

# INVESTIGATING THE ROLE OF BIOTIC VERSUS ABIOTIC PROCESSES IN THE GENERATION OF ACID SULFATE SOILS IN COASTAL NSW

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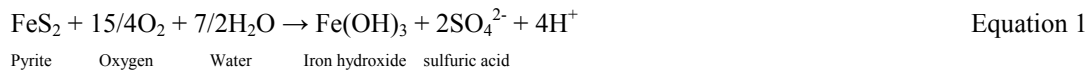
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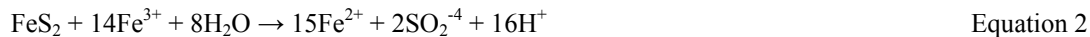
## INTRODUCTION

Acid Sulfate Soils (ASS) is the common name given to soils containing iron sulfides. Sulfides formed in these soils are relatively stable under anoxic conditions. However, if environmental conditions change and oxygen is introduced into the system, sulfides are oxidised to produce sulfuric acid leading to detrimental impacts on ecological systems, economic structures and infrastructure. The acidification of many estuarine ecosystems along the eastern coast of Australia has been attributed to ASS. In Australia, the ASS of most concern are those that have formed within the past 10,000 years (Holocene). This correlates with the last major sea level rise, which saw low-lying coastal fringes inundated with sea water (White *et al.* 1997, Sammut 2000, White & Melville 2000).

Oxidation of pyrite is a slow reaction that occurs *in situ*. The oxidation of pyrite can be described by the reaction (Aaso 1999-2000, Sammut 2000):



Although O<sub>2</sub> will oxidize pyrite, ferric iron is a much more effective oxidant via the reaction:



The rate-limiting step in the rapid oxidation of pyrite by Fe<sup>3+</sup> is the oxidation of ferrous iron (Fe<sup>2+</sup>), though in acidic conditions (pH < ca. 4), ferrous iron (Fe<sup>2+</sup>) is relatively stable. Iron-oxidizing bacteria can increase the rate of Fe<sup>2+</sup> oxidation significantly, and consequently increase the reaction rate of equation 2 (Ward *et al.* 2004). This paper focuses on the role that iron- and sulfur-oxidising bacteria play in the oxidation and weathering of pyrite in an acid sulfate soil site in an attempt to estimate the relative impact of biotic versus abiotic effects in generation of acidity.

## FIELD SITE

Acid sulfate soils were first identified in Australia in the Lower Macleay River Catchment, on the mid-north coast of New South Wales (NSW) (Walker 1963). These are the result of an episode of estuarine sediment deposition, which on exposure to oxidation have the potential to become extremely acidic. Similar sediments are widespread on Australian coastal floodplains, particularly Queensland and NSW, and the areas with the most severe acid concerns are now being recognised as 'hot spots' requiring urgent remedial attention.

## FIELD WORK

Field work was undertaken in April and July 2004 in the Macleay River Catchment NSW on a privately owned cattle farm, Illalong. The study area is located within the Holocene estuarine sediments of the Clybucca Creek catchment (Atkinson 1999). Illalong is situated on alluvial deltaic back-swamps and has an extensive flat swamp floor of predominantly low elevation (0-1 m). Water and sediment samples were collected from several locations at Illalong. Water samples were collected from surface water when available and stored in clean HDPE bottles. Acidity (pH) and electrical conductivity (EC) were measured in the field. Sediment samples were obtained from 7 locations by hand augering. Sediment samples were placed in plastic bags and sealed to limit evaporation and oxidation. Sample characteristics, including color, were described in the field. At three sites, sediments were also collected using a PVC coring device which allowed the sediment profile to remain intact. The coring device consisted of an inner and outer PVC tube. The inner tube collected the core, and the outer was left in place to serve as a temporary piezometer.

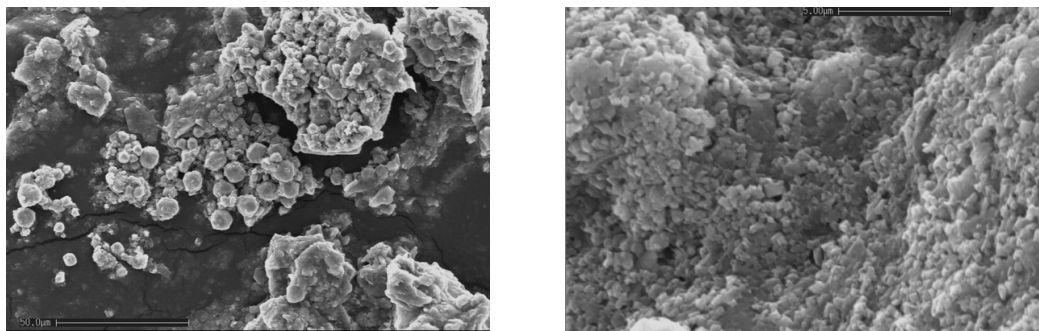
At several of the sites, clean pyrite crystals were placed in the field to allow for microbial colonization and oxidation. Pyrite samples placed in the field in April were collected in July for microscopic analysis.

## METHODS

The primary aim of the project involved determining how biological (microbial) processes affect the generation of acid sulfate soils. To address this, sediment was characterised and water samples collected. A series of experiments were conducted, designed to determine abiotic versus biotic generation of acidity and the presence of chemolithotrophic bacteria. Sediment samples were characterized for acidity and electrical conductivity based on standard 1:5 sediment-water extracts. The presence of pyrite was determined using a peroxide test developed by van Beers (1962). Metal concentration in the extracts was analysed by ICP-AES. Based on the results of these tests, a subset of the sediment samples was characterized by SEM and XRD. A series of microcosm experiments were set up with sediment samples collected in the field. Samples were selected from sites that were identified as ASS environments. Two biotic samples and two abiotic samples were prepared for each site. Abiotic controls were created by autoclaving. Glass flasks were initially autoclaved for 15 minutes. 5 g of sediment were added and autoclaved for an additional 30 minutes under high pressure before 150 ml of filtered de-ionised water was added and the flask was placed on the orbital shaker. The orbital shaker was maintained at 200 rpm throughout to keep flasks aerated. Aliquots were removed over time and analysed for acidity and metal concentration. Enrichment culture experiments were set up using standard media (ATCC) for acidophilic Fe and S oxidizing bacteria.

## RESULTS

The transition from oxidizing to reducing conditions with depth in the sediment column was apparent from field observations. The humic gleys found at the study site were acid to neutral soils with significant amounts of organic matter throughout the dark A-horizons. Subsoils were characterised by their rusty and ochreous streaks and mottles in a pale grey matrix. A layer of aragonite-rich shells was found at a depth of 80 cm. Beneath this layer, a blue-grey glauconite-rich clay occurred. The characteristic yellow mottles of jarosite, a partial oxidation product of pyrite, were observed at varying depths throughout the sediment column. Jarosite typically forms at pH below 3.7 in strongly oxidised conditions (White *et al.* 1997). Results of the sediment-water extracts showed that in many parts of the pasture, the sediment was near neutral pH. However, some of the sediment was extremely acidic (pH 3-5) in the upper parts of the profiles. pH increased with depth to near neutral below 40-80 cm due to buffering of acidity by dissolution of the shell layer. Although jarosite was abundant, comprising 1-10% of the sediment, pyrite was not. Estimates of pyrite content on the sediment was  $\ll 0.1\%$  by the peroxide test, this was confirmed by SEM and XRD analysis of sediments

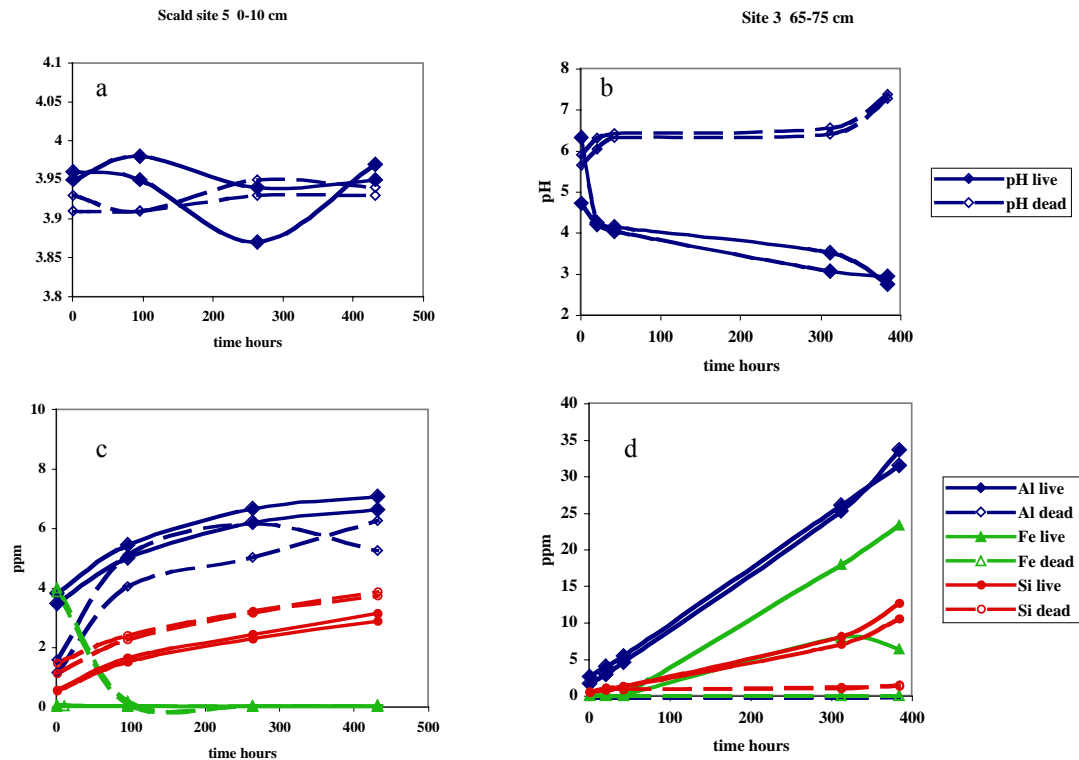


**Figure 1:** SEM image of framboidal pyrite extracted from within a sealed shell (80 cm) in the wet pasture (**left**) and crystalline jarosite from a jarosite mottle (**right**).

Results of the microcosm experiments showed that in many of the samples there was no significant change in solution pH over time and no significant difference in metal concentration in solution between the live versus dead experiments (Figure 2a, b) over a few weeks duration. Even if the chemolithotrophic microbes are present, they do not appear to be metabolically active (producing acidity) in the microcosms over the course of the experiments. This may reflect the fact that in this system, in the upper part of the sediment column in the 'oxidized zone', the pyrite has already been largely oxidized, as evident from the abundant jarosite mottles that are found throughout the sediment column in this site.

Although most of the microcosm experiments showed no significant difference in solution chemistry for the biotic versus abiotic treatments, for one of the samples there was a large difference in acid production (Figure 2b, d). For the samples taken from the wet pasture above the shell layer, pH decreased from near neutral to

ca. pH 4 within 2 days in the biotic experiments, and to pH ca. 3 within 2 weeks, suggesting active pyrite oxidation, whereas, in the abiotic experiment with this sediment sample there was no significant change in pH over time. In addition, there was an increase in Fe, Al, and Si concentration in the biotic experiments, indicating pyrite oxidation and silicate mineral dissolution due to the increased acidity (Figure 2d). Microbial enrichment culture experiments in S and Fe oxidizing media with sediments from this location showed evidence of microbial cell growth over a period of several weeks.

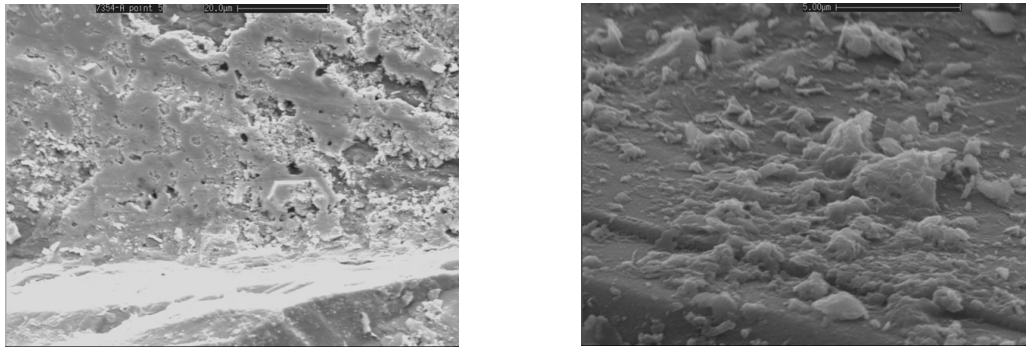


**Figure 2:** pH (a, b) and dissolved Al, Fe, and Si (c, d) in microcosm experiments over time for scald surface (left) and 65-75 cm deep in wet pasture (right).

In order to investigate microbially mediated pyrite alteration under field conditions (see for example Rojas-Chapana *et al.* 2004), clean pyrite samples were placed in several locations and allowed to react for several months. Samples were then retrieved and examined for evidence of microbially-mediated alteration of the surface. The pyrite surfaces showed evidence of pitting, oxidation, and secondary mineral precipitation (Figure 3). Pyrite samples also showed evidence of individual microorganisms attached to surfaces and formation of biofilms.

## CONCLUSION

Acidophilic bacteria are an intrinsic part of the acid sulfate soil environments studied in this project. The impact that these bacteria have on pyrite oxidation is dependent on the environmental parameters around them. The abiotic versus biotic experiments provided conclusive evidence for the effect that biotic organisms have on ASS. For biotic samples that underwent pyrite oxidation, dramatic decreases in pH were recorded. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis of solution extracts from microcosm experiments showed that significant amounts of major cations were released into solution as a result of pyrite oxidation. Culturing experiments revealed the presence of both Fe-oxidising and S-oxidising bacteria in the pastured sites (site 3 and site 4). These bacteria were limited to the oxidised regions of the profile (< 80 cm). Slow changes in pH observed in the abiotic versus biotic experiments, and the subsequent slow growth rates for iron- and sulfur-oxidising bacteria in culture mediums are attributed to the limited quantity of pyrite found in soil samples (<< 1%).



**Figure 3:** SEM images of pyrite surfaces. **Left:** etch pits and secondary mineral phases on pyrite surface. **Right:** an accumulation of secondary mineral phases, microbial cells, and organic polymers

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