Ni exposure	Selenocysteine-containing peroxiredoxin PrxU	R4187_S62_R4188_S63_02620	*Desulfovibrio desulfuricans ND132*
	Outer membrane protein P6	R4187_S62_R4188_S63_02748	*Desulfomicrobium baculatum DSM 4028*
	Bacterioferritin	R4187_S62_R4188_S63_06127	*Desulfomicrobium baculatum DSM 4028*
	Outer membrane efflux protein BepC	R4187_S62_R4188_S63_07083	*Desulfomicrobium baculatum DSM 4028*
	Rubredoxin-oxygen oxidoreductase	R4187_S62_R4188_S63_10797	*Desulfomicrobium baculatum DSM 4028*
	Rubredoxin-oxygen oxidoreductase	R4187_S62_R4188_S63_95586	*Desulfomicrobium baculatum DSM 4028*
	Rubredoxin-oxygen oxidoreductase	R4187_S62_R4188_S63_84067	*Desulfomicrobium baculatum DSM 4028*
Co exposure	ATP synthase subunit beta	R4187_S62_R4188_S63_55496	*Pseudomonas simiae*
	ATP synthase subunit alpha	R4187_S62_R4188_S63_55499	*Pseudomonas veronii 1YdBTEX2*
	ATP synthase subunit beta	R4187_S62_R4188_S63_22536	*Marinobacterium sp. ST58-10*
	Transcription termination/antitermination protein NusG	R4187_S62_R4188_S63_74555	*Pseudomonas veronii 1YdBTEX2*
	Leucine-%2C isoleucine-%2C valine-%2C threonine-%2C and alanine-binding protein	R4187_S62_R4188_S63_03572	*Desulfomicrobium baculatum DSM 4028*
	putative FAD-linked oxidoreductase	R4187_S62_R4188_S63_56986	*Desulfomicrobium baculatum DSM 4028*
	putative FAD-linked oxidoreductase	R4187_S62_R4188_S63_09244	*Desulfomicrobium baculatum DSM 4028*
	Spermidine/putrescine-binding periplasmic protein	R4187_S62_R4188_S63_08733	*Desulfomicrobium baculatum DSM 4028*
	Spermidine/putrescine-binding periplasmic protein	R4187_S62_R4188_S63_55657	*Desulfomicrobium baculatum DSM 4028*

exclusively detected only on the presence of Ni. Contrary, in a previous study, expressed autolysis-inducible proteins and cell wall autolysis by-products that bounds Ni where detected on a pure culture of *Desulfotomaculum* sp. (Fortin et al. 1994). Nevertheless, this study used a mixed culture unlike the aforementioned study, which shows that the Ni effect can be found in other SRB species and thus, it could allow the development of a more versatile and efficient sulphate reducing bioreactor system for Ni recovery.

Conclusions

The results presented in this study shows that in the presence of Ni, SRB generates extracellular proteins that selectively complex Ni, while Co does not generate this response and tends to precipitate with sulphide. The modification of the removal behaviour of Ni and Co through SRB culture is a sustainable solution to selectively recover both metals from mine drainages, thus providing an environmental and economic benefit.

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References

Alexander J. B. Zehnder BAH, Thomas D. Brock, and Karl Wuhrmann (1980) Characterization of an Acetate-Decarboxylating, Non-Hydrogen-Oxidizing Methane Bacterium Arch Microbiol 124:1-11

Beech IB, Cheung CWS (1995) Interactions of exopolymers produced by sulphate-reducing bacteria with metal ions Int Biodeterior Biodegrad 35:59-72 doi:10.1016/0964-8305(95)00082-G

Cord-Ruwisch R (1985) A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria J Microbiol Methods 4:33-36

Crundwell FK, Moats MS, Ramachandran V, Robinson TG, Davenport WG (2011) Cobalt – Occurrence, Production, Use and Price chapter 28:357-363 doi:10.1016/b978-0-08-096809-4.10028-0

Esposito G, Veeken A, Weijma J, Lens PNL (2006) Use of biogenic sulfide for ZnS precipitation Sep Purif Technol 51:31-39 doi:10.1016/j.seppur.2005.12.021

Flett DS (2004) Cobalt-Nickel Separation in Hydrometallurgy: a Review Chemistry for Sustainable Development 12:81-91

Fortin D, Southam G, Beveridge TJ (1994) Nickel sulfide, iron-nickel sulfide and iron sulfide precipitation by a newly isolated Desulfotomaculum species and its relation to nickel resistance FEMS Microbiol Ecol 14:121-132 doi:10.1111/j.1574-6941.1994.tb00099.x

Gillan DC (2016) Metal resistance systems in cultivated bacteria: are they found in complex communities? Curr Opin Biotechnol 38:123-130 doi:https://doi.org/10.1016/j.copbio.2016.01.012

Guibaud G, Comte S, Bordas F, Dupuy S, Baudu M (2005) Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel Chemosphere 59:629-638 doi:http://dx.doi.org/10.1016/j.chemosphere.2004.10.028

Hennebel T, Boon N, Maes S, Lenz M (2015) Biotechnologies for critical raw material recovery from primary and secondary sources: R&D priorities and future perspectives New Biotechnology 32:121-127 doi:http://dx.doi.org/10.1016/j.nbt.2013.08.004

Lenz M, Kolvenbach B, Gygax B, Moes S, Corvini PFX (2011) Shedding Light on Selenium Biomineralization: Proteins Associated with Bionanominerals Appl Environ Microbiol 77:4676-4680 doi:10.1128/aem.01713-10

Moreau J, Weber P, Martin M, Gilbert B, Hutcheon I, Banfield J (2007a) Extracellular Proteins Limit the Dispersal of Biogenic Nanoparticles Science (Washington) 316:1600-1603

Moreau JW, Weber PK, Martin MC, Gilbert B, Hutcheon ID, Banfield JF (2007b) Extracellular Proteins Limit the Dispersal of Biogenic Nanoparticles Science 316:1600-1603 doi:10.1126/science.1141064

Nehdi M, Tariq A (2007) Stabilization of sulphidic mine tailings for prevention of metal release and acid drainage using cementitious materials: a review Journal of Environmental Engineering and Science 6:423-436 doi:10.1139/s06-060

Ogata H, Lubitz W, Higuchi Y (2016) Structure and function of [NiFe] hydrogenases J Biochem 160:251-258 doi:10.1093/jb/mvw048

Sánchez-Andrea I, Stams AJM, Weijma J, Gonzalez Contreras P, Dijkman H, Rozendal RA, Johnson DB (2016) A case in support of implementing innovative bio-processes in the metal mining industry FEMS Microbiol Lett 363 doi:10.1093/femsle/fnw106

Schneider T, Riedel K (2010) Environmental proteomics: Analysis of structure and function of microbial communities Proteomics 10:785-798 doi:10.1002/pmic.200900450

Sima M, Dold B, Frei L, Senila M, Balteanu D, Zobrist J (2011) Sulfide oxidation and acid mine drainage formation within two active tailings impoundments in the Golden Quadrangle of the Apuseni Mountains, Romania Journal of hazardous materials 189:624-639 doi:10.1016/j.jhazmat.2011.01.069

Simonnot M-O, Vaughan J, Laubie B (2018) Processing of Bio-ore to Products. In: Van der Ent A, Echevarria G, Baker AJM, Morel JL (eds) Agromining: Farming for Metals: Extracting Unconventional Resources Using Plants. Springer International Publishing, Cham, pp 39-51. doi:10.1007/978-3-319-61899-9_3

Thurtell D, Gibbons M, Philalay M, Drahos N, Martin K, Moloney J, Nguyen T (2018) Resources and Energy Quarterly March 2018. Australian Goverment, Office of the Chief Economist

Villa-Gomez DK, Lens PNL (2017) Metal recovery from industrial and mining wastewaters using sulphate-reducing bioreactors. In: Rene ER, Sahinkaya E, Lewis A, Lens P (eds) Sustainable Heavy Metal Remediation: Volume 2: Case studies, vol 9. Springer Cham, Switzerland. doi:10.1007/978-3-319-61146-4_3

Villa-Gomez DK, Papirio S, van Hullebusch ED, Farges F, Nikitenko S, Kramer H, Lens PN (2012) Influence of sulfide concentration and macronutrients on the characteristics of metal precipitates relevant to metal recovery in bioreactors Bioresource technology 110:26-34 doi:10.1016/j.biortech.2012.01.041

Zhuang W-Q, Fitts JP, Ajo-Franklin CM, Maes S, Alvarez-Cohen L, Hennebel T (2015) Recovery of critical metals using biometallurgy Curr Opin Biotechnol 33:327-335 doi:http://dx.doi.org/10.1016/j.copbio.2015.03.019