

BIOSIGNATURES OF COLD SEEP CARBONATES TO HELP UNDERSTAND THE CARBON CYCLE WITHIN THE OCEAN WORLDS. A. de Dios-Cubillas¹, D. Carrizo¹, L. Sánchez-García¹, I. López² and O. Prieto-Ballesteros¹, ¹Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid, Spain, ²Department of Biology, Geology, Physics and Inorganic Chemistry, King Juan Carlos University, Móstoles, Madrid, Spain. (adedios@cab.inta-csic.es)

Introduction: Enceladus' ocean is shielded from the cryogenic temperature and radiation of space thanks to the overlying icy shell. Nonetheless, the relatively warmer "tiger stripes" fractures, localized near the south pole, might connect the liquid reservoir to the outer layers activating the plumes erupt ion [1]. The ion and neutral mass spectrometer and the cosmic dust analyzer onboard Cassini spacecraft were able to analyze the plume composition, identifying not only water (H₂O), methane (CH₄), carbon dioxide (CO₂), ammonia (NH₃), molecular nitrogen (N₂) and molecular hydrogen (H₂) [2], but also sodium salts (NaCl, NaHCO₃/Na₂CO₃) and silica particles at E-ring in which Enceladus orbits [3-5].

The ejected materials that were measured give us an idea of the interior chemistry, but may not represent the exact ocean composition. There could be fractionation processes during materials rising [6], whereas in the ocean interior and on the seafloor the carbon sequestration process may occur via methane clathrate hydrates formation [7] and/or carbonation process when dissolved carbon dioxide reacts with serpentine in the rocky core [8].

Rock mantle degassing could provide gasses including carbon dioxide [3], while hydrogen and methane could be also released during the serpentinization of the olivine-rich core rocks [9], increasing ocean alkalinity [10] and turning the environment to favorable for chemolithoautotrophic life, such as methanotrophic archaea [11].

A comparable geyser activity may occur on Europa, since some observations from the Hubble and the Galileo spacecraft indicate that plumes can emerge today from the equatorial and southern latitudes [12, 13].

Considering gas seepage, clathration and carbonation processes, a carbon cycle may develop in these moons' oceans with some similarities to what is happening in terrestrial cold seep systems located along continental margins. Terrestrial cold seeps are sites where methane-enriched fluids coming from sediment deposits or dissociated clathrates layers discharge into the upper water column [14]. Clathrate hydrates stabilize at high pressure and low temperature regimes. Part of the liberated carbon is sequestered in some cold seep environments as authigenic carbonate, during anaerobic oxidation of methane (AOM) mediated by

consortia of methanogenic archaea and sulfate-reducing bacteria (SRB) [15]. Whether the gas is derived from clathrate hydrate dissociation that transitionally retained the gas, the carbonate is called as clathrite [16].

We are studying the biogeochemistry of carbonates from two different cold seeps, a chimney from the Gulf of Cadiz and a clathrite from the Hydrate Ridge (offshore from Oregon), with the aim to understand the potential trajectories of the carbon in the oceans of ocean moons. We analyze how the carbon tracks could affect to the strategy of the search for biosignatures in future space missions to the icy worlds.

Methodology: Mineralogical characterization of the samples was determined by X-ray diffractometer, infrared spectroscopy and optical petrographic microscope. Geochemically, organic carbon ($\delta^{13}\text{C}$) composition of carbonates was measured by isotope-ratio mass spectroscopy (IRMS), carbon and oxygen isotopic compositions by stable isotope analysis and the content of total organic carbon (TOC %) was performed using an elemental analyzer. Finally, lipid biomarkers (nonpolar, polar and acidic fractions) and their carbon isotopic composition were analyzed by gas chromatography-mass spectrometry (GC-MS) coupled to the IRMS.

Results and discussion: A summary of the results is featured on Table 1. According to the stable isotopic values, the carbonate chimney and clathrite were formed under deep water mass from different carbon source, thermogenic [17] and biogenic methane [18] respectively. The particular geological setting of clathrate decomposition explains the breccia fabric of clathrite [19].

The environment played a key role during carbonate formation and preservation, which might ultimately affect the detection of lipid biomarkers, based on their mineral and organic matter composition. Calcite and aragonite minerals are evidence from sulfate-poor and sulfate-rich sediments [20]. The contribution of allochthonous material is represented by quartz, montmorillonite, muscovite and chlorite-serpentine minerals, along with saturated carboxylic acids. In the Cadiz sample, hematite, goethite and oxi-hydroxides are indicative of aerobic oxidation [21], while a series of alkyl-benzenes of thermal degradation [22, 23]. Such kind of processes could alter the profile of biomarkers. For instance, anaerobic degradation products of phytol

(pristine and phytane) were only identified, that could likely indicate SRB-bacterial mediation [24], also supported by the presence of framboidal pyrite aggregates [25]. On the contrary, in the Pacific sample not only these organic compounds were also detected, but also characteristic lipid biomarkers with a highly depleted $\delta^{13}\text{C}$ values that indicate the syntrophic relationship of SRB (*iso/anteiso*- $\text{C}_{15:0}$ and MUFA $\text{C}_{16:1w7}$) with methanogenic and methanotrophic archaea (PMI, crocetane and archaeol).

		Carbonate chimney	Clathrite
Mineralogy analyses	Aragonite		x
	Magnesium calcite	x	
	Calcite magnesian	x	x
	Dolomite ferroan	x	
	Quartz	x	x
	Chlorite-serpentine		x
Montmorillonite	x		
Petrographic description	Fabric	Mudstone/wackestone texture	Monogenetic intraformational breccia
	Grains	Quartz, bioclasts, peloids, intraclasts and minor glauconite, muscovite, pyritic framboids and hematite pseudomorphs of pyrite	Heterometric angular micrite clasts
	Matrix/cement	Micrite matrix	Radial-acicular aggregates of aragonite
	Observations	Reddish brown colour along its external and central hole surfaces because of the precipitation of Fe oxide and hydroxide minerals	Intergranular porosity
Bulk geochemical composition	TOC	0.5 %	9 %
	$\delta^{13}\text{C}_{\text{org}}$	-22.6 ‰	-49.1 ‰
	$\delta^{13}\text{C}_{\text{inorg}}$	-12.5 ‰	-44.8 ‰
	$\delta^{18}\text{O}$	4.0 ‰	3.6 ‰
	n-alkanes	2.44 $\mu\text{g}\cdot\text{g}^{-1}$ -28.8 ‰	4.28 $\mu\text{g}\cdot\text{g}^{-1}$ -28.7 ‰
	<i>Pristane</i>	0.11 $\mu\text{g}\cdot\text{g}^{-1}$ -30.3 ‰	0.22 $\mu\text{g}\cdot\text{g}^{-1}$ -32.4 ‰
	<i>Phytane</i>	0.14 $\mu\text{g}\cdot\text{g}^{-1}$ -24.9 ‰	n.d.
	<i>Crocetane</i>	n.d.	1.38 $\mu\text{g}\cdot\text{g}^{-1}$ -104.9 ‰
	<i>PMI</i>	n.d.	0.98 $\mu\text{g}\cdot\text{g}^{-1}$ -113.2 ‰
	<i>C_{25:1}</i>	n.d.	-106.8 ‰
	n-alkanoic acids	6.5 $\mu\text{g}\cdot\text{g}^{-1}$	8.15 $\mu\text{g}\cdot\text{g}^{-1}$
	$\text{C}_{15:0}$	n.d.	-87.7 ‰
	$\text{C}_{16:0}$	-28.4 ‰	-37.1 ‰
	$\text{C}_{18:0}$	-28.9 ‰	-31.5 ‰
	Unsat. Acids	0.7 $\mu\text{g}\cdot\text{g}^{-1}$	11 $\mu\text{g}\cdot\text{g}^{-1}$
	$\text{C}_{16:1w7}$	n.d.	-59.1 ‰
	i-/a- alkanolic acid	0.1 $\mu\text{g}\cdot\text{g}^{-1}$	0.96 $\mu\text{g}\cdot\text{g}^{-1}$
	i $\text{C}_{15:0}$	n.d.	-84.4 ‰
	a $\text{C}_{15:0}$	n.d.	-87.6 ‰
	n-alkanols	0.57 $\mu\text{g}\cdot\text{g}^{-1}$ -28.4 ‰	6.50 $\mu\text{g}\cdot\text{g}^{-1}$ -50.5 ‰
<i>dihydrophytol</i>	0.34 $\mu\text{g}\cdot\text{g}^{-1}$ -31.9 ‰	2.46 $\mu\text{g}\cdot\text{g}^{-1}$ -109.3 ‰	
<i>archaeol</i>	n.d.	11.53 $\mu\text{g}\cdot\text{g}^{-1}$ -117.7 ‰	
<i>sn-2-hydroxyarchaeol</i>	n.d.	3.63 $\mu\text{g}\cdot\text{g}^{-1}$ -112.6 ‰	
<i>cholesterol</i>	0.07 $\mu\text{g}\cdot\text{g}^{-1}$	0.51 $\mu\text{g}\cdot\text{g}^{-1}$ -96.3 ‰	

Table 1. It is divided horizontally into three main areas according to types of analyses performed. The first area shows all minerals identified by X-ray diffraction and IR spectroscopy. A cross marks which of them is part of mineralogical composition of each sample. The second one is a summary of petrographic description. Finally, the third one shows the TOC content, samples isotopic composition ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$), and concentration and the stable carbon isotopic ratio of lipid biomarkers (non-polar, acidic and polar fractions), in that order.

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References: [1] Spencer et al. (2006) *Science*, 311, 1401-1405. [2] Waite et al. (2017) *Science*, 356, 155-159. [3] Glein et al. (2015) *Geochimica et Cosmochimica Acta*, 162, 202-219. [4] Khawaja et al. (2019) *MNRAS*, 489, 5231-5243. [5] Spahn et al. (2006) *Science*, 311, 1416-1418. [6] Fifer et al. (2020) *LPI*, 2326, Abstract 2727. [7] Bouquet et al. (2015) *Geophysical Research Letters*, 42, 1334-1339. [8] Glein and Waite (2020) *Geophysical Research Letters*, 47. [9] Sleep et al. (2004) *PNAS*, 101, 12818-12823. [10] Kotlarz et al. (2020) *Astrophysical Bulletin*, 75, 166-175. [11] McKay et al. (2008) *Astrobiology*, 8, 909-919. [12] Jia et al. (2018) *Nature Astronomy*, 2, 459-464. [13] Arnold et al. (2019) *Geophysical Research Letters*, 46, 1149-1157. [14] Greinert et al. (2010) *Marine Geology*, 272, 6-25. [15] Elvert and Suess (1999) *Naturwissenschaften*, 86, 295-300. [16] Kennett and Fackler-Adams (2000) *Geology*, 28, 215-218. [17] Wang et al. (2015) *Marine and Petroleum Geology*, 68, 192-205. [18] Peckmann and Thiel (2004) *Chemical Geology*, 205, 443-467. [19] Bohrmann et al. (2002) *Proc. Fourth Int. Conf. Gas Hydrates*, 102-107. [20] Greinert et al. (2001) *American Geophysical Union*, 124, 99-113. [21] Magalhães et al. (2012) *Sedimentary Geology*, 243-244, 155-168. [22] McCollom and Simoneit (1999) *Orig. Life Evol. Biosph.*, 29, 167-186. [23] McCollom (2013) *Reviews in Mineralogy and Geochemistry*, 75: 467-494. [24] Grossi et al. (1998) *Organic Geochemistry*, 29, 845-861. [25] León et al. (2006) *Deep-Sea Research II*, 53, 1464-1481.