National Aeronautics and Space Administration

FY23 Topic Areas Research and Technology Development (TRTD)

Preservation and viability of microorganisms in vitreous Mg-bearing salt hydrates on Europa

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Strategic Focus Area: Ocean Worlds

Objectives: To determine the potential of both structural preservation and viability of microorganisms in salty ices likely to be encountered on the surface of Europa.

Background: Vitreous (non-crystalline) Mg-bearing salt hydrates should be present on Europa based on published experiments. These materials have the potential to preserve viable microbial life by avoiding the physical damage caused by crystallization. Stable glasses on (or near) the surface may thus represent the best place to find evidence of preserved life on Europa until the subsurface ocean can be directly accessed.

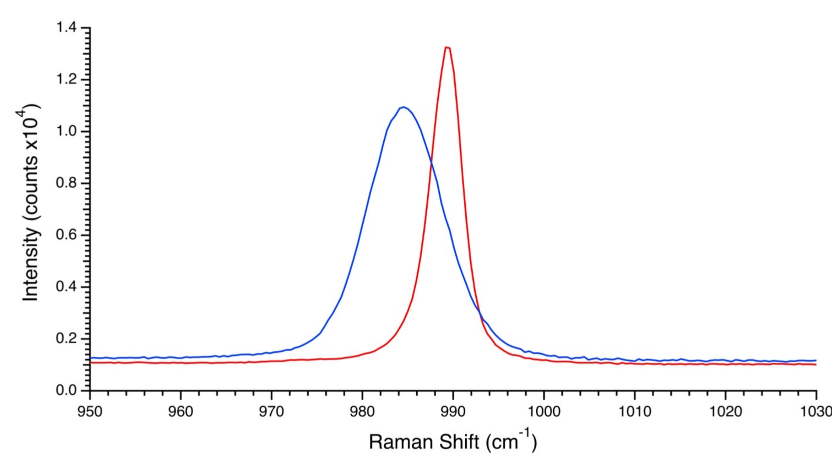

Figure 1. The characteristic sulfate feature of vitreous $MgSO_4$ hydrate (blue) and crystalline $MgSO_4$•$11H_2O$ (meridianiite; red).

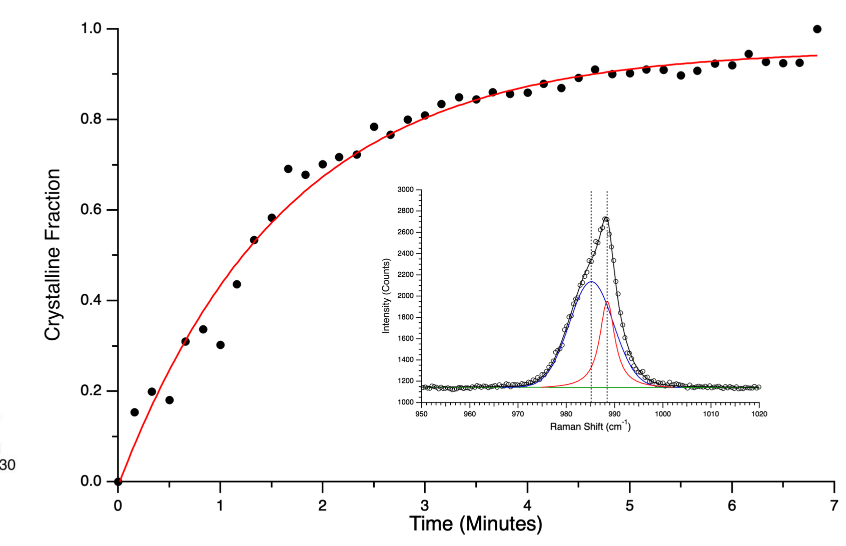

Figure 2. Crystalline fraction as a function of time at 200 K. Crystalline fraction derived at each time step by fitting the to the glass and crystalline line shapes (inset).

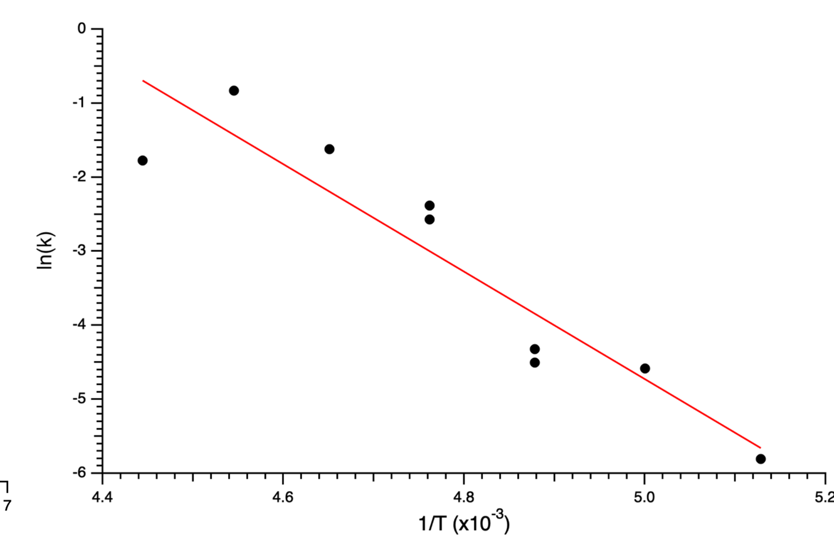

Figure 3. Arrhenius plot of the experimental data (black dots). The best fit of the Arrhenius relation to the experimental data is shown as a red line.

Approach and Results:

Freezing Rate Experiments: 5 μL drops of $MgSO_4$ solutions at various concentrations were pipetted onto a glass slide at room temperature mounted within a cryogenic optical stage. Samples were then cooled at fixed rates to 100 K and examined with Raman spectroscopy to determine whether the resulting ice was vitreous or crystalline (Figure 1). In this manner we have determined the minimum freezing rate that results in glass formation. The results showed that glasses form at cooling rates as low at 10 K/min depending on the concentration and species. Details are published in Johnson and Vu (2022).

Crystallization Kinetics Experiments: 5 μL drops of 2 M $MgSO_4$ solutions were pipetted onto a microscope slide precooled to 100 K. After setting the cryostage to a given temperature, the sulfate v_1 symmetric stretch Raman feature was monitored as a function of time to record the transition from glass to crystal. The transition was identified by the change from the characteristic broad sulfate feature of glassy $MgSO_4$ hydrate (blue in Figure 1) and the sharp, slightly shifted, sulfate feature of crystalline $MgSO_4$•$11H_2O$ (meridianiite; red in Figure 1). At a given temperature, fits were made to the respective sulfate feature to determine the vitreous vs crystal population. Plotting this as a function of time yields an exponential curve (Figure 2), which in turn gives a rate constant for the reaction at that temperature. Arrhenius analysis of the kinetics (Figure 3) then gave the activation energy for the reaction to be 60.4 kJ/mol suggesting this process will not occur spontaneously on Europa. Details are published in Johnson *et al*. (2023).

Viability Experiments: Involved freezing salt solutions with known concentrations of *Pseudoalteromonas haloplaktis and Marinobacter santoriniensis* (washed in 0.1 M $MgSO_4$) by pipetting onto a microscope slide within the cryostage. Experiments were conducted by freezing samples at the 50 K/min to produce vitreous salt hydrates, followed by annealing at 260 K to induce crystallization. Once frozen, glass/crystal formation is confirmed by monitoring the sulfate v_1 symmetric stretch Raman feature. Samples are then thawed quickly at 298 K and recovered for analysis via cultivation and Colony Forming Unit (CFU) screening to assess viability, Scanning Electron Microscopy (SEM) for morphological examination, and DHM to assess motility. CFU results show a statistically significant preservation effect comparing glass to crystalline samples (Figure 4) while SEM images show significantly more cell lysing in the crystalline vs glass samples (Figure 5). Details are published in Parker *et al*. (2023).

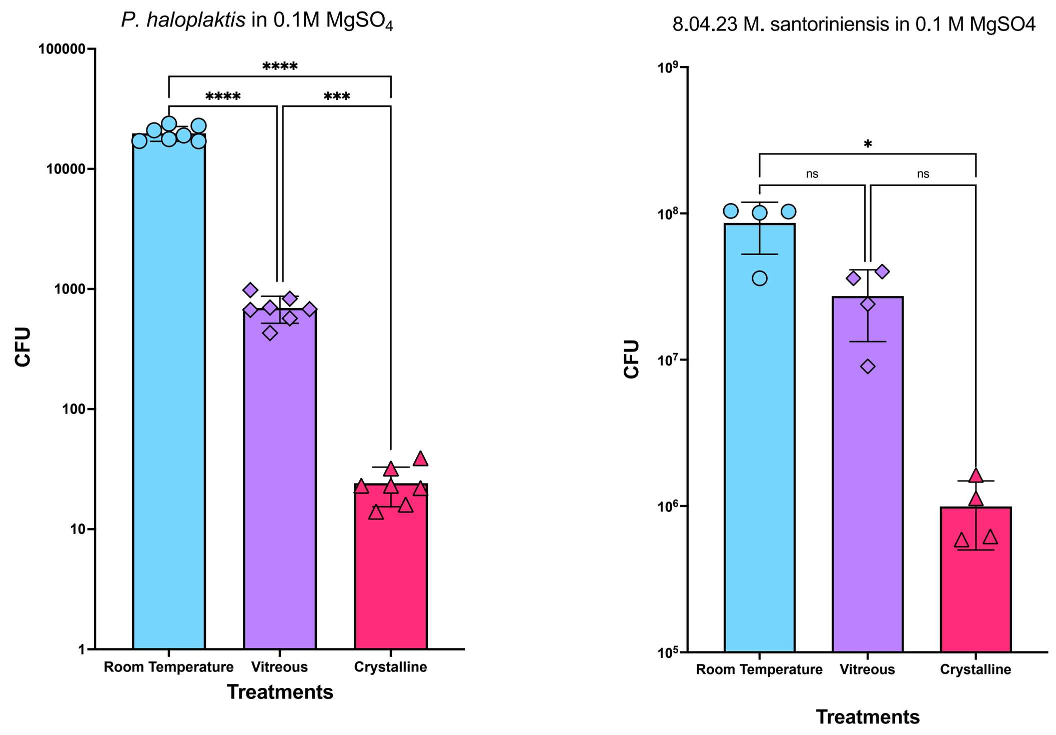

Figure 4. Results of CFU screening of three samples in 0.1M $MgSO_4$: room temperature control, vitreous sample, and a crystalline sample. After recovery of the thawed samples, cultivation and CFU screening showed significantly more viable organisms in the vitreous samples over the crystalline samples.

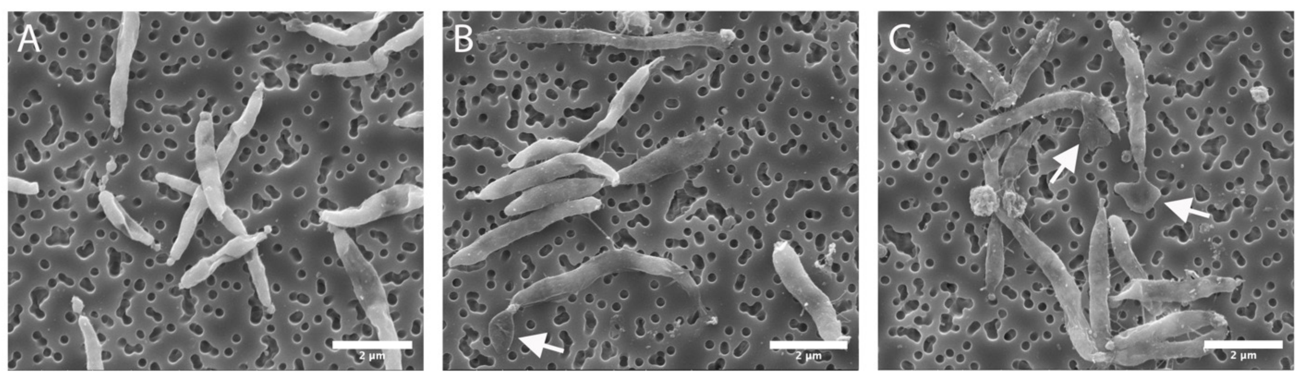

Figure 5. SEM images after samples were frozen and then thawed. (A) Room temp control: the cells are bright and 'inflated' with prominent curvature, indicating that the majority of these cells retained intact cellular membranes. (B) Glass sample: includes intact bright/ 'inflated' cells mixed with grey flattened/ 'deflated' cells; evidence of cytoplasmic release (white arrow). (C) Crystalline sample: nearly all cells appear deflated with numerous examples of cytoplasmic release (white arrows) indicating widespread cell lysing.

Significance/Benefits to JPL and NASA:

Detecting viable microorganisms on an Ocean World would be the type of overwhelmingly conclusive evidence that could convince even skeptics of the presence of life elsewhere in our Solar System. This work will inform *in situ* search strategies for biomarkers and potentially even preserved endogenic microorganisms both in terms of where to look (*e.g.*, vitreous Mg-bearing salt hydrate deposits) and how to detect and determine viability.

Publications:

- P.V. Johnson and T.H. Vu, "Formation of Vitreous Salt Hydrates Under Conditions Relevant to Europa," The Planetary Science Journal, 3:151 (7pp), 2022 July.
- P.V. Johnson, T.H. Vu, and R.Hodyss, "Crystallization Kinetics of Vitreous Magnesium Sulfate Hydrate and Implications for Europa's Surface" The Planetary Science Journal, 4:7 (5pp), 2023 January.
- C.W. Parker, T.H. Vu, T. Kim, and P.V. Johnson, "Vitreous Magnesium Sulfate Hydrate as a Potential Mechanism for Preservation of Microbial Viability on Europa," The Planetary Science Journal, accepted for publication August 8, 2023.

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