Abiotic Sulfur Cycle and Implications for the Emergence of Life: An Experimental Study at Carnegie Institution for Science

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Sulfur is a crucial element for biochemistry, both as an electron acceptor/donor for various metabolic processes as well as a component of amino acids and cofactors. Sulfur minerals (*e.g.* various insoluble sulfides and sulfates) in marine sediments and the oceanic and continental crust store enormous amounts of S, which provide redox equivalents for many biological and abiological processes. Moreover, pyrite has been suggested to influence the attachment and self-organization of the building blocks of life in submarine environments (Wächtershäuser 1988; Afrin *et al*., 2020). S is an unusual element in having nine potential redox states ranging from +6 (H_2SO_4), to 0 (e.g., S_0, S_8) to -2 (*e.g.* H_2S), and the chemical behavior of each redox state can vary widely. For example, the solubilities of transition metal sulfides and alkaline earth metal sulfates can vary markedly as a function of pH and co-solute concentration, and the various oxidation state S species have markedly different Henry's Law behavior.

Simple sugars in presence of nucleophilic species (usually amines, but also sulfides) produce complex chemical set of reactions collectively known as the Maillard reaction (Weber 2005; Delidovich *et al*., 2014). These reactions can sometimes result in the production of self-assembled, organic micron-sized spherules that researchers have proven to be capable of growth over time (Rand *et al*., 2011; Weber 2005). These microspherules are of interest to prebiotic chemists because they present plausible cell-like containers that could have stored and protected the building blocks of life in prebiotic setting. Such microspherules could be considered examples of protocells, which help explain how cells emerged from simple molecules and evolved into modern cellular life.

During my visit to Carnegie Institution for Science, Dr. Cleaves and I designed a range of experiments with the purpose of recreating and studying the Maillard reaction under diverse conditions using a wide variety of reagents to study the assembly and growth of these microspherules. We used a variety of simple sugars, thiol and amine nucleophiles, and several divalent transition metals to examine the production of microspherules over a pH range of 1-11. An Opentrons-OT2 pipetting robot (see Figure 1) was programmed to fill 96 well plates, with each well containing a novel reaction carried out at 275 µL scale (see Figure 2). From each source plate, two dilution sets were assembled to study the relationship between molar concentration and micro spherule production rates. The solutions were then covered in protective films to prevent further oxygenation and were laid to rest for multiple days.

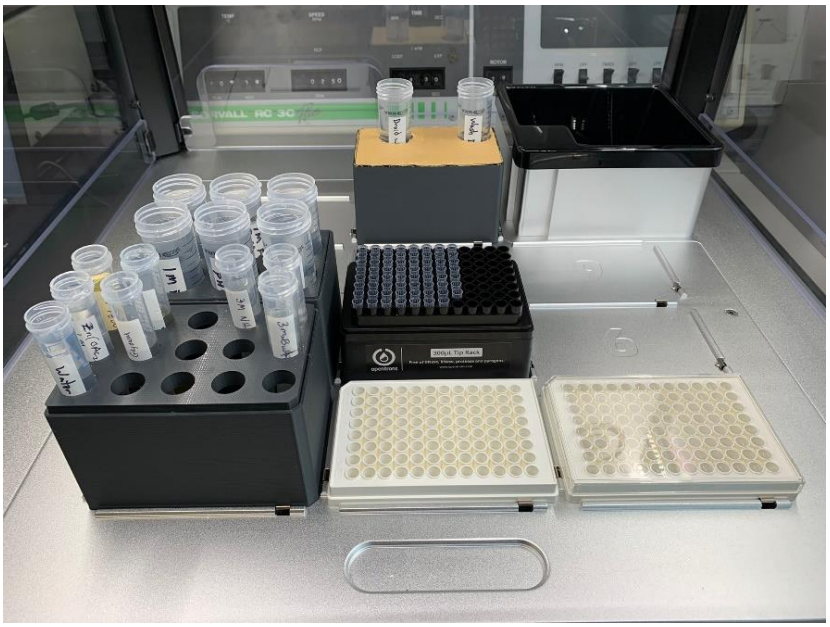

Figure 1: Deck setting for Opentrons-OT2 automated liquid handling robot.

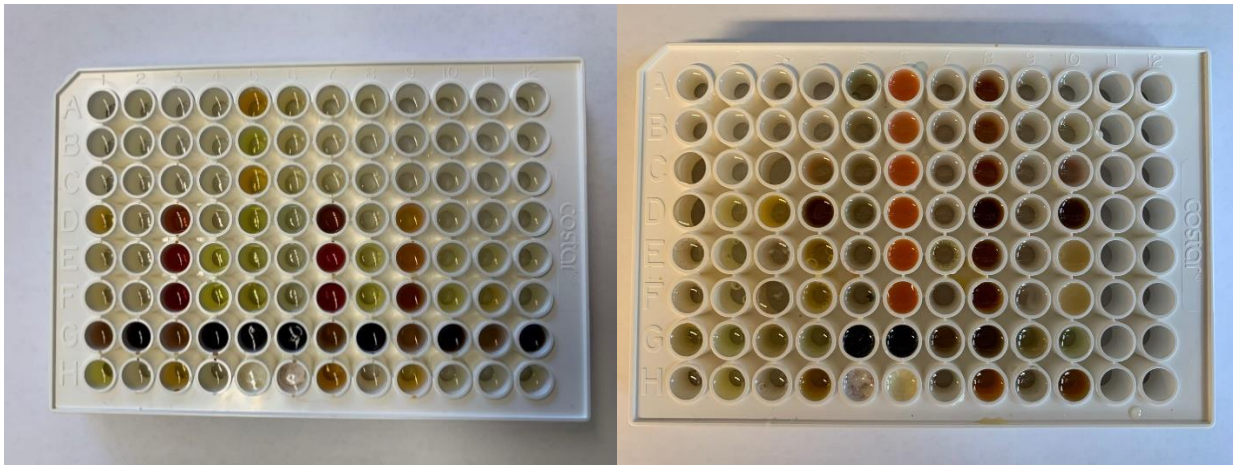

Figure 2: Two exemplary 96 well plates, with each well containing a unique reaction solutions with a specific combination of nucleophile, sugar, and transition metal at specific pH value. Plate from the left is composed of dihydroxyacetone and sodium pyruvate sugars with a variety of nucleophiles and reagents. Plate from the right is composed of xylose and glyoxal sugars with variety of nucleophiles and reagents.

We began recording UV/visible spectra of the plates immediately after mixing reagents using a Tecan Infinite 200 PRO plate reader between 340 and 600 nm, then collected further spectra after 1, 2, 7 and 14 days. Sample results for the reaction of glyoxal and sodium hydrosulfide as a function of time are shown in Figure 3.

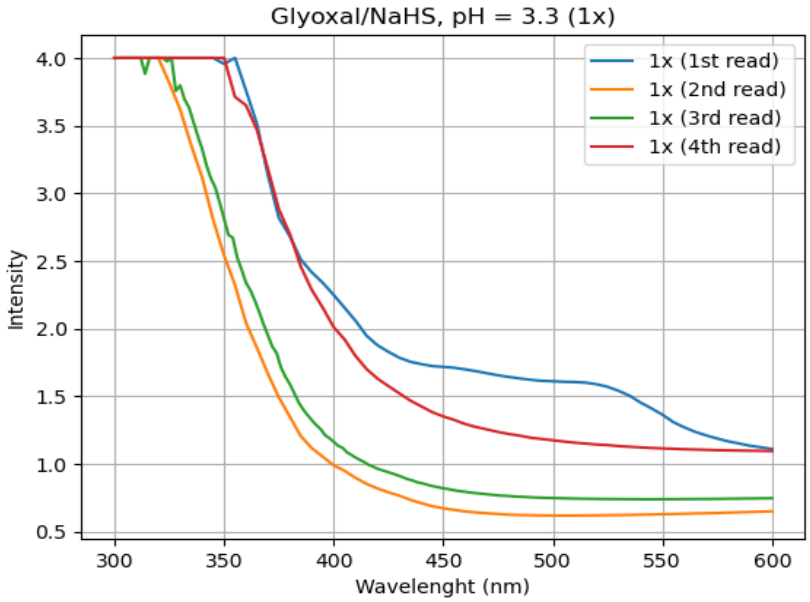

Figure 3: Sample UV/visible spectra of glyoxal and sodium hydrosulfide reaction in source plate. Microspherules produced from this reaction are pictured in Figure 4D.

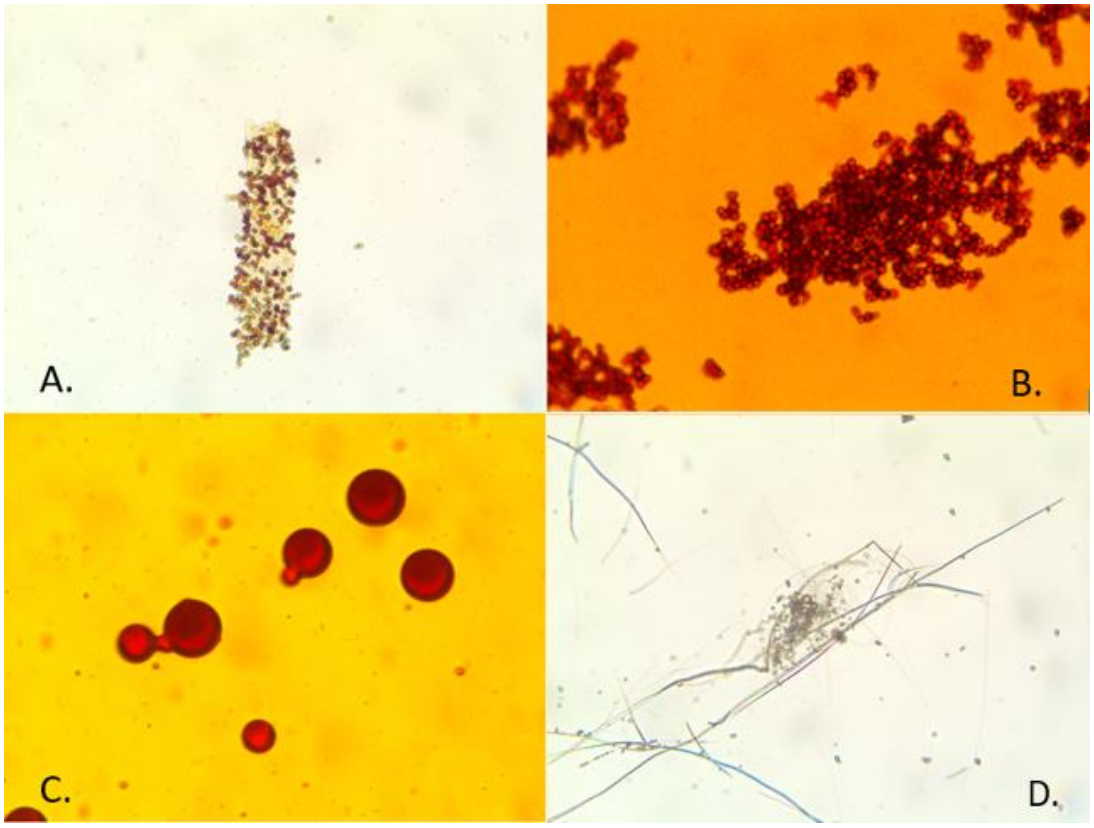

Figure 4: Organic microspherules produced in Maillard reactions starting from diverse reagents and reaction conditions. A) glyoxal with butylamine at pH = 3.3, size approximately 8 µm, B) xylose with butylamine and zinc acetate at pH = 7.0, size approximately 25 µm, C) dihydroxyacetone with butylamine at pH = 11.0, size approximately 190 µm, D) glyoxal and sodium hydrosulfide at pH = 3.3, size approximately 8 µm.

At the end of 14 days, 5 µL aliquots from each well were visually inspected using a AmScope T600A-PCS-8M digital microscope. We were able to identify microspherule products of different colors, sizes, morphologies, and conglomerate densities from several reaction wells (see Figure 4), some of which are similar to those reported by Weber (2005) and Rand *et al.* (2011).

Microspherules found in S-containing nucleophile solutions showed slight growth at the time of inspection (see Figure 4D), contrasting higher growth and conglomeration of other solutions at the same time span. However, S-containing nucleophile solutions showed productions of thin filaments that aligned microspherules in chain-like formations. Our next aims are to analyze the spectrophotometry data and to refine the chemical reactions involved in each solution. Further experimental designs are already underway for future studies, examining various other physical and chemical factors of prebiotic relevance.

References:

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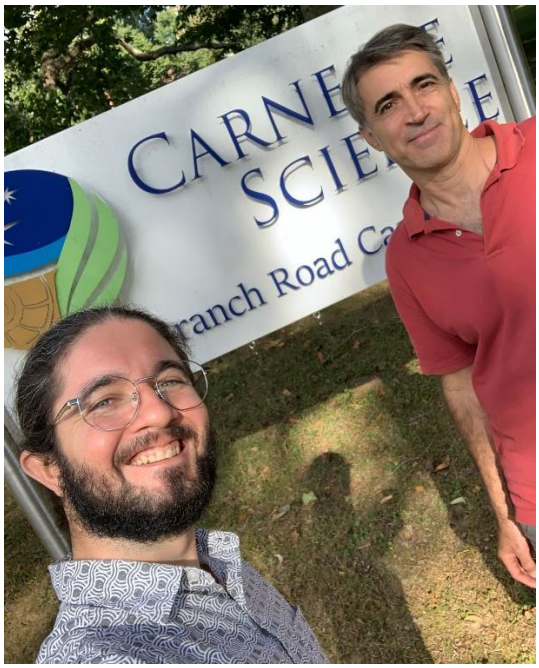

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