

ANAEROBIC CARBON MONOXIDE OXIDATION UNDER VERY DIVERSE CONDITIONS AS A MODEL FOR SUB-SURFACE MARS. G. M. King,¹ A. DePoy¹, and M. R. Myers¹ ¹Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, gkingme@gmail.com.

Introduction: Two distinct enzyme systems support anaerobic carbon monoxide oxidation. One depends on molybdenum, and occurs in bacteria and archaea typically considered aerobes or facultative anaerobes. Some of these microbes can couple CO oxidation to nitrate or perchlorate under anoxic conditions [1]. The second system depends on nickel, and occurs in a wide range of obligate anaerobes, including acetogens, methanogens, sulfidogens, and hydrogenogens, some of which are hyperthermophiles [2]. The Ni-dependent system is evolutionarily very old, and likely represents one of the earliest catabolic systems [3]. While much is known about the microbiology and biochemistry of this system, relatively little is known about the ecological conditions that support its activity. Since CO could support anaerobic metabolism in the near-surface or deep sub-surface of Mars, we have initiated an analysis of conditions that support Ni-dependent CO oxidation in terrestrial systems to assess the range of possibilities that might be relevant for Mars.

Methods: We collected soils from forests, managed agricultural systems, lake and stream sediments, hot springs, geothermally heated soils, marine muds and sands, salt-saturated muds, and soil developing on weathered basaltic lava flows. Samples were transferred to 60-cm³ serum bottles for incubations with CO added at approximately 10 ppm final concentrations to headspaces with air or deoxygenated N₂ for incubations at temperatures near ambient field values. In addition, parallel sets of samples were incubated with N₂ headspaces containing about 25% CO at ambient temperatures or at 60 °C up to 84 °C. CO concentrations were monitored over time by analyzing headspace sub-samples using gas chromatographs equipped with TCD or mercury vapor detectors. Hydrogen and methane production by samples containing 25% CO was also monitored with TCD-GC or FID-GC.

Results and Discussion: At headspace concentrations of 10 ppm, CO was readily consumed without a lag by most samples under both oxic and anoxic conditions, usually at similar rates. This suggested that populations of Mo- and Ni-dependent CO oxidizers are widely distributed, metabolically active, and capable of responding rapidly even in samples that might be considered sub-optimal for one process or the other, e.g., oxic soils for anaerobic CO oxidation, and anoxic muds for aerobic oxidation.

Responses to additions of 25% CO were more informative about the distribution and activities of Ni-dependent CO oxidizers. At ambient temperatures, including 10 °C, many sample types consumed CO with lags of varying lengths. In contrast, activity at 60 °C occurred with little or no lag in some cases, or after extensive lags in others. Somewhat surprisingly, activity was low or not observed at all in geothermally heated soils and sediments with in situ temperatures > 60 °C, even though many Ni-dependent CO oxidizers are thermophiles, some of which have been isolated from hot springs. At both ambient temperatures and 60 °C, CO uptake was typically associated with hydrogen accumulation, indicative of the water-gas shift reaction rather than acetogenesis. Exceptions occurred in sediments and soils containing high sulfate concentrations where sulfidogens likely consume hydrogen if not CO. Coupling to methanogenesis appeared negligible. Novel observations from this study include anaerobic CO oxidation at 25% concentrations and 10 °C, oxidation in some salt-saturated muds and soils, and CO uptake in solfotara soils at 80 °C with a pH of 2.5.

Conclusions: Anaerobic, presumably Ni-dependent CO oxidation occurred at low CO concentrations across a wide range of ecological contexts; similar results were observed for incubations with 25% CO levels, but activity was greatest and appeared to be induced by incubations at 60 °C, likely due to germination of thermophilic, spore-forming Firmicutes. The results suggest that sub-surface communities on Mars with access to a geothermal CO source could persist under many different, plausible conditions.

References: [1] King G. M. (2015) *Proc. Natl. Acad. Sci.*, 112, 4465–4470. [2] Yoneda Y. et al. (2015) *FEMS Microbiol. Ecol.*, 91, doi: 10.1093/femsec/fiv093. [3] Adam P.S. et al. (2018) *Proc. Natl. Acad. Sci.*, 115, www.pnas.org/cgi/doi/10.1073/pnas.1807540115.