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Geochemical challenges to methanogenesis in serpentinizing systems: Implications for the habitability of ocean worlds

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Serpentinization, the aqueous alteration of ultramafic rocks, is potentially prevalent in the subsurface of Mars and the "ocean worlds" of the outer solar system. Methanogenesis is considered a model metabolism for life in such environments, and we studied the activity of a methanogen isolated from the actively-serpentinizing Samail Ophiolite (Sultanate of Oman) to inform our understanding of this potential. Serpentinization produces fluids that are hyperalkaline, H_2-rich, and poor in dissolved inorganic carbon. At alkaline pH, Ca and Mg limit the aqueous concentration of the electron acceptor of methanogenesis through formation of carbonate minerals. Ca and Mg ions may also influence biological functions such as through enzyme cofactors and cell wall stability. To assess the influence of Ca and Mg on methanogenesis, we used a batch culture approach to evaluate methane production response of *Methanobacterium* NSHQ4 to varying concentrations of Ca and Mg under alkaline conditions.

Our first experiment investigated the influence of carbonate mineral formation driven by the concentrations of Ca and Mg at pH 9.5. Methane production was two orders of magnitude higher in solutions treated with 0.9 millimolar Ca and 20 millimolar Mg (carbonate mineral saturation) than in treatments with 1.2 micromolar Ca and 1.9 micromolar Mg (below mineral saturation). These results suggest that *Methanobacterium* NSHQ4 is more metabolically active with high concentrations of Ca and Mg and/or in the presence of carbonate minerals. To test if the concentration of Ca influences growth without mineral formation, pH was set to 8.2, the Ca concentration was varied by three orders of magnitude (1 to 100 micromolar) and NaCl was set to buffer salinity. A positive correlation between methane production and Ca concentration was observed. Combined, these results suggest Ca concentration influences the activity of *Methanobacterium* NSHQ4. Overall, our work emphasizes the importance of considering the concentration of major ions and their influence on substrate availability when evaluating the habitability of ocean worlds.