My early career award was focused on carrying out experiments to assess bicarbonate uptake by a serpentinized fluid methanogen, *Methanobacterium NSHQ4*, isolated from the Samail Ophiolite in Oman. Serpentinized fluids can have incredibly low concentration of CO_2, a substrate for methanogenesis (metabolic redox reaction, $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$). Therefore, they provide a unique opportunity to understand potential biological adaptations to low CO_2 conditions, like bicarbonate utilization. Furthermore, serpentinized fluids on Earth serve as an analog for other ocean worlds in our outer solar system like the moons Enceladus and Europa. Insights we gain about life in serpentinizing systems on Earth can provide valuable insights into the habitability of these moons as well as help guide future life detection missions.

In my application I proposed I would conduct tracer experiments with *Methanobacterium NSHQ4* and analyze the isotopic composition of methane produced by the organism on an instrument called a Picarro. The GeoMicrobial (GEOM) Co-culturing Lab has adapted their Picarro instrument for such analyses, which gave me the unique opportunity to conduct these experiments. One experiment conducted assessed the activity kinetics of CO_2 for *M. NSHQ4*. Initial results suggest that the affinity for CO_2 of *M. NSHQ4* is approximately 2 orders of magnitude higher than that of a methanogen from a typical carbon replete environment. Higher CO_2 affinity may be due more efficient uptake and utilization of CO_2, or it could be utilization of both CO_2 and bicarbonate by *M. NSHQ4*. The second experiment was a part of a collaboration with Sabrina Elkassas, a graduate student in the Oceanography program at MIT and in the joint Woods Hole Oceanographic Institute graduate school program working under the advisement of Dr. Julie Huber. Sabrina visited the GEOM lab at CU and implemented my experimental protocol to assess CO_2 activity kinetics of a methanogen of the species, *Methanocalculus natronophilus*, an alkaline soda lake methanogen. While working is still ongoing, our preliminary results suggest that for activity, *M. natronophilus* requires at least 40 times more inorganic carbon than our serpentinized fluid methanogen, *M. NSHQ4*. These results suggest CO_2 availability plays a critical role in the adaptation and evolution of methanogens on Earth and may play a critical role in our ability to predict the habitability of and ultimately detect life on other ocean worlds in our solar system. We plan to submit an abstract on this work to the American Geophysical Union (AGU) 2025 conference.

In addition to the tracer experiments I was able to help design and shadow experiments conducted with a chemostat system (shown in **Figure 1**) developed by Dr. Sebastian Kopf and graduate student, Harpreet Batther, to assess the influence of inorganic carbon availability on important potential biosignatures, namely carbon and hydrogen isotopic composition of methane and lipids. Through this work Harpreet Batther discovered that carbon limitation can produce isotopically enriched $^{13}CH_4$, which was previously thought to be indicative of abiotic methane. This emphasizes the importance of careful examination of methane isotopes when trying to distinguish methane produced through abiological processes and biologically produced methane.

[A] **[B]**

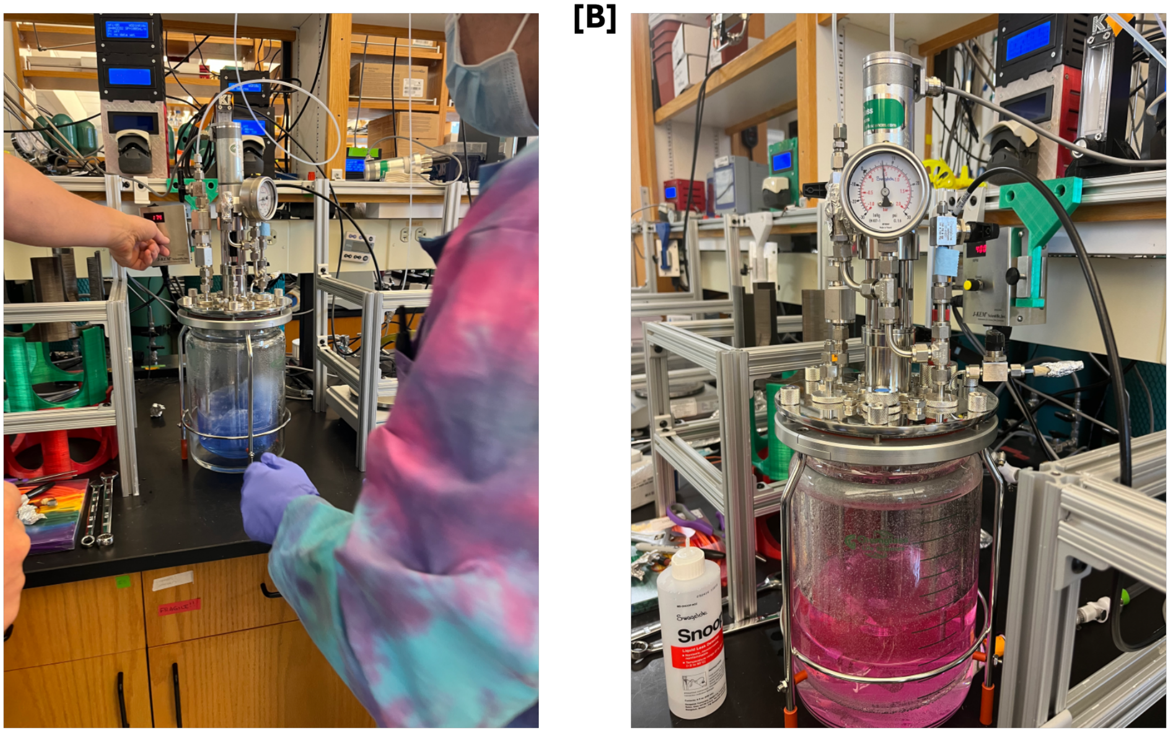

Figure 1. Dr. Sebastian Kopf and Harpreet Batther set up a chemostat cultivation system for methanogens at University of Colorado, Boulder in the GeoMicrobial (GEOM) Co-cultivation Lab. **[A]** Dr. Kopf (left) and Harpreet Batther (right) test the stirring capabilities of the chemostat. **[B]** The microbial growth media in the chemostat turned pink from blue after a reductant to remove oxygen was added. Methanogens can only survive under conditions without oxygen.

As a result of my time at CU, I was able to foster new collaborations and build new connections. I got to know an Interdisciplinary Consortia for Astrobiology Research (ICAR) team focused understanding biological compartmentalization and its role in the evolution of complex life on Earth. In building this relationship, I was presented with a job opportunity for a Research Associate position with Alexis Templeton's lab to examine the ways in which biological compartmentalization may play a role in the survival of *Methanobacterium NSHQ4* in serpentinized fluids. I transitioned to this position in January 2025 and maintain my collaborations with Tori Hoehler at NASA Ames through Blue Marble Space Institute of Sciences.