

INVESTIGATING ABIOTIC NITROGEN REACTIONS WITH ORGANICS IN MARS-RELEVANT IRON MINERAL SYSTEMS. L. M. Barge, J. M. Weber, E. Flores, A. A. Fraeman, J.-P. Jones, E. Martinez, S. M. Perl,
¹NASA Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena CA 91109
(laura.m.barge@jpl.nasa.gov)

Introduction: The Curiosity rover has detected organic matter in 3-billion year old mudstones at Gale crater perhaps containing carboxyl groups, N-bearing groups, and organic-sulfur compounds [1,2]; nitrates / nitrites have also been detected on the surface [3,4]. A variety of reactive minerals are present in the vicinity of where the organic matter was detected, including iron oxides and oxyhydroxides, phyllosilicates, and trace amounts of iron sulfides [5,6]. Some of these iron minerals are reactive and are known to be capable of driving nitrate/nitrite reduction to produce species such as ammonia (NH_4) [7,8], which can react with simple organics (e.g. carboxylic acids such as pyruvate) to abiotically produce amino acids [9]. The Martian surface and near-subsurface might therefore provide a reactive setting to drive abiotic organic reaction networks that produce products similar to those found in biological metabolic cycles [9,10]. Here, we report experiments simulating the abiotic organic reactions that could occur in Mars analog iron oxyhydroxide mineral systems in the presence of various nitrogen species (nitrate, nitrite, and ammonia).

Methods: All reactions were carried out in a nitrogen-filled glove box, and all solutions were prepared with ultrapure water that had also been purged with nitrogen or argon gas to remove any dissolved oxygen. The iron hydroxide minerals were prepared using $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ with 50 mM total iron concentration in each vial. The amount of Fe(II) and Fe(III) varied depending on the desired mole fraction of Fe(II):Fe(III). Here we tested 100% Fe(II) and Fe(II):Fe(II) = 1:1; in previous work this procedure produced magnetite [9] which is an analog for the original iron phase precipitated at Gale crater [11]. Nitrate, nitrite, or ammonia were introduced into the reaction by addition of sodium nitrate, sodium nitrite, or ammonium chloride. The organic precursors were α -keto acids (glyoxylic acid and/or pyruvate); organic precursors were added at a total concentration of 2.5 mM. Water was added to dissolve all reactants, and sodium hydroxide (NaOH) was added to precipitate with the iron as iron hydroxide minerals. Each solution was titrated to pH = 10. Experiments were placed in a water bath at 70°C and sampled at t=0, 24h, 48h, 72h.

Organic reactions were monitored via ^1H Nuclear Magnetic Resonance spectroscopy. Five 1-mL aliquots were taken at each time point while agitating the bottles to ensure an even distribution of liquid and solid in

each sample to which 0.5 mL of 1 M NaOH was added to precipitate out the iron from solution since iron interferes with NMR analysis. Experiment samples were spiked with authentic samples to verify the presence/absence of the amino acid and α -hydroxy acid products.

Iron colorimetry analysis was conducted on the solid and liquid portions of pH 10 pyruvate experiments with nitrate or nitrite to determine how much the iron mineral oxidized throughout the experiment. For the reactions, one extra sample was taken at t=0 to which 0.5 mL of H₂O was added, the sample was centrifuged, and the supernatant was transferred to a new Eppendorf tube. Another 3 extra samples were taken at t=0 to which 0.5 mL of 2.5 M HCl was added to dissolve the solid. All samples were diluted to 2 mL where 1 mL was taken for Fe(II) and 1 mL was taken for Fe(total). To the Fe(II) samples, 100 μL of dH₂O was added and to the Fe(total) samples, 100 μL of 0.8 M ascorbic acid was added to reduce Fe(III) to Fe(II). After 10 minutes, 100 μL of 1 M HCl, 100 μL of 1 M sodium acetate, and 2 mL of 0.3% 1,10 phenanthroline were added to every sample while agitating the vials in between each reagent; the total assay volume was 3.3 mL. The samples were set aside for 10 minutes to let the color fully develop and were analyzed with a Thermo Fisher GENESYS 30 Visible spectrophotometer set to a wavelength of 510 nm.

Results: Our results indicate that the N species and its oxidation state in a Mars analog mineral system has a significant effect on abiotic organic chemistry. In the presence of ammonia (NH₄Cl), both pyruvate and glyoxylate reacted with iron hydroxides to form amino acids (alanine or glycine) and/or α -hydroxy acids (lactate or glycolate). In ammonia experiments, the iron mineral did not noticeably oxidize throughout the experiment. Using either nitrate or nitrite as the N species yielded identical results in glyoxylate reactions with iron hydroxide, forming only the α -hydroxy acid (glycolate), and no amino acid. However, in pyruvate experiments, nitrate vs. nitrite yielded distinct results. Nitrate led to lactate formation as expected, but nitrite led to two new ^1H NMR peaks that do not correspond to any previously observed product in these reactions (and the expected amino acid or α -hydroxy acid products were not observed at all). Nitrate and nitrite also had a significant effect on the mineral iron oxidation state: in nitrate experiments, about 60% of the Fe(II)

was oxidized by the end of the experiment, and in nitrite experiments, almost all of the Fe(II) was oxidized at the end of the experiment (**Figure 1**).

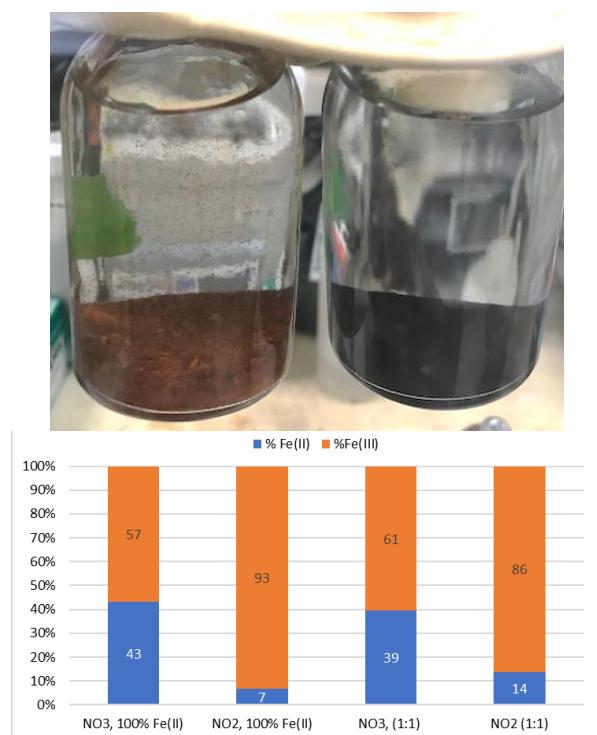


Figure 1: (Top L) Pyruvate reaction with nitrite and iron hydroxides (Fe(II):Fe(III) = 1:1) and (Top R) glyoxylate reaction with nitrate and iron hydroxides (Fe(II):Fe(III) = 1:1), after 72 hours. The color change in the nitrite-containing experiment indicates complete iron oxidation, as opposed to the nitrate-containing experiment where the mineral remains dark. %Fe(II) in the precipitates was measured via colorimetry and in all cases, nitrate experiments yielded ~60% iron oxidation and nitrite experiments yielded ~90-100% iron oxidation (data from pH 10 pyruvate experiments shown).

Implications / Conclusions: Nitrogen and iron redox cycling was observed to affect organic synthesis under Mars-relevant mineral / aqueous conditions. Starting with simple organic precursors (pyruvate and glyoxylate), the organic reaction pathways that occurred depended on which nitrogen species was present - nitrate, nitrite, or ammonia. With nitrate, some of the iron in the minerals was oxidized and α -hydroxy acids were synthesized. With nitrite, α -hydroxy acids and an unknown organic product were synthesized, and almost all of the iron in the mineral was oxidized rapidly. With ammonia, amino acids and α -hydroxy acids were synthesized, and the iron minerals did not oxidize significantly.

Nitrate and nitrite can theoretically also reduce to ammonia on iron minerals (or Fe(II)), and this could link different branches of this pathway. Though nitrate was observed to undergo redox reactions with the mineral-bound Fe(II) in our experiments, it did not create any nitrogen species that reacted with the organic precursors. If nitrite had been reduced to ammonia on Fe minerals in our reactions, we would have expected to see the formation of the amino acids similar to that which occurs in the ammonia experiments. Therefore, we hypothesize that nitrite is reacting with Fe(II) to form a reduced N product other than ammonia that reacts with pyruvate, to produce the unknown organic. More study is needed to understand the abiotic redox cycling of nitrogen and its reactions with organics in reactive iron mineral systems, and to better constrain the likely abundances of different N species in planetary environments.

Acknowledgements: This work was supported by a JPL Research & Technology Development Innovative Spontaneous Concept.

References: [1] Eigenbrode et al. (2018) *Science* 360, 1096–1101. [2] Freissinet et al., (2015) *JGR Planets*, 120, 3. [3] Stern et al (2015) *PNAS* 112: 4245–4250. [4] Navarro-González, et al. (2019) *JGR Planets*, 124, 94–113. [5] Vaniman et al. (2014) *Science* 343, 6169, 1243480. [6] Rampe et al., (2017) *EPSL*, 471. [7] Hansen et al. (1994) *GCA* 58:2599–2608. [8] Summers et al. (2012) *Astrobiology*, 12, 2. [9] Barge et al. (2019) *PNAS*, 116 (11) 4828–4833. [10] Muchowska et al. (2019) *Nature* 569, 104–107. [11] Tosca et al. (2018) *Nature Geoscience* 11, 635–639.