Hydrometallurgy 125–126 (2012) 34–41

Contents lists available at SciVerse ScienceDirect

Hydrometallurgy

journal homepage: www.elsevier.com/locate/hydromet

Microbial community dynamics during a demonstration-scale bioheap leaching operation

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ARTICLE INFO

Article history:
Received 6 February 2012
Received in revised form 23 April 2012
Accepted 3 May 2012
Available online 9 May 2012

Keywords:
Bioleach communities
Complex sulfide ore
Demonstration-scale bioheap
Mesophiles
Thermophiles

ABSTRACT

In the present work the microbial community of a low grade nickel ore demonstration-scale bioheap was examined under varying weather (outside air temperature between +30 and −39 °C) and operational conditions over a period of three years in Talvivaara, Finland. After the start-up of heap irrigation, oxidation of pyrrhotite and pyrite increased the heap temperature up to 90 °C. Leach liquor temperatures varied between 60 and 15 °C over the operation period, affecting the progress of sulfide ore oxidation.
The microbial communities were profiled by polymerase chain reaction (PCR) — denaturing gradient gel electrophoresis (DGGE) followed by partial sequencing of 16S rRNA gene. Large temperature gradients prevailed resulting in the simultaneous presence of active mesophilic and thermophilic iron- and/or sulfur-oxidisers in the heap. As mineral oxidation progressed microbial diversity decreased and *Acidithiobacillus ferrooxidans* became increasingly dominant. The number of bacteria in the leach liquors was in the range of 10^5–10^7 cells mL^{-1}. After one year of bioheap operation several ore samples were drilled from the heap and *A. ferrooxidans*, *Acidithiobacillus caldus,* an uncultured bacterium clone H70 related organism, *Ferrimicrobium acidiphilum* and a bacterium related to *Sulfobacillus thermosulfidooxidans* were found. Cell counts from the ore samples varied between 10^5 and 10^7 cells g^{-1} ore sample. The archaeal species present in leach liquors were novel and related to uncultivated species. During the secondary leaching phase the leaching community remained steady. *A. ferrooxidans* dominated, and an uncultured bacterium clone H70-related organism and *Leptospirillum ferrooxidans* were present.

1. Introduction

Heap bioleaching of low-grade sulfide ores has become an important process for metal recovery (for reviews, see: Rawlings, 2002; Watling, 2008). During the last twenty years the process has been optimized successfully including ore crushing, agglomeration, aeration, leach liquor distribution and stacking stages (Brierley and Brierley, 2001). Leach liquor pH can be adjusted before irrigation. Temperatures are affected by the composition and concentration of the sulfidic minerals because of exothermic oxidation reactions. Aeration and irrigation rates affect evaporation and heat dissipation (Ehrlich, 2001; Rawlings, 2002; Watling, 2006).

Microorganisms present in bioheaps are mainly ferrous iron- and sulfur-oxidizing chemolithotrophs, although some heterotrophs have been reported (Hallberg and Johnson, 2001). The regeneration of ferric iron (Fe^{3+}) and proton release (H^+) are essential for metal sulfide oxidation and dissolution of valuable metals. As sulfuric acid is produced by the oxidation of sulfur, these organisms generate an acidic growth environment. Many of the chemolithotrophic acidophiles are sensitive to organic matter and thus heterotrophic acidophiles detoxify the bioleaching environment. A small fraction of the bioleaching microorganisms is found in the leach liquor, while most of the microorganisms adhere to the mineral surfaces (Rohwerder et al., 2003; Crundwell, 1996). Studies of microbial communities inhabiting commercial reactor based, bioleaching processes have been successfully carried out in recent years (Pradhan et al., 2008). However, microorganisms inhabiting industrial bioheaps and dumps have gained less attention (Demergasso et al., 2005).

The aim of the present work was to study the microbial community structures and their dynamics during a demonstration-scale complex sulfide ore (17 000 tons) bioheap leaching operation. Spatial and temporal changes in microbial communities were monitored and included strong fluctuations.

1.1 . Talvivaara ore deposit

Talvivaara complex multi-metal black schist ore deposit is located in central-eastern Finland with 1550 million tons of classified

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doi:10.1016/j.hydromet.2012.05.001

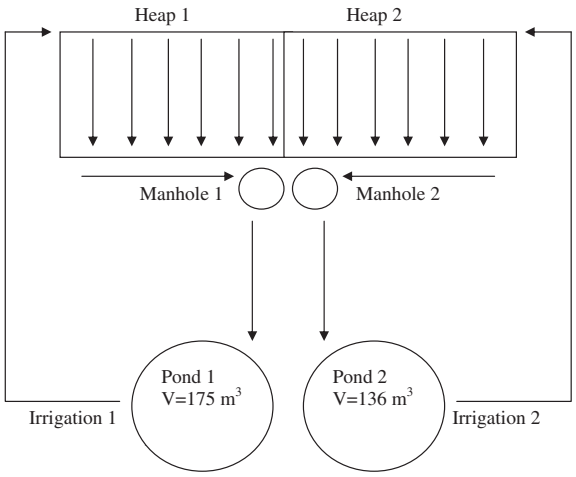

Fig. 1. Diagram of the sampling points of the Talvivaara bioheaps with the direction of the liquid flow marked with arrows. Each heap had its own liquid circulation. The amount of the ore of Heap 1 was 10 255 tons and for Heap 26 703 tons, respectively.

resources (Talvivaara, 2012). The mineral composition of the sulfides used in the demonstration-scale bioheaps was 61.2% pyrrhotite [$(Fe_{1-x})(S_2)$, where X = 0.7–0.9], 24.3% pyrite (FeS), 5% pentlandite [$(Fe,Ni,Co)_9S_8$], 6.5% alabandite (MnS), 2.4% chalcopyrite ($CuFeS_2$) and 1% sphalerite [(Zn,Fe)S]. Valuable metal contents were as follows: 0.27% Ni, 0.56% Zn, 0.14% Cu and 0.02% Co (for detailed description see Riekkola-Vanhanen, 2007). Prior to the bioheap demonstration, laboratory scale studies had demonstrated the amenability of Talvivaara ore to bioleaching (e.g. Puhakka and Tuovinen, 1986a, b, c; Riekkola-Vanhanen and Heimala, 1999; Wakeman et al., 2008; Halinen et al., 2009a, b). After a year of bioleaching 65% of nickel and 60% of zinc were leached. After 48 months, 99% of nickel and zinc were leached.

2. Materials and methods

2.1. Design and start-up of the demonstration heaps

During summer 2005, a 17 000 ton demonstration plant was constructed at the Talvivaara mine site (Fig. 1). A representative ore sample was mined, crushed to 80% −8 mm, agglomerated and stacked in a two-part heap (8 m high, 30 × 120 m). Heap 1 was agglomerated with sulfuric acid solution (pH 1.8) including inoculum (described below). Heap 2 was agglomerated with sulfuric acid solution only. Irrigation of the heaps was started in August 2005. The irrigation flow rate was at the beginning 10 L m^{-2} h^{-1} on Heap 1 and 20 L m^{-2} h^{-1} on Heap 2. It was decreased later to 5 L m^{-2} h^{-1} on both heaps.

Leach liquors were collected by subsurface drains below the heaps and directed to manholes. From the manholes liquors flowed to pregnant leach solution (PLS) ponds and back to irrigation (Fig. 1). The operational volumes of ponds 1 and 2 were 175 m^3 and 136 m^3, respectively. Ten percent side stream was removed continuously for metal recovery and replaced with well water. After the start-up of irrigation, the oxidation of pyrrhotite and pyrite increased the heap temperature up to 90 °C. Leach liquor temperatures remained always at above 15 °C over the operation period, even during the boreal winter.

2.2. Inoculation of Heap 1

The iron and sulfur-oxidizing enrichment culture was originally enriched from mine site water samples on Fe^{2+}, S^0 and Talvivaara ore powder at pH 1.8 (Halinen et al., 2009a). The enrichment culture was grown in laboratory to the volume of 4.5 m^3 (Geological Survey of Finland (GTK), Outokumpu). It was transported to the mine site and pumped into a microbial pond (MP) with initial water volume of 40 m^3. Most of the water used originated from on-site drilled well (temperature 5 °C). Liquid pH in the pond was adjusted to 1.8 with sulfuric acid and pulp concentration (w v^{-1}) was set to 1% prior to inoculation. After the inoculation ammonium sulfate concentration was increased stepwise to 0.4 g L^{-1} using 25% (v v^{-1}) stock solution and 500 kg of elemental sulfur was added. No liquid heating or cover was used. The volume of 40 m^3 was increased to 150 m^3 with well water. Inoculation of Heap 1 was accomplished during agglomeration and by irrigating the heap by acidic microbial solution, total inoculum volume being 99 m^3. Heap 2 was not inoculated.

2.3. Secondary bioheaps

On February 2007 after 18 months of operation, the heaps were reclaimed and restacked to the secondary bioheap. Irrigation rate was 2 L m^{-2} h^{-1}. No aeration was provided. Bioleaching of copper and cobalt was continued (data not shown). Minor amounts of nickel and zinc were bioleached, probably from the parts that were not reached during the primary phase.

2.4. Sampling

First samples for microbiological analyses were taken from the microbial pond (MP), where the inoculum was grown, and from the manholes (MH 1 and 2) that collected the irrigation and rain water that percolated trough the heaps. Next samples were taken after 3 months of bioleaching. Samples (50 mL) from manholes and ponds (P 1 and 2) were collected thereafter every month. In July 2006 pond samples were changed to irrigation samples (IR 1 and 2). Sampling was continued when primary bioheaps were reclaimed to the secondary bioheaps. Fig. 1 shows the sampling points and the sampling and analysis program was as presented in Fig. 2.

2.5. Cell counts

Total cell counts were estimated from the samples with 4′, 6-diamidino-2-phenylindole (DAPI) staining technique using epifluorescence microscopy. Microbes were detached from the ore samples according to methods described in Halinen et al. (2009a). 15 g of the ore sample was mixed with 40 mL of sterile Zwittergent-washing solution (0.38 g L^{-1} ethylene glycol tetraacetic acid, 3.35^{-4} g L^{-1} Zwittergent, 3.73 g L^{-1} KCl, pH adjusted to 2.5 with 2 M HCl). The mixture was shaken and sonicated 5 × 1 min in order to detach microorganisms from ore particles. Thereafter, the sample was allowed to settle for about 30 min to prevent the small ore

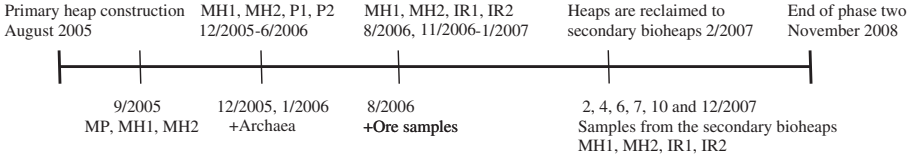

Fig. 2. Timescale of sampling. MP = microbial pond, MH1 = manhole 1, MH2 = manhole 2, P1 = pond 1, P2 = pond 2, IR1 = irrigation 1, IR2 = irrigation 2.