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Enhancing the activity of iron-oxidising bacteria: A case study with process liquors from heap bioleaching of a complex sulphide ore

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ABSTRACT

In the present study, six process liquors (PL1 - PL6) originating from heap bioleaching of a complex sulphide ore were examined to reveal factors limiting microbial activity in the bioheaps. PL4 had the lowest iron oxidation activity even though its indigenous iron-oxidising community was diverse (*Acidithiobacillus, Leptospirillum*, *Acidiferrobacter*, and *Sulfobacillus* species). Shake flask experiments at 27 °C revealed that ferrous iron (Fe^{2+}) and aluminium (Al^{3+}) concentrations up to 16 and 12 g/L, respectively, were not inhibitory for the iron-oxidising microorganisms in PL4. In addition, Al^{3+} concentrations of ≤6 g/L were shown to enhance iron oxidation rates. High correlation between increased concentrations of cadmium (Cd), sulphate (SO_4^{2-}), and vanadium (V) and decreased iron oxidation rates was detected when comparing process liquors 1–6. Moreover, possible nutrient limitation in PL4 was delineated by selectively supplementing it with macro- and micronutrients. Supplementation of 320 mg/L of nitrogen (as NH_4^+) to PL4 significantly increased iron oxidation rates from 20 mg/L/h (no nutrient supplementation) to 160 mg/L/h and would likely also enhance the heap bioleaching process. Additionally, microorganisms growing in high inhibitory ion concentrations (e.g. Cd^{2+}) were shown to be more sensitive to nitrogen deficiency than microorganisms growing in more dilute liquors.

1. Introduction

Heap bioleaching is used worldwide to extract metals particularly from low-grade sulphide ores (Brierley, 2008). Effective mineral oxidation inside the heaps is dependent on the activity of iron- and sulphur-oxidising microorganisms (Akcil et al., 2007). Studies on Talvivaara multimetal black schist ore deposit located in Sotkamo, Finland have demonstrated that the bioheaps have diverse indigenous microbial communities (Halinen et al., 2009a, 2009b, 2012). The heap leaching solutions contain several iron-oxidising microorganisms that have been shown to actively oxidise iron at high manganese (15.6 g/L Mn), aluminium (10.3 g/L Al), zinc (4.20 g/L Zn), nickel (2.64 g/L Ni), magnesium (1.2 g/L Mg), copper (0.37 g/L Cu), and cobalt (0.08 g/L Co) concentrations released during the bioleaching (Halinen et al., 2009a). Additionally, the indigenous iron-oxidising microorganisms have successfully been used to precipitate excess iron from barren heap leaching solutions (Nurmi et al., 2009) and to remove dissolved iron and sulphate from effluent streams obtained from the multimetal ore heap bioleaching process (Nurmi et al., 2010).

High metal concentrations present in heap bioleaching processes can lead to metal accumulation inside the microbial cells and subsequently inhibit the activity of the iron-oxidising microorganisms (Dopson et al., 2003). Some metals, such as iron (Fe) and Mn, act as important trace elements in biochemical reactions. Other metal cations, such as mercury (Hg^{2+}), cadmium (Cd^{2+}), and silver (Ag^+), have no physiological function due to their high toxicity to microbial cells (Nies, 1999). The toxic effect of a metal cation is also highly dependent on its oxidation state. Trivalent cations, such as Al^{3+}, may become toxic to microorganisms due to their replacement of essential divalent metal complexes in the cells, as higher valence complexes are thermodynamically more stable (Amonette et al., 2003). Moreover, microorganisms do not have active transport systems for trivalent ions as they do for mono- and divalent cations (Williams, 1999), making trivalent cations more difficult to detoxify.

In addition to soluble metals, a wide range of macro- and micronutrients affect the activity of iron-oxidisers. Sulphide ores generally contain adequate amounts of micronutrients (e.g. trace metals). However, bioleaching process solutions may need to be supplied with macronutrients such as ammonium (NH_4^+) and potassium (K) (du Plessis et al., 2007). After carbon, nitrogen is the second most important element for biomass formation as it is an integral constituent of many proteins and nucleic acids. Some iron-oxidisers, such as *Acidithiobacillus ferrooxidans* (Levicán et al., 2008; Valdés et al., 2008; Yan et al., 2015), *Leptospirillum ferrooxidans* (Norris et al., 1995), and *L. ferrodiazotrophum* (Tyson et al., 2005), are able to fix nitrogen (N_2) from the atmosphere

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and thus grow diazotrophically. Although it has been previously reported that e.g. *L. ferriphilum* is not able to fix atmospheric N_2 (d'Hugues et al., 2008; García-Moyano et al., 2008; Tyson et al., 2004), recent studies have shown that some strains are able to switch to N_2 fixation if NH_4^+ becomes scarce in the leaching environment (Galleguillos et al., 2013; Issotta et al., 2016). Iron-oxidisers incapable of N_2 fixation are dependent on the uptake of dissolved nitrogen compounds from the leaching environment. Moreover, bioleaching microorganisms cannot acquire phosphate (PO_4^{3-}) from air, unlike carbon or in some cases nitrogen, and the nutrient is therefore sometimes supplied as a fertiliser to the bioleaching solutions (Rawlings, 2007).

As the indigenous iron-oxidising microorganisms are essential for both the Talvivaara bioleaching process and the control of waste streams produced during bioleaching, it was necessary to study their iron oxidation activity in the actual process liquors originating from the heap bioleaching process. Microbial community compositions in the liquors were delineated and their growth conditions studied. Moreover, different physicochemical factors affecting iron oxidation such as nutrient sufficiency and heavy metal inhibition were explored using shake flasks. The aim was to reveal factors limiting microbial activity in the bioheaps and to find ways to enhance the activity of the indigenous bioleaching microorganisms in order to further enhance the bioleaching process.

2. Materials and methods

2.1. Process liquors from the mine site

The six process liquors (PL) studied in this work were collected on the same day from different parts of a heap bioleaching process of a complex sulphide ore as described in Table 1. The chemical composition of the liquors at the time of the sampling was as presented in Table 2. The samples were stored at 4 °C and used in the experiments without sterilisation. PL4 was studied most thoroughly due to its decreased iron oxidation activity revealed in shake flask assays described in Section 2.2.

2.2. Shake flask experiments

In the present study, six shake flask experiments (Table 3) were conducted to reveal factors limiting microbial activity in the bioheaps and to find ways to improve the iron oxidation activity (i.e. the iron oxidation rate; see Section 2.5) of the indigenous bioleaching microorganisms in order to enhance the overall bioleaching process. In **Experiment 1 "Comparison of process liquors"**, the possible inhibitory effects of process liquors 1–6 on the indigenous microorganisms were studied by incubating 95% (v/v) of each liquor with 5% (v/v) of an iron-oxidising enrichment culture (see Section 2.4). The aim was to compare the iron oxidation activity of process liquors 1–6 with respect to their chemical composition (Table 2) in order to delineate the possible inhibitory factors in the bioheaps. Experiment 1 revealed that PL4 had the lowest iron oxidation activity and subsequently, iron oxidation in a dilution series of PL4 in mineral salts medium (MSM; see Section 2.3) was examined to further quantify the liquor's inhibitory factors and/or nutrient limitation (**Experiment 2 "Dilution series with PL4"**). The experiment was conducted by supplementing 40–100% (v/v) of PL4 with 0–1% (v/v) of trace elements solution (TES; see Section 2.3) and MSM to adjust the final culture volume to 100 mL (0–49% v/v).

As bioleaching environments may contain high ferrous iron (Fe^{2+}) concentrations, the resistance of the indigenous iron-oxidising microorganisms towards Fe^{2+} was examined by supplementing diluted PL4 with Fe^{2+} stock solution so that the initial Fe^{2+} concentration in the cultures was 5–16 g/L (**Experiment 3 "Fe^{2+} resistance"**). Fe^{2+} concentration of 5 g/L was considered as a control, as the concentration was lower than in the iron-oxidising enrichment culture (5.6 g/L; see Section 2.4) or in any of the process liquors (6.1–15 g/L; see Table 2). Moreover, the effect of high aluminium (Al^{3+}) concentrations towards the activity of the indigenous microorganisms was studied due to the metal's trivalent nature by supplementing diluted PL4 with 3–12 g/L of Al^{3+} added as $Al_2(SO_4)_3 \cdot \sim 14\ H_2O$ (**Experiment 4 "Al^{3+} toxicity"**). No aluminium was added to the control flask.

Experiment 5 "Delineating limiting nutrients" was conducted by incubating PL4 1) on its own, 2) with MSM and TES, 3) with MSM, and 4) with TES. The aim was to reveal if the iron oxidation activity of the indigenous microorganisms was decreased by nutrient deficiency and whether PL4 was lacking mineral salts (macronutrients), trace elements (micronutrients), or both. Experiment 5 revealed that the mineral salts content of PL4 was insufficient to support the iron oxidation activity of the indigenous microorganisms. Nitrogen (N) and sodium (Na) were potentially lacking from PL4 based on its chemical composition data (see Table 2). Phosphorus (P) and potassium (K), on the other hand, were selected for further studying as they are essential for microbial growth. Therefore, PL4 was supplemented separately with i) N as $(NH_4)_2SO_4$, ii) P as K_2HPO_4, iii) Na as $Na_2SO_4 \cdot 10\ H_2O$, and iv) both Na and K as $Na_2SO_4 \cdot 10\ H_2O$ and KCl, respectively (**Experiment 6 "Macronutrient limitation"**). The initial concentrations of the compounds corresponded to their initial concentrations in Experiment 5 (containing 49% v/v MSM) to allow comparison between Experiments 5 and 6.

The shake flask experiments were carried out in duplicate at 27 °C, at 150 rpm mixing. The experiments are summarised in Table 3 using the following variables: addition of process liquors or selected metals/nutrients; whether or not the iron-oxidising enrichment culture (see Section 2.4) was added as inoculum; nutrient content (see Section 2.3); and the possible controls included in the experiments. Culture pH was adjusted to 1.7 before autoclaving with concentrated H_2SO_4 in all the

Table 1
Origin of process liquors 1–6.

Process liquor	Origin
1–4	Primary heap pads (equipped with air piping and irrigation with recycled leach solution)
5	Secondary heap pad (containing reclaimed and re-stacked ore from the primary heap pads)
6	Raffinate pond

Table 2
Chemical composition of process liquors 1–6.

		Process liquor					
		1	2	3	4	5	6
pH		3.15	2.34	2.17	2.05	3.53	1.60
Redox (vs. Ag/AgCl)	mV	310	389	419	385	279	322
Aluminium	mg/L	1400	3600	9000	6100	3100	71
Calcium	mg/L	620	530	610	630	630	480
Cadmium	mg/L	3.2	5.2	16	16	6.3	0.37
Cobalt	mg/L	9.5	21	84	47	24	0.42
Copper	mg/L	6.3	45	200	89	1.9	0.38
Total iron	mg/L	16,000	8500	10,000	16,000	14,000	6500
Ferrous iron	mg/L	15,000	7200	8400	14,000	13,000	6100
Magnesium	mg/L	5600	4700	9000	9400	7000	5400
Manganese	mg/L	8100	4000	5400	6400	5300	3500
Molybdenum	μg/L	140	84	96	110	99	59
Nickel	mg/L	1200	970	2300	2400	1500	31
Nitrogen	mg/L	2.6	<1.0	1.5	<1.0	1.0	<1.0
Selenium	μg/L	<200	<200	<200	<200	<200	<200
Silicon	mg/L	120	130	140	140	120	4.8
Sodium	mg/L	470	290	59	58	270	2200
Sulphate	mg/L	72,000	64,000	130,000	130,000	78,000	49,000
Vanadium	μg/L	2300	6600	37,000	34,000	4900	820
Zinc	mg/L	3500	2300	5100	6000	3400	18