

**PROSPECTIVE MECHANISMS OF PYRITE OXIDATION IN
ANOXYGENIC ENVIRONMENTS ANALYZED BY THE FERROZINE
ASSAY**

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Prospective Mechanisms of Pyrite Oxidation in Anoxygenic Environments Analyzed by the Ferrozine Assay

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Pyrite survived extensive redox weathering in the Archean (4.0-2.5 Ga) due to lack of readily available atmospheric oxygen in coastal, shallow-water environments. However, instances of oxidized detrital pyrite are still found that date as far back as 3.22 Ga. Three hypotheses have been proposed to explain oxidation of pyrite in the Paleoproterozoic (3.6-3.2 Ga): (1) Oxygenic photosynthesis, (2) Anoxygenic phototrophy, and (3) Photooxidation. Each of these three hypotheses provide ample, yet debatable amount of evidence to support pyrite oxidation. In this research, we investigated prospective oxidation mechanisms of pyrite in the Paleoproterozoic Era utilizing the ferrozine assay. It was determined that it is possible to quantify the amount of pyrite oxidized in an anoxygenic environment utilizing the ferrozine assay.

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NOMENCLATURE

| | |
|------------------|---|
| Fe^{2+} | Ferrous iron |
| Fe^{3+} | Ferric iron |
| Pyrite | FeS_2 |
| Ferrozine | Monosodium salt hydrate of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid |
| c | concentration |
| ϵ | Molar absorptivity |
| A | Absorbance (unitless) |

CHAPTER I

INTRODUCTION

The Archean eon ocean is known to be a reducing environment with large quantities of ferrous iron (Holland, 1973). This implies that Fe (II) must have been oxidized to Fe (III) in absence of atmospheric oxygen.

Three models have been proposed to explain pyrite oxidation in this highly anoxic environment (Konhauser et al., 2007). Photo-oxidation: The first model suggests photooxidation as the primary driving force for iron oxidation (Braterman et al. 1983). In this abiotic model, UV and visible light near the ocean surface expel electrons from Fe (II), thereby reducing it to Fe (III) (Francois, 1986). Oxygenic photosynthesis: The second proposed model suggests biologically mediated oxygenic photosynthesis as the primary pathway for iron oxidation (Tice and Lowe 2004). Anoxygenic photoferrotrophy: The third model suggests microbially mediated anoxygenic photoferrotrophy as an alternative to pyrite oxidation in absence of readily available oxygen. It is the aim of this paper to investigate further the photooxidation of pyrite, and to analyze if a catalyst such as rutile would speed up the photooxidation process by forming hydrogen peroxide from radiolytic dissociation of water (Wacey et al. 2011).

CHAPTER II

METHODS

Three solutions were prepared, a ferrozine, buffer, and a reducing agent were prepared along with a couple of standards with Fe(II) and Fe(III) were prepared. To run the ferrozine assay we had to make a ferrozine solution, a ferrozine buffer at a PH of 9.5, reducing agent as well as the standard Fe(III) stock solution.

The first reagent ferrous stock solution i.e. Fe(II) stock solution method was made to a 100 ppm (parts per million) meaning that 10 mg of solute is dissolved in 0.1 L of nanopure water. In more detail, 51.388 mg of ferrous sulfate heptahydrate was dissolved in 100 mL of water in a volumetric flask. This resulted in making our Fe(II) stock solution. The second reagent was ferrozine iron reagent which contains thiol meaning it can be reduced easily. Thiols are known to be reducing agents. This 0.01 M ferrozine iron reagent was prepared in ammonium acetate where 0.508g of ferrozine and 0.7708 g of ammonium acetate were added to 0.1L of nanopure water. The third reagent is the 10M ferrozine buffer. To make that 77.08 g of ammonium acetate was dissolved in 100 mL of nanopure water. Then ammonium hydroxide was added dropwise with consistency to 100mL of nanopure water and the pH was measured until a 9.5 was obtained. The fourth reagent was making a reducing agent that was 10% w/v meaning 10 grams of hydroxylamine added to a 100mL of solution. The 100mL of solution is the result of 60 mL of water and 40 mL of hydrochloric acid. The fifth reagent is 1% v/v Fe III stock solution that is made by adding 0.74576g of ferrous sulfate heptahydrate with a molar weight of 414.32

g/mole to 0.1 of water. Then, 0.1 L of 5M hydrochloric acid is added to that summing a total volume of 0.2 L of Fe(III) stock solution.

The procedure went as follows we measured two absorbance readings using a Beckman-Coulter DU 730 spectrophotometer. The first Absorbance value, A1, was recorded after adding 1 ml of the Fe(III) stock solution to 100 μ l of Ferrozine. The second absorbance value, A2, was measured when a known amount of the mixture from the prior step was mixed with 150 μ l of reducing agent. Once this compound was left for around 10 minutes to allow for reduction of Fe(III). After the wait is over, 50 μ l of the buffer is then added to adjust the PH levels in the cuvette. Then the absorbance value is recorded once more at a wavelength of 562 nm as A2. The reason we have ferrozine in our method is this compound allows us to measure the Fe(II) attached to ferrozine without it being oxidized in the lab. Fe(II) can get easily oxidized and therefore needs to be bound to another organic compound in order to quantify it and constructing a calibration curve.

CHAPTER III

RESULTS

Ferrozine interacts with iron to form a dark purple/ crimson compound. The absorbance values are collected at a wavelength range between 550-600nm, where the maximum absorbance is recorded at a wavelength of 562nm. Fe(III) reacts with the Ferrozine, thereby changing the color of the ferrous compound from its original yellow color. The following absorbance values are measured both before and after the reduction step and buffer using Beer's law. Once the reducing agent and buffer have been added, the Fe(III) is reduced to Fe(II), which increases the concentration of the Fe(II)-ferrozine compound.

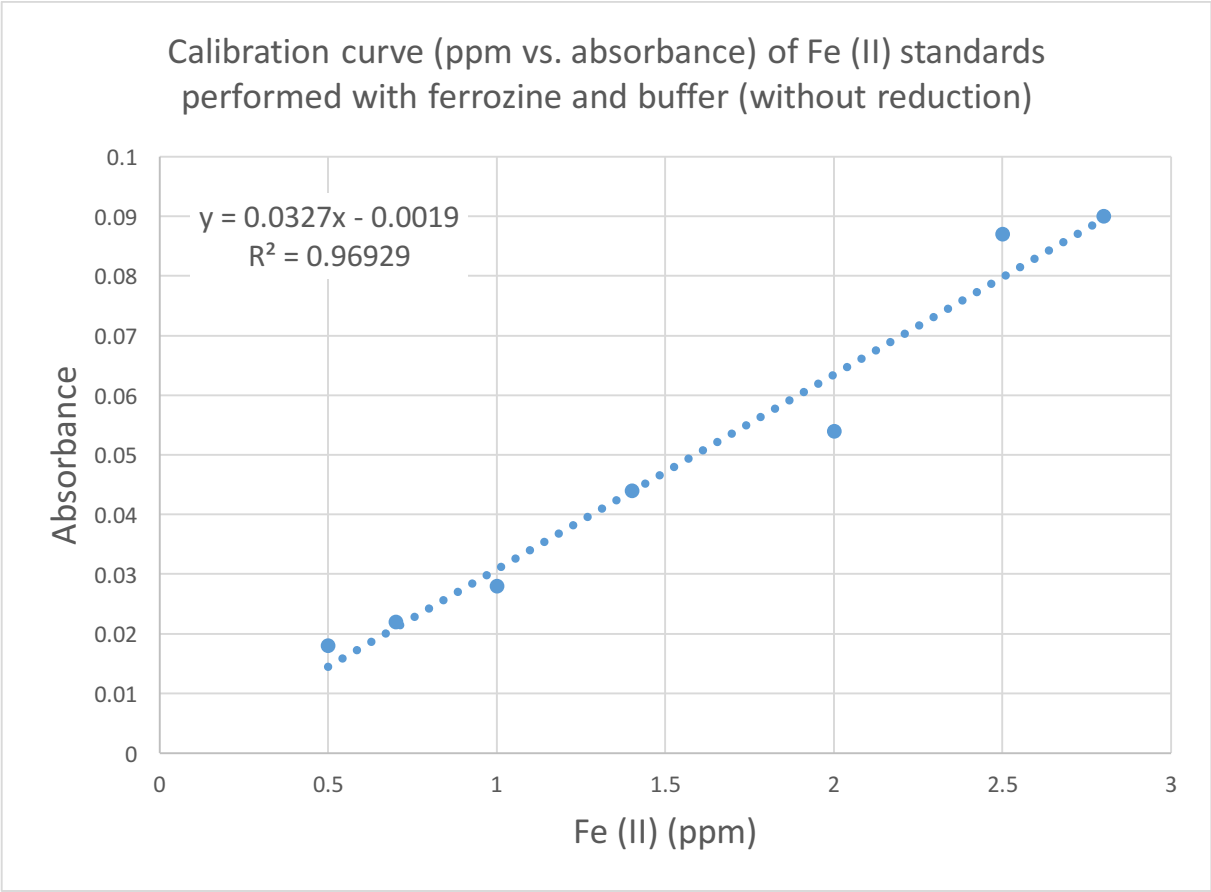


Figure 1: Calibration curve of Fe(II) standards performed with Ferrozine and Buffer (before the reduction step). This graph has a coefficient of determination 0.96929, indicating a strong linear relationship.

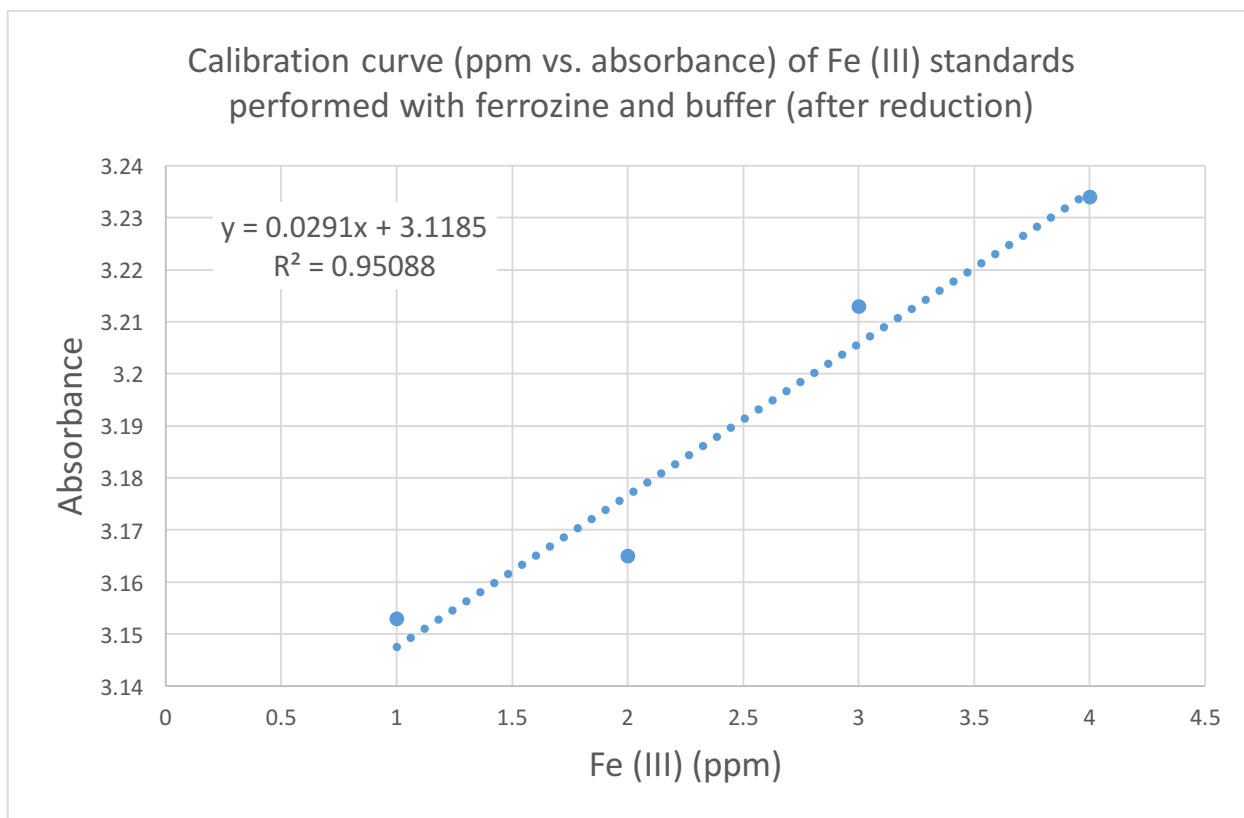


Figure 2: Calibration curve of Fe(III) standards performed with Ferrozine and Buffer (after the reduction step). This graph has a coefficient of determination 0.95088, indicating a strong linear relationship.

Ferrozine assay calibration curves (ppm vs. absorbance) of Fe(II) and Fe(III) standard curves before and after the reduction are depicted as absorbance as a function of concentration. (Figure 1 & 2). Spectrophotometer analyses was carried out on Fe(II)-ferrozine compound in cuvettes both before and after the reduction using hydroxylamine. Both Figure 1 and Figure 2 show strong linear relationships with coefficients of determination of 0.96292 and 0.95088 respectively. Each displays a linear relationship that nearly transects the origin. This result is expected based on prior ferrozine assay determination of coefficients (

CHAPTER IV

CONCLUSION

The oxidation of pyrite in anoxygenic conditions may hold the keys to developing biosignatures necessary for discovering the existence of life outside of earth. The three models proposed for oxidation of pyrite remain the crucial background necessary to study the prospective development of a new biosignature.

The future research experimentation of photooxidation in an anoxygenic environment must occur in order to understand the whole scope of pyrite oxidation. This experiment could consist of multiple trials involving UV light exposure on test tubes filled with pyrite and water; control samples would be used that are not exposed to UV light. If the method proves successful, additional trials with rutile and pyrite could be complete to test the possible catalytic properties of rutile. The measurement of oxidation would rely on the ferrozine assay outlined in this work. If the oxidation of pyrite is successful, the correlation and regression statistics would be analyzed to determine the rate of oxidation of pyrite and the rate constant values associated with the rutile catalyst.

REFERENCES

Braterman, P.S., A.G Cairns-Smith, and R.W. Sloper, Photooxidation of hydrated Fe^{2+} — Significance for banded iron formations, *Nature*, 303:163-164, (1983).

François, L M. "Extensive deposition of banded iron formations was possible without photosynthesis." *Nature*, 320.6060:352-354, (1986).

François, L M. "Reducing power of ferrous iron in the Archean Ocean, 1. Contribution of photosynthetic oxygen." *Paleoceanography* 1.4:355-368, (1986).

François, L M. "Reducing power of ferrous iron in the Archean Ocean, 2. Role of FeOH^+ photooxidation." *Paleoceanography* 2.4:395-408, (1987).

Holland, H.D., The oceans: A possible source of iron in iron formations, *Econ. Geol.*, 68:1169-1172, (1973).

Konhauser, K. O., Amskold, L., Lalonde, S. V., Posth, N. R., Kappler, A., and Anbar, A., Decoupling photochemical Fe(II) oxidation from shallow-water deposition. *Earth and Planetary Science Letters*, 258:87–100, (2007).

Tice, Michael M., and Donald R. Lowe. "Photosynthetic Microbial Mats In The 3,416-Myr-Old Ocean." *Nature* 431.7008:549-552, (2004).

Wacey, D. "Earliest Microbially Mediated Pyrite Oxidation In Similar To 3.4 Billion-Year-Old Sediments." *Earth And Planetary Science Letters* 301.1-2 (n.d.):393-402, (2011).