CO-PARTITIONING OF MICROBIAL BIOMASS WITH CRYOGENIC PHASES DURING RAPID FREEZING OF ENCELADUS-RELEVANT FLUIDS. M. G. Fox-Powell[1], C. R. Cousins[2], B. Stephens[1], C. Dazley[1], D. Slade[1], G. Richards[1], K. Olsson-Francis[1]. [1]AstrobiologyOU, The Open University, Walton Hall, Milton Keynes, UK, mark.fox-powell@open.ac.uk. [2]School of Earth and Environmental Sciences, University of St Andrews, St Andrews UK.

Introduction: The plumes emanating from the south polar region of Saturn's moon Enceladus were studied by the Cassini mission, and have revealed evidence for ongoing hydrothermal activity [1], organic chemistry [2], and redox conditions favorable for microbial metabolism [3] in the ocean below.

Salt-rich icy particles encountered by Cassini in the plumes and the associated E-ring were interpreted to originate as rapidly frozen droplets of ocean spray [4]. Seawater aerosols on Earth can contain high densities of microorganisms [5], thus it follows that if extant microbial communities exist within Enceladus's ocean, cells may become incorporated into these particles and ejected into space where they can be sampled by future spacecraft [6].

The potential fate of biomass within frozen icy particles is not understood. Under conditions of gradual cooling, microorganisms have been observed to associate with inorganic phases precipitated between ice crystals [7]. However, under the conditions of flash-freezing expected in the Enceladus plumes, crystallization of specific salt phases can be kinetically inhibited [8] with unknown implications for the capture and preservation of potential microorganisms. For example, rapid flash-freezing of simulated Enceladus ocean fluids has been shown to generate a solute-rich glass at ice grain boundaries that decomposes to microcrystalline salts upon warming [8].

Here, we used cryogenic imaging alongside sublimation extraction techniques to study the interaction of microorganisms with precipitated inorganic phases during freezing of simulated Enceladus ocean fluids, at contrasting cooling rates. Our findings reveal how putative microbial biomass may be expressed in the Enceladus plumes, and carry implications for its potential detection using current and future spacecraft sampling strategies.

Methods: We synthesized a fluid designed to simulate Enceladus ocean chemistry, based on Cassini measurements, using the pH 9 recipe from [8]. Four pure strains of microorganism were selected to capture a range of cell sizes, morphologies, cell surface properties and phylogenetic affiliations. Strains included: *Sphingopixis alaskensis*, a marine bacterium with ultra-small cell volume (<0.1 μm^3); *Escherechia coli*, a mesophilic gram-negative rod-shaped bacterium (cell volume 0.6–0.7 μm^3); *Marinococcus halophilus*, a halophilic gram-positive coccoidal bacterium (cell volume 0.5–0.9 μm^3); and *Methanothermococcus okinawensis*, a thermophilic, methane-producing archaeon (cell volume 0.6–1.8 μm^3) similar to those considered as models for Enceladus, isolated from a Pacific deep-sea hydrothermal vent. We also included a mixed community, enriched under anaerobic conditions over three successive generations in a medium based on our Enceladus ocean fluid, taken from the Strokkur hydrothermal pool in Geysir, Iceland.

Cells were suspended in simulated Enceladus ocean fluid, and droplets with radii between 10 µm and 1 mm were cooled at two contrasting rates: rapidly quench cooling in liquid nitrogen, which produced cooling rates on the order of 10^1-10^2 K s^{-1}, and gradual cooling at 0.01 K s^{-1}. This approach allowed us to identify unique effects of rapid flash-freezing.

The immediate partitioning of microorganisms alongside ice and non-ice phases within frozen droplet interiors was imaged at 120 K via cryo-scanning electron microscopy. In samples that contained solute-rich glass, the association of microorganisms with glasses was examined at cryogenic conditions, and samples were subsequently warmed to initiate devitrification (cold crystallization).

Mineralogy of crystalline precipitates and the associations between microcrystalline salts and microorganisms was analyzed using electron microscopy and X-ray diffraction (XRD) following extraction of non-ice phases from the ice matrix by sublimation. This extraction technique has been shown to preserve the salt microstructure originally accumulated at ice grain boundaries, allowing it to be analyzed at ambient temperatures [8].

Results and discussion:

Heterogeneity of salt microstructure. Flash-freezing segregates simulated Enceladus ocean fluids into ice and non-ice materials distributed heterogeneously at the >10 µm scale throughout a droplet interior. At the sub-10 µm scale, crystal textures formed from flash freezing showed homogenous distributions that were similar regardless of the presence or absence of biomass. This suggests a cold crystallization route to formation in both cases, and contrasts with gradually cooled fluids, where the presence of biomass resulted in extreme

heterogeneity in mineral distributions (Fig. 1). Ongoing work imaging frozen droplet interiors at cryogenic temperatures will track the fate of cells at all stages of the cold crystallization process.

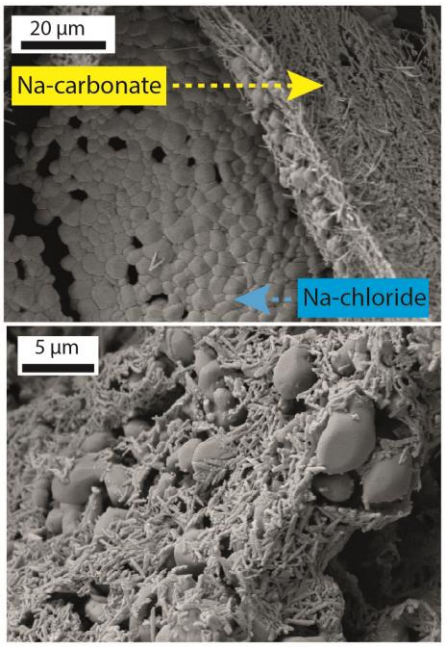

Figure 1. Scanning electron micrographs of salt phases precipitated from fluids seeded with microbial cells from community enrichments. Upper: gradual freezing (0.01 K s^{-1}). Lower: flash freezing (~60 Ks^{-1}).

Microbial biomass influences cryogenic mineral formation. XRD analyses of cryogenic salt phases shows that the presence of microbial biomass during freezing influences bulk mineralogy (Fig. 2). In the absence of biomass, nahcolite ($NaHCO_3$) is kinetically inhibited during flash freezing by the cold crystallization pathway. However, in the presence of microorganisms, nahcolite does form during flash freezing, and the resulting mineralogy instead resembles that of gradually cooled control fluids (Fig. 2). This indicates that the presence of microorganisms kinetically favors the precipitation of nahcolite. Moreover, this finding provides a route by which the presence of biomass at Enceladus could influence the mineralogy of icy particles in the plumes. Given that mineralogy is also sensitive to cooling rate and fluid pH [8], the non-destructive analysis of mineralogy within plume particles at Enceladus should be prioritized in future observations.

Our results reveal complex, cooling rate-dependent interactions between microbial biomass and rapidly

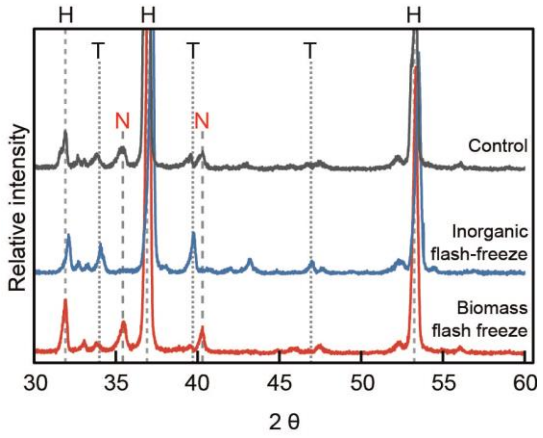

Figure 2. X-ray diffraction patterns from salt mineral assemblages produced by freezing in the presence and absence of microbial cells from community enrichments. 'Control' trace represents samples cooled gradually in the absence of biomass. H: halite (NaCl); T: Trona (Na_2CO_3•$NaHCO_3$•$2H_2O$); N: Nahcolite ($NaHCO_3$)

precipitating inorganic phases during freezing of Enceladus ocean fluids. Ongoing work is focused on determining specific associations between microorganisms and crystalline material, and the behavior of solute-rich glasses in the presence of microorganisms. If glasses do form at Enceladus, they have the potential to perfectly preserve microorganisms. However, if glasses do not form, or decompose into microcrystalline salts, biological structures may be destroyed. The association of microbial cells with specific inorganic phases has implications for interpreting secondary ion fragments in spacecraft impact-ionization mass spectra [9], and in the planning for future plume sampling missions that target *in situ* analyses, or more ambitiously, cryogenic sample return.

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