

MICROBIAL STUDY OF THE MARINE SHORE PORPHYRY COPPER TAILINGS DEPOSIT BAHIA DE ITE, PERU¹

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Abstract. Preliminary results of a microbiological study of the marine shore tailings disposal at the Bahia de Ite (tailings from Toquepala and Cuajone porphyry copper mines, Peru) are presented. The goal of the study is to understand the microbiological communities and its distribution in the tailings profiles before and after the installation of a wetland on the marine shore tailings deposit. The tailings have low sulfide (~2 wt% pyrite equivalent) and low carbonate contents. Molecular based methods, mainly terminal restriction fragment length polymorphism (T-RFLP), cloning and sequencing as well as cultivation methods were applied to characterize the microorganisms. Iron/sulfur oxidizing bacteria (e.g. *Leptospirillum*, *Acidithiobacillus* and *Sulfobacillus*-like) and heterotrophs (e.g. *Acidiphilium* and *Acidobacterium*-like) were present in the still oxidizing tailings. The maximum bacteria, as determined by total cell count, were detected at the oxidation front where sulfide minerals as pyrite and oxygen are both available. These tailings are characterized by a low biomass and a low bacterial diversity. In the remediated zone, due to the less extreme condition, the bacterial diversity was higher and a dominance of heterotrophs and sulfate reducing bacteria (SRB) were expected in this zone.

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Introduction

The microbiology of mining environment has become a research topic of great importance (Johnson and Hallberg, 2003). Interaction between microbes and chemical parameters is a key factor for a better understanding of mine waste environment, and for solution and prevention propositions. Microorganisms play an important role in the processes of sulfide oxidation and reduction in mine waste systems. According to redox conditions, they can participate in the formation and/or neutralization of acid mine drainage. Acidophilic microorganisms (bacteria and archaea) are able to catalyze Fe^{+2} iron oxidation, increasing the rate of supply of Fe^{+3} iron to oxidize metal sulfides (Baker and Banfield, 2003; Johnson and Hallberg, 2003). Others microorganisms can reduce Fe and sulfide. The goal of this study is to understand the biogeochemical processes resulting from the construction of a wetland on the oxidizing tailings (Diaby et al., 2006). In the here presented study, molecular techniques, mainly Terminal restriction enzyme fragment length polymorphism (T-RFLP) were used to investigate the microbial communities involved in the oxidation and reduction processes in the tailings deposited at the Ite bay, Southern Peru. Microbial communities along a depth profile of the remediated (represented by SI4) and un-remediated zone (represented by SI10 and SI11) of the deposit were investigated.

Methodology

Sampling was taken using percussion soil sampling equipment and the samples were frozen until processing. Molecular methods were used to characterize bacterial communities. DNA was extracted using a soil DNA isolation kit from MoBio Laboratories Inc. The extracted DNA served as template for amplification of the 16S rRNA genes by PCR using Eubacterial specific primers. The PCR products were analyzed by T-RFLP to highlight the relative abundance and predominance of bacterial groups.

Preliminary results

Biomass distribution

The DNA extraction does not work in all the samples analyzed. Only the samples of the oxidation zone and the oxidation front gave quantifiable DNA which was used for further analysis. This is due to the fact that these areas contain the optimal condition of bacterial development (oxygen and sulfides). In the un-remediated zone, some of the acidophilic bacteria are autotrophic, and therefore they are able to synthesize biomass by utilizing CO_2 as carbon source. They obtain energy for carbon fixation and growth through sulfide and/or Fe^{+2} oxidation. The presence of these acidophiles promotes development of heterotrophic bacteria in the same area as they can use the organic compounds excreted by the autotrophs (either from living or dead biomass).

The sulfide minerals in the tailings were oxidized gradually from surface towards depth, a process which is increased by microbiological activity (Ehrlich, 1998). Dold *et al* (2005) showed that all of the biological and geochemical evidences points to the complete oxidation of Fe^{+2} to Fe^{+3} in the oxidation zone and the exhaustion of sulfide minerals at the surface of the tailings lead to the movement of the majority of bacteria towards the richer zone. This creates an oxidation front that migrates downwards in the tailings stratigraphy. Thus, the bacteria follow this front to find their nutrients, i.e. sulfides.

In the remediated zone, where reducing conditions were installed, bacteria were detected mainly at the interface water/sediment which offers to them the optimal living area, i.e. it is a zone rich in SO_4^{-2} and Fe^{+3} which can be reduced for the metabolism of the organisms.

Bacterial composition

The Terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis give the relative abundance and can indicated the relative dominance of groups of bacteria. The number of nucleotides obtained which is specific for each bacterium can give indication about the species expected. Four samples of the un-remediated zone (Fig. 1) and one of the remediated zones (Fig. 1) are presented to show the bacterial diversity in the tailings of Ite bay. Terminal Restriction Fragment Length Polymorphism (T-RFLP) carried out on the SI11 samples (85 and 150 cm) showed the predominance of terminal restriction fragments with 370 bp and 153 bp (Fig. 1, A, B); whereas the SI10 samples showed dominance of terminal restriction fragments with 204 bp (Fig. 1 C) and 435 bp (Fig. 1 D). These terminal restriction fragments are close to other obtained by digestion with the same enzyme (HhaI) and described by Diaby et al 2005. They correspond to Fe or Fe/S oxidizing bacteria. 370 bp is the finger print of *Leptospirillum*-like bacteria known to be an exclusive Fe oxidizer and usually found in mining environment. 204 bp is a finger print characteristic of the Fe/S oxidizer and Fe reducer, *Acidithiobacillus ferroxidans*. In the remediated zone (Fig. 1 E), due to the less extreme condition, the bacterial diversity was higher and dominated by terminal restriction fragment with 91, 92, 94 bp which might correspond to the same specie. Due to the reducing condition, sulfate reducing bacteria (SRB) are expected in this zone.

Preliminary Conclusion

The tailings reserve of Ite bay showed a great difference between the microbiological communities of the remediated and the un-remediated zone. This is due to the difference of environmental conditions. In the un-remediated zone where sulfides minerals are still present in contact with oxidizing condition, presence of Fe oxidizers and Fe/S oxidizing bacteria are expected, whereas SO_4^{-2} reducing bacteria and other anaerobes will dominate the wetland. This wetland (remediated zone) is less extreme, with pH around neutral value and anoxic conditions. It showed more relative bacterial diversity than the un-remediated zone. The tailings reserve of Ite bay is an example showing the change of bacterial composition and diversity in mining environment according to oxidation and reduction processes.

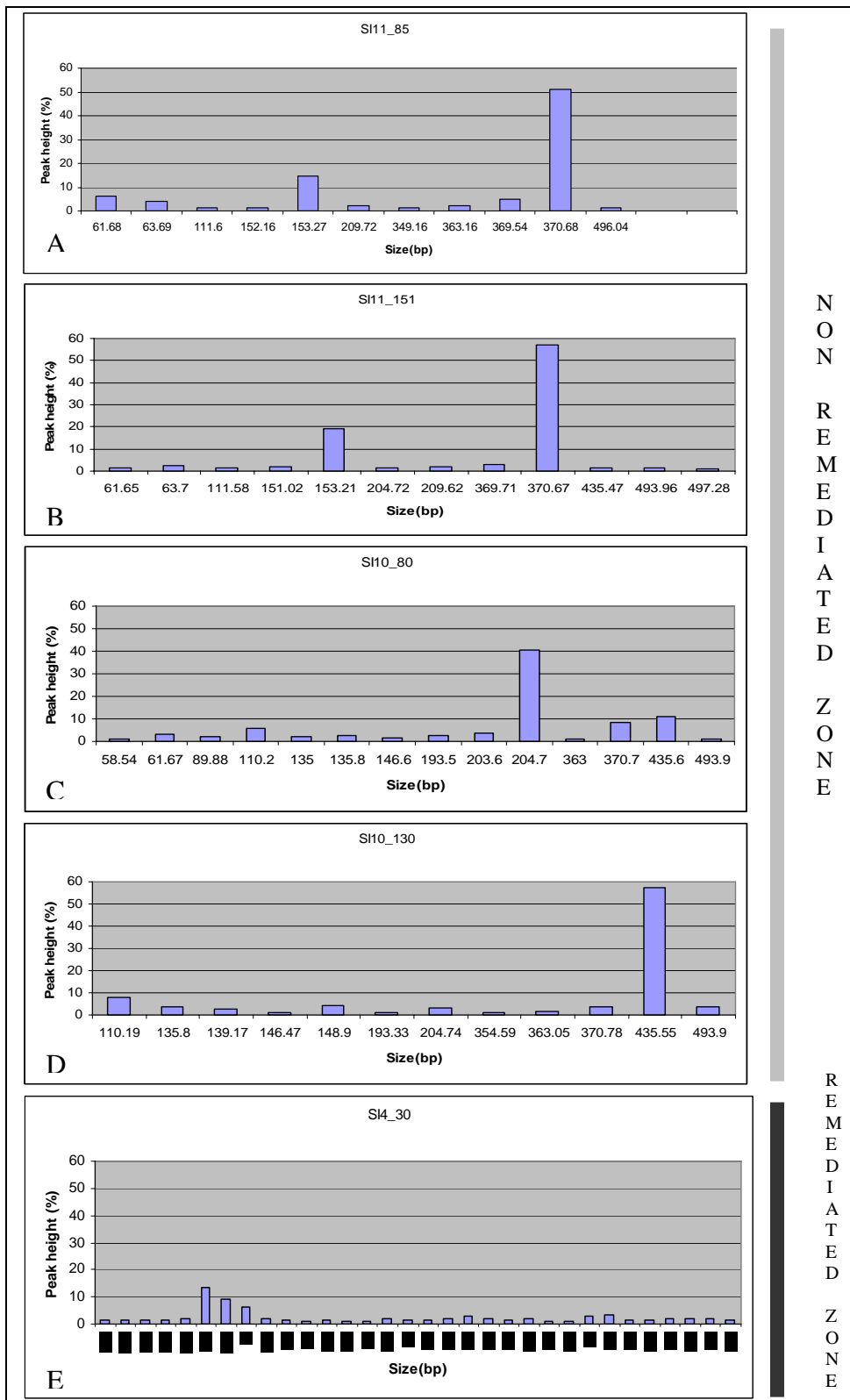


Figure 1: T-RFLP of SI11 (85 and 150 cm of depth) and SI10 (80 and 130 cm of depth) samples of un-remediated zone, and SI4 (30 cm of depth) of remediated zone, amplified with Eubacterial 16S rRNA primers and digested with the restriction enzyme HhaI

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