MICROBIAL EFFECTS OF NATURAL PHOSPHATE ROCK (NPR) ADDITION TO MINING WASTES[1]

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Abstract. Various treatments have been proposed to attenuate and eventually inhibit the generation of acid mine drainage (AMD) or acid-rock drainage (ARD). Previous work performed by Boojum Research Ltd. indicated that the addition of a mining waste product from the TexasGulf (North Carolina) phosphate mine showed some promising results when pyritic waste rocks from a northern Quebec Cu /Zn mine were mixed with NPR during an outdoor weathering experiment that lasted 989 days. Scanning electron microscopy (SEM) observations of the waste rock surfaces submitted to NPR addition in the weathering experiment indicated the presence of both iron and phosphorus in bacterial biofilms, whereas rock surfaces in the control experiments (i.e, without NPR) showed microbial corrosion. In order to revive the biofilms on the rock surfaces, samples from the weathering experiments with NPR were submerged in a growth medium adjusted to pH 7.2 for 7 days (which was similar to the pH measured in the effluents from the drum experiment). ESEM (Environmental Scanning Electron Microscopy) observations revealed microbial colonization and fine grain precipitation on the microbial cells present in the biofilms. To further assess the interactions between NPR, bacteria and the waste rocks, fresh cut surfaces of waste rocks were mixed with NPR and acidophilic bacteria at pH 2.2. NPR addition first neutralized the acidity of the growth medium by supplying soluble phosphate and carbonate and prevented the development of corrosion pits on the fresh rock surfaces. The reactivity of the waste rock surfaces appears to depend on the microbial activity at the surface, i.e., whether bacteria produced a biofilm or not. It is also clear that the presence of NPR favored the development of biofilms.

Additional Key Words: inhibition of acid rock drainage, mine waste management.

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Introduction

Acid mine drainage (AMD) and acid rock drainage (ARD) generated from the oxidation of metal sulfides present in mining wastes are a serious environmental problem facing the mining sector. The environmental impacts of AMD and ARD include the acidification of surface and ground waters and the release of toxic heavy metals to the surrounding aquatic and terrestrial ecosystems. Recent studies have shown that iron(III) phosphate coatings on pyritic waste rocks can be prepared by soaking the mine waste in a solution of hydrogen peroxide, potassium dihydrogen phosphate and sodium acetate (Day et al., 2000; Evangelou, 1994; Georgopoulou et al., 1996). The precipitation of Fe(III) phosphate minerals limits the concentration Fe^{3+} in solution, a powerful oxidizing agent of metal sulfides (Renton et al.,1988). Although these studies have added significantly to the understanding of the interaction of pyrite with phosphate, the surface interaction with microbes was not addressed.

An experiment with pyritic Cu/Zn waste rocks from a mine in northern Quebec was initiated by Boojum Research Ltd., following a report of the Northern Mining Magazine about a Gibraltar's dump in British Columbia that stopped producing metal leachate. An investigation of the leach dump (Scott, 1991) revealed that the rocks were coated with a precipitate that prevented metal leachate. Scanning electron microscopy (SEM/EDS) observations of the same rocks indicated that the rock's coatings contained phosphate and jarosite. This practical example suggested that the precipitation of phosphate minerals onto waste rock surfaces could indeed stop the generation of acid. As a result, a detailed investigation of the effectiveness of Natural Phosphate Rock (NPR) addition to waste rocks (Cu-Zn wastes) was undertaken by Boojum Research Ltd. from 1992 to 1995. ARD was reduced in the systems containing NPR, likely in part due to the formation of secondary mineral coatings onto the rock surfaces (Kalin et al., 1998). However, the exact mechanisms responsible for the attenuation of acid generation and mineral formation were not addressed along with the role of microorganisms on the stability of the precipitates.

We report here microscopic observations of the waste rock surfaces using a SEM/EDS and an ESEM (Environmental Scanning Electron Microscope) and we explore the possible microbial and chemical mechanisms taking place at the NPR and waste rock surfaces. Results from the present study may lead to a methodology useful in the management of ARD from waste rock piles.

Materials and Methods

Previous waste rock drum experiments (1992-1995)

The waste rocks used in the drum experiments were from a Cu/Zn mine in Northern Quebec, located in the Archean Abitibi Greenstone belt. Distinct ore zones were associated with the sulphide minerals which had an S content ranging from 20 to 80 %. Natural phosphate waste rocks, from the Aurora phosphate district in North Carolina (NPR), were obtained from TexasGulf in Raleigh N.C. NPR (diameter of 1-10 mm) were composed of calcium phosphate (36%) and calcium carbonate (48%) (Kalin et al., 1998). Waste sulfide rocks were placed in drums and mixed with known quantities of NPR. NPR was either added as a distinct layer in the drums or mixed throughout. Control drums were also studied whereby no NPR was added. All drums were left outside to weather for 989 days (see Kalin et al., 1998).

Biofilm activation experiment

In order to investigate the biofilms that formed on the rocks from the NPR waste rock drum experiments, selected rock samples were immersed in a PYTP medium adjusted at pH 7.2 (Siering and Ghiorse, 1996) for 7 days. The selected medium allowed the revival of neutrophilic bacteria present within the dry biofilm. The surfaces were observed using an environmental scanning electron microscope (ESEM).

Biofilm formation experiments

To assess the relationship between the waste rock, NPR and bacteria under ARD conditions, fresh cut waste rock samples were immersed in a medium suitable for acidophilic iron and sulfur oxidizing bacteria. On set of samples was placed in Erlenmeyer flasks containing NPR, whereas the second set did not contain NPR. Each system was acidified to pH 2.2 with H_2SO_4 and inoculated with tailings containing acidophilic sulfur and iron oxidizers. The flasks were gently shaken at room temperature in order to support microbial colonization of the rock surface. The pH of the solutions was recorded over time. The freshly colonized surface of the waste rocks was analyzed by SEM/EDS after 9 and 37 days.

Results

Previous waste rock drum experiments

Figure 1 shows the surface of the waste rocks in the absence of NPR (a) and in the presence of NPR (b) after 989 days. In the absence of NPR addition, corrosion pits caused by microbes were visible on the waste rock surface (see arrows on Figure 1a), whereas fractured thick biofilms (caused by desiccation) and bacteria were observed on the waste rocks mixed with NPR (see arrows on Figure 1b).

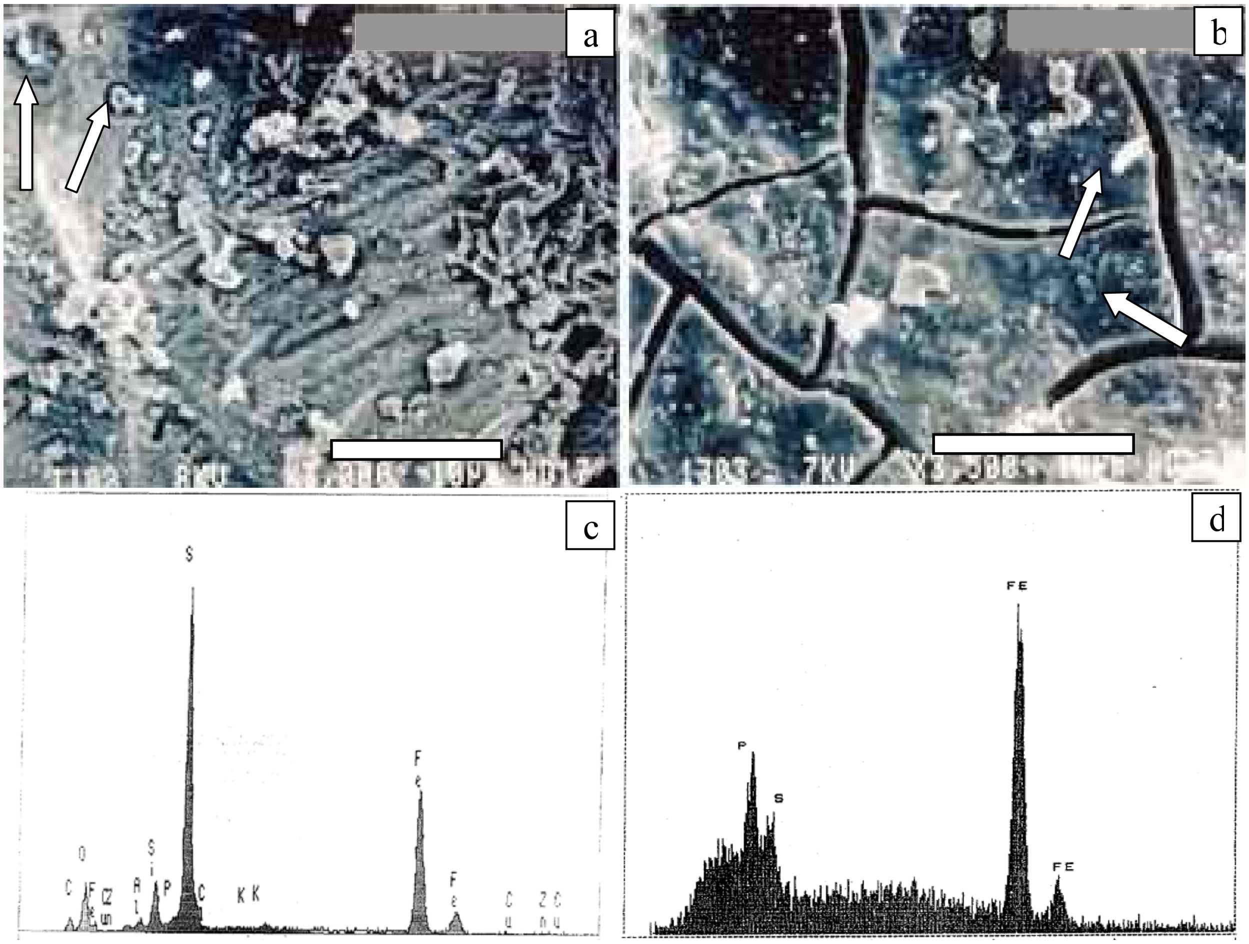

Figure 1. SEM images of the surface of the waste rocks in the drum experiments in the absence (a) and presence of NPR (b) and the associated EDS spectra (without NPR (c) and with NPR (d)) after 989 days. Surface corrosion was observed in the absence of NPR (see arrows on 1a), whereas desiccated biofilms and bacteria were recognized on the surface of the rock in the presence of NPR (see arrows on 1b). The corroded surface was mainly composed of S and Fe (1c) whereas the biofilms formed by the NPR addition were composed of P and Fe (1d), indicating the presence of iron phosphate precipitates. (scale bars: 10 um)

Biofilm activation and formation experiments

Figure 2 shows the surface of pyrite-rich waste rocks collected from the colonization experiments after 7 days at pH 7. ESEM imaging of the samples was done without drying the samples and allowed the observation of the samples under moist atmosphere. Microbes originally present in the drum were revived in the medium and reformed biofilms on the waste rock surface. Biofilms containing microbes and fine grained precipitates were formed on most of the rock surface (Figure 2a and 2b). The use of a neutral pH growth medium was chosen as this was the pH of the effluents of the drums with NPR. High magnification observations (Fig. 2b) indicated that the cocci forming bacteria were surrounded by fine granular aggregates suggesting that biomineralization occurred on the microbial cell surfaces. EDS analysis of the biofims indicated the presence of Fe and S but not the presence of P (Figures 2c).

In the biofilm formation experiments at low pH and in the presence of NPR, microbial colonies developed inside corroded cavities on the NPR grain surface after 9 days. In addition, fine Fe-rich grains occasionally precipitated on the NPR surfaces. In the absence of NPR, etched pits and microbes were observed on most of the waste rock surface after 37 days, whereas etched pits were absent from the surface of the rocks in the NPR samples, even though bacteria were present. The addition of NPR to the acidic systems increased the pH of the systems to neutral pH conditions, whereas, the systems without NPR remained acidic.

Discussion

North Carolinas phosphate ores possess a good sorption capacity for heavy metals, such as lead, cadmium and zinc, which makes them applicable for the amendment of acid mine drainage (Chen et al., 1997). Their efficiency was tested in the drum experiments (performed by Boojum Research Ltd.) and results indicated that NPR addition was indeed an efficient tool to attenuate acidity and ARD (Kalin et al., 1998). Our experiment with fresh rock surfaces, acidophilic bacteria and NPR also corroborated the results of the drum experiments, i.e., acidity was neutralized in the presence of NPR addition and the surface of the sulfide-rich waste rocks showed little evidence of bacterial and chemical corrosion. Our observations also indicated that the morphology of the biofilms was different in the presence and in the absence of NPR. In the absence of NPR, the waste rock surface was apparently corroded by bacteria because cavities developed near the microbial cells (Figure 1a), whereas biofilms formed on the surface of the waste rocks in the presence of NPR. EDS analysis suggested that biomineralization, i.e., Fe-phosphate precipitation, occurred within these biofilms.

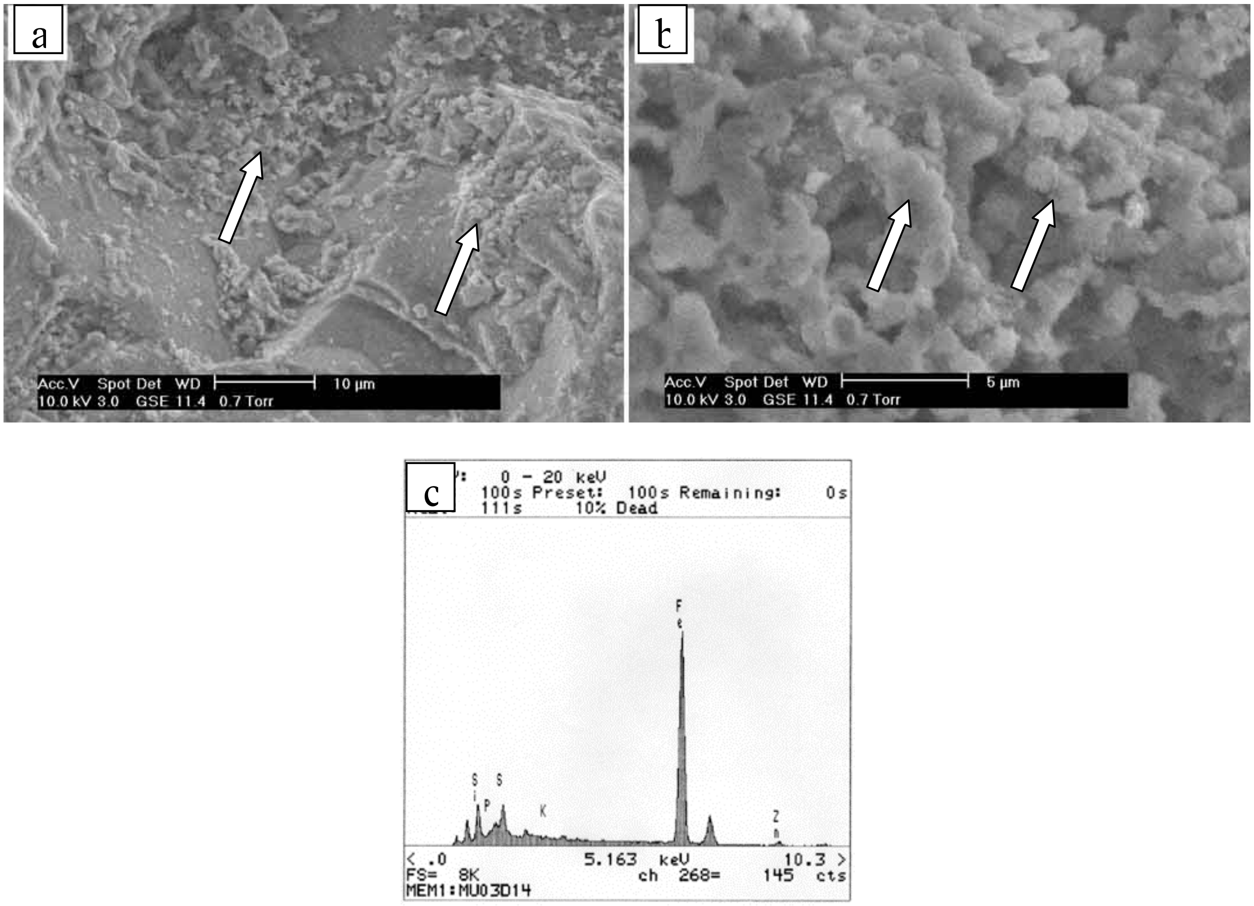

Figure 2. ESEM images of the revived biofilms (at pH 7) on the surface of pyrite-rich waste rocks isolated from the drum experiments. After 7 days, bacteria originally present in the drums were revived and they reformed biofilms on the waste rock surface (see arrows in 2a). Fine precipitates could also be seen on the rock surface and in close association with the cells (see arrows in 2b), suggesting that biomineralization could occur on the bacterial cell walls and exopolymers. EDS analysis of the precipitates in the biofilm indicated the presence of Fe and S, but P was not detected (c). Scale bars are 10 um for 1a and 5 um for 2b.

Many studies have reported that iron-oxidizing bacteria, such as *Acidithiobacillus ferrooxidans,* can corrode the surface of pyrite due to the oxidation of Fe^{2+} to Fe^{3+} (e.g. Razzell and Trussell, 1963). In the presence of NPR, no cavities were however observed near the cells and biofilms were present (Figure 1b). This indicates that in the presence of NPR, bacteria directly formed biofilms on the surface of the waste rocks and ultimately immobilized iron and phosphorus on their cell walls and exopolymers. Several studies have reported the formation of amorphous iron phosphates similar to strengite ($FePO_4 \cdot 2H_2O$) on microbial cell walls (Akai et al., 1999; Konhauser et al., 1994). The mechanisms suggested by those authors is that Fe^{2+} is oxidized by bacteria, and that Fe (III) phosphates are formed using PO_4 ions derived from the nearby environment (Akai et al., 1999). Another major source of phosphate may be acquired from the bacterial cells themselves (Konhauser et al., 1994), because bacterial cell walls possess two phosphate rich lipid bilayers containing either phospholipid or lipopoly-saccharides (Beveridge, 1989).

The microbial exopolymers could also act as a template for iron phosphate precipitation. In fact, recent work by Tashiro and Tazaki (1999) indicated that exopolymers could indeed play an important role in biomineral formation. They illustrated the process and mechanism of Fe-hydroxide formation on exopolymers by *in situ* direct observations. They suggested that exopolymers possess a micro-environment different from the bulk conditions (such as redox potential) which favors the formation of iron hydroxides. Ueshima and Tazaki (2001) suggested that microbial exopolymers can also act as an ionic binder for the formation of Fe-layer silicates in Si-rich solution. In the case of P-rich solution, it is possible that iron phosphate could be formed in a similar manner.

The phosphate content of exopolymers is most likely higher than the bulk concentration in most environments and it is possible that it favors the nucleation of phosphate-rich minerals. Phosphorus is also known as an essential nutrient for microbial growth (Fenchel and Blackburn 1979), and bacteria have the ability to store large quantities of phosphate (Beveridge, 1989). The main role of microbes in the formation of phosphate minerals is the mobilization of phosphate out of the organic detrital matter (Ehlich, 1999). Under super-saturation conditions, the liberated phosphate can react with metal cations and precipitate as phosphate minerals. In soils, microbes can also promote the authigenic formation of $FePO_4 \cdot 2H_2O$ (strengite), $Fe_3(PO_4)_2 \cdot 8H_2O$ (vivianite) (Adams and Burkhart 1967) and $AlPO_4 \cdot 2H_2O$ (Variscite) (Ehlich, 1999).

Our results showing biofilms composed of microbial cells surrounded by fine grains (Figure 2b) strongly indicate that mineral formation was induced by the presence of bacteria. However, the exact chemical microenvironment surrounding the microbial cell wall (i.e. within the exo-polymer matrix), such as the pH, and the chemical composition of the minerals are still unknown. The bacteria present within the biofilm were most likely iron and/or sulfur oxidizers, because the medium was designed to enhance their growth (i.e., low pH, high sulfate concentration and presence of sulfide rocks). Acidity generation resulting from the chemical and microbial oxidation of sulfide minerals was in fact observed in both the drum experiment and the laboratory experiment when NPR was absent.

Our results also indicate that acidophilic bacteria preferentially attached themselves to the surface of NPR grains. Mineral weathering can be accelerated in the presence of organic acids (Barker and Banfield, 1996, Welch et al., 1999, 2002), but organic acids (i.e., bacterial exudates) and humic substances can also inhibit the formation of minerals (such as short-range ordered aluminosilicates) (Inoue and Huang, 1984, 1990). In the present study, microbes proliferated on the NPR surface because they were likely capable of leaching phosphorus out of the NPR surfaces. This is in agreement with the study of Rogers et al. (1998) who reported that feldspars with apatite inclusions were heavily colonized by native microbial populations and the grains showed obvious signs of weathering. In addition, Welch et al. (2002) suggested that the production of organic ligands in micro-environments could accelerate apatite dissolution by one to two orders of magnitude compared to inorganic abiotic conditions if microbes can lower the pH of their environment. In our laboratory experiment, NPR addition resulted in dissolution of phosphate and neutralization of the acidity of the growth medium within 10 days. This suggests that phosphate from the NPR minerals allowed bacterial proliferation on the waste rock surface and the potential formation iron-phosphate precipitates.

Calcium phosphate, such as apatite, can be weathered by bacteria and microbial exudates, but the stability of iron phosphate minerals in the presence of bacteria remains unknown. Iron phosphates, such as strengite ($Ksp = 10^{-26}$), are chemically more stable than calcium phosphates, such as hydroxyapatite ($Ksp = 10^{-17}$) (Stumm and Morgan, 1996). The study of the long term stability of the iron phosphate coatings on waste rock surfaces is then necessary in order to fully assess the use of NPR waste rock piles.

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