

## Are $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ robust biosignatures in Mars-relevant geothermal systems?

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Relic hydrothermal systems are a key target in the search for evidence of past microbial life on Mars, as analogous environments on Earth are known to support chemolithotrophic life with a high preservation potential in the geologic record [1].

Hydrothermal systems can provide basic elements for life and redox couples for metabolism [2], and traces of these processes can be recorded in stable isotope ratios. We explored the utility of biological isotope fractionation as a biosignature in hot spring deposits using two geochemically distinct Icelandic geothermal systems: Kerlingarfjöll and Kverkfjöll. By investigating contrasting systems, differences in microbial biogeochemistry can be identified, allowing for organic carbon  $\delta^{13}\text{C}$  and inorganic  $\delta^{34}\text{S}$  values to be assessed as potential isotopic biosignatures.

Kerlingarfjöll pools have temperatures from 20 to 60°C with circum-neutral pH, while Kverkfjöll pools have temperatures of 16.8 and 20°C, pH of 1-3. Kverkfjöll has very high concentrations of  $\text{SO}_4^{2-}$  (0.61 to 171.04 mM), whereas Kerlingarfjöll  $\text{SO}_4^{2-}$  ranges from 0.91 to 9.8 mM. In both locations, geothermally-sustained ephemeral pools host lithotrophic microorganisms adapted to anoxic, oligotrophic environments.

Total Organic Carbon (TOC) weight % abundances have values ranging from 0.11 to 0.24 % at Kerlingarfjöll, and 0.34 to 0.42 % at Kverkfjöll. The  $\delta^{13}\text{C}_{\text{TOC}}$  values at Kerlingarfjöll vary from -16.8 to -23.5 ‰, and from -19.2 to -23.5 ‰ at Kverkfjöll, showing evidence for biological  $\text{CO}_2$  fixation. Sedimentary pyrite  $\delta^{34}\text{S}$  values are more negative at Kerlingarfjöll (-4.45 to -2.34 ‰) compared to Kverkfjöll (-2.36 to -0.70 ‰), nonetheless complicating the distinction between biological from volcanic processes.

By coupling geochemical data with 16S rRNA phylogenetic DNA assays and known biological sulfate reduction rates, we provide constraints on the minimum required  $\delta^{34}\text{S}$  fractionation to produce detectable biosignatures.

[1] Shulze-Makuch, D. et al. (2007). *Icarus* 189, 308-324

[2] Hays, L. et al. (2017). *Astrobiology* 17(4), 363-400.