PRESERVATION OF MICROBIAL VIABILITY IN VITREOUS MAGNESIUM SULFATE HYDRATE: IMPLICATIONS FOR EUROPA. P. V. Johnson[1], C. W. Parker[1], T. H. Vu[1], and T. Kim[1], [1]Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA 91109 (Paul.V.Johnson@jpl.nasa.gov).

Introduction: Recently published experiments have demonstrated that flash-freezing of putative Europa Ocean brines containing Na^+, Mg^{2+}, Cl^- and SO_4^{2-} results in the formation of vitreous Mg-bearing salt hydrates [1]. These findings present an intriguing possibility in terms of finding evidence of life on Europa as vitrified phases are known to protect cellular structures from damage that can occur during crystallization. Therefore, if there are in fact endogenic microorganisms present in the subsurface ocean, one could imagine a scenario where they could be entrained in glasses formed by brines erupting in plumes and depositing on the surface where they could be preserved and potentially remain viable. Discovery of such viable microorganisms on Europa would provide near irrefutable evidence of life elsewhere in our Solar System.

Further work to better understand the conditions under which Mg-bearing brines form vitreous salt hydrates upon freezing has been presented by Johnson and Vu [2]. Here, the formation of vitreous salt hydrates is explored as a function of cooling rate, salt species, and ionic concentration. These results showed that vitreous $MgSO_4$ and $MgCl_2$ hydrates form when sub-molar solutions of these species (i.e., at concentrations on the order of that expected in Europa's Ocean) are cooled at rates as low as 11 K/min and 8 K/min, respectively. This means that vitreous hydrate formation is not limited to extreme, flash freezing scenarios like plume deposition, but could be relevant to other brine emplacement mechanisms making them more likely to be found on Europa's surface. For example, brines could slowly freeze at the bottom of the ice crust forming vitreous salt hydrates which then, through convection in the ice crust, could be emplaced on the surface.

Further bolstering the case for the relevance and potential importance of vitreous salt hydrates on Europa are the kinetics experiments reported by Johnson et al [3]. Here, the authors show that the activation barrier for the conversion of vitreous $MgSO_4$ hydrate to crystalline $MgSO_4$•$11H_2O$ (meridianiite) at 100 K is 60 ± 9 kJ/mol. As discussed in the paper, the shortest characteristic lifetime for the vitreous $MgSO_4$ hydrate supported by this result is ~38 million years, i.e., on the order of the age of Europa's surface (~60–100 Ma). Although this work did not investigate the effects of radiation or impacts, it is safe to conclude that once formed on Europa, vitreous $MgSO_4$ hydrate will not spontaneously crystallize without some external trigger mechanism.

Given that the works summarized suggest that vitreous Mg-bearing salt hydrates are likely to be formed on Europa and are expected to remain for extended periods of time, the remaining question is whether or not such materials do in fact have a preservation effect on microorganisms at temperatures relevant to the surface. Here we present experiments designed to explore this question. Specifically, we report experiments that compare the viability of microorganisms in $MgSO_4$ solutions after being frozen and cooled to 100 K under conditions that produce both vitreous and crystalline salt hydrates.

Experimental Methods: Viability experiments were performed using *Pseudoalteromonas haloplaktis* as a representative microorganism. *P. haloplanktis* is a motile bacterium found in Antarctic coastal sea water. It is both psychrophilic and halophilic, and was thus chosen as an analog for a potential endogenic Europan microorganism.

Six glass microscope slides were prepared by etching eleven separate 5 mm diameter spots onto each slide using large grit sand paper. 15 μL aliquots of a 0.1 M $MgSO_4$ solution containing ~5×10^7 cells/ml were then pipetted onto each pre-etched spot. Of the six slides, two remained at room temperature throughout the course of the freezing experiments to act as controls. Samples were frozen after glass slides were sequentially mounted within a microscope cryostage (Linkam LTS420), which then cooled the slides to 100 K. Two slides were cooled at 50 K/min resulting in the formation of vitreous $MgSO_4$ hydrate. The remaining two slides were frozen similarly to first form the glass, then annealed at 260 K to induce the formation of crystalline $MgSO_4$•$11H_2O$ (meridianiite). The slides were then re-cooled to 100 K.

Upon reaching 100 K, Raman spectroscopy was used to confirm the state of $MgSO_4$ hydrate in the frozen samples by examining the ν_1 sulfate symmetric stretch. Vitreous $MgSO_4$ hydrate was identified by its characteristic broad peak (FWHM of 10 cm^{-1}) centered at 985 cm^{-1}, whereas crystalline $MgSO_4$ hydrate (i.e. meridianiite) was identified by its distinctive sharp (FWHM of 4 cm^{-1}) peak centered at 989 cm^{-1} (Figure 1) [2]. After verifying vitreous/crystalline nature of the sample at 100K, slides were quickly transferred to a temperature-regulated 'hot' plate held at 298 K. Once

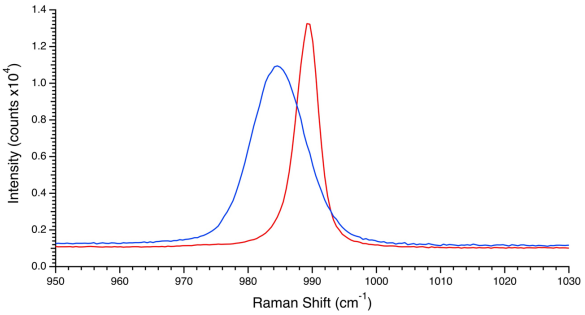

Figure 1. The characteristic broad sulfate feature of vitreous $MgSO_4$ hydrate (blue) and the sharp, slightly shifted, sulfate feature of crystalline $MgSO_4 \cdot 11H_2O$ (meridianiite; red) are shown above.

melted, samples were recovered for analysis via cultivation and Colony Forming Unit (CFU) screening to assess viability, Scanning Electron Microscopy (SEM) for morphological examination, and Digital Holographic Microscopy (DHM) to assess motility.

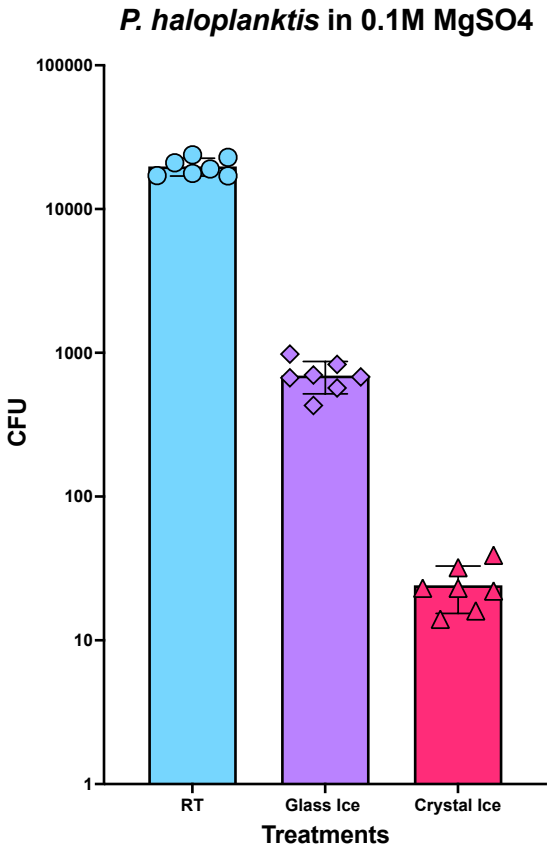

Figure 2. The figure shows the results of CFU screening of three samples in 0.1M $MgSO_4$: room temperature control, vitreous (glass ice) sample, and a crystalline sample. After recovery of the thawed samples, cultivation and CFU screening showed significantly more viable organisms in the vitreous samples over the crystalline samples.

Results: Results of CFU screening are shown in Figure 2. As expected, the room temperature control samples yielded the most viable organisms with an average of 1.98 x 10^4 CFUs. The average number of viable organisms in the vitreous samples was 6.94 x 10^2 CFUs, while the average for the crystalline samples was 2.41 x 10^1 CFUs. These results show a statistically significant higher CFU count in the vitreous versus crystal samples.

Figure 3 shows SEM images taken of recovered samples from room temperature controls, and the vitreous and crystalline freezing experiments. These results are consistent with the CFU results. Cells in the room temperature control show intact cell membranes while the crystalline sample shows widespread cell lysing. The vitreous samples are a mix of intact and non-intact cells.

DHM analysis showed depressed (to non-existent) cell motility in all samples including the room temperature controls. This result suggests that a previously motile *P. haloplanktis* is rendered non-motile in $MgSO_4$ brine.

Conclusions: These experiments support the hypothesis that vitreous salt hydrates can promote the preservation and viability of microorganisms upon freezing of relevant solutions at Europa surface temperatures.

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References: [1] Vu T. H. et al. (2020) *Icarus*, 349, 113746. [2] Johnson and Vu (2022) *Planet. Sci. J.*, 3, 151. [3] Johnson *et al.* (2023) *Planet. Sci. J.*, in press.

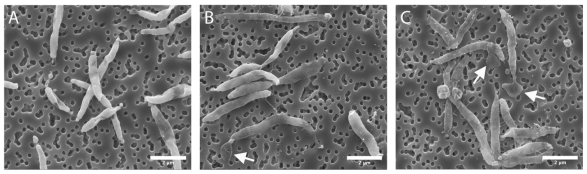

Figure 3. SEM images after samples were frozen and then thawed. (A)-room temp control: the cells are bright and 'inflated' with prominent curvature, indicating that the majority of these cells retained intact cellular membranes. (B) Vitreous sample: includes intact bright/ 'inflated' cells mixed with 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 lysing.