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Characterizing extant biosignatures: A case for nitrogen isotopes

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Biosignatures are identifiable features of past or extant biological activity (Des Marais et al., 2008). Biosignature identification and analysis related to key metabolic processes and bioessential elements is needed for life detection efforts and to understand the history of life on Earth. Stable isotopes can be utilized as biosignatures of enzymatic activity within the ancient rock record. Previous analysis of Archean-aged sedimentary rocks for nitrogen isotopes ($\delta^{15}N$) biosignatures reveals that biological nitrogen fixation likely evolved early in Earth's history, around 3.2 billion years ago (Stüeken et al., 2015). Biological nitrogen fixation utilizes the metalloenzyme nitrogenase (reviewed in Rucker & Kaçar, 2023). While it is known that nitrogenase activity is impacted by different growth conditions, to what degree this impact is reflected in the biosignature produced by microbes is not well known. Specifically, how variable atmospheric composition affects the isotopic signature of nitrogenase requires establishing new laboratory-based methods that allow for tractability.

To address the current gap in knowledge, we constructed a batch bioreactor system in which nitrogen-fixing microbes can be grown under various conditions. The model aerobic nitrogen-fixing proteobacteria, *Azotobacter vinelandii*, was used in the growth experiments. Wild-type *A. vinelandii* was grown in the bioreactors under variable headspace gas compositions, and the cell pellets were analyzed via isotope ratio mass spectrometry for the $\delta^{15}N$ of cell biomass. Cultures of *A. vinelandii* were also grown in standard Erlenmeyer flasks to compare with the bioreactors. This study supports the development of a new laboratory method for understanding N-isotope biosignatures and their range, combining molecular and microbiology techniques. This work will aid future life detection and habitability studies by illuminating the effects environmental conditions can have on the biological isotopic fractionation of a bioessential element. This research was supported by Metal Utilization and Selection across Eons (MUSE), a NASA Interdisciplinary Consortium for Astrobiology Research (ICAR) grant.