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Simulating microbial processes in extraterrestrial, aqueous environments.

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Abstract

Finding evidence of life elsewhere in the Solar System is dependent on understanding biotic processes that could occur within potentially habitable environments. Here, we describe a suite of high-pressure flow-through chambers that have been developed to investigate biotic and abiotic processes within simulated sub-surface martian and icy moon environments.

Keywords

Simulation chamber, icy moon, Mars, sub-surface, habitability

Introduction

Finding evidence of liquid water is crucial for understanding when, where, and under what conditions past or present life may have existed in the Solar System, and it is now known that a wide variety of aqueous environments exist beyond the Earth, for example on Mars and the icy moons (e.g., Arvidson and Catalano, 2018; Glein et al., 2018; Lasue et al., 2019; Matson et al., 2009; Monteux et al., 2016; Ramirez and Craddock, 2018). Of particular importance, from a habitability perspective, are water-rock interactions, such as serpentinisation (e.g., Bridges and Schwenzer, 2012; Holm et al., 2015; Melwani Daswani et al., 2016; Schwenzer and Kring, 2009), since they control local chemical conditions. As water circulates, rock dissolution occurs, liberating elements including those that may be bio-essential, and secondary minerals are precipitated. This results in changes to the chemical composition of the fluid and the redox state of elements, and produces a chemical gradient (e.g., Barge and White, 2017; Bridges and Schwenzer; 2012; Hand et al., 2007; Schulte et al., 2006). Such conditions may be conducive to chemosynthetic life forms, which relies on harnessing energy from redox reactions to produce cellular energy, so understanding these environments is important for investigating the limits of habitability and identifying bio-signatures that could be used as evidence of life.

We have developed a simulation facility with the aim of studying abiotic and biotic processes within the sub-surface environments of Mars and the icy moons, Europa and Enceladus. With evidence for present or past bodies of water (e.g., Grotzinger et al., 2015; Hsu et al., 2015; Jia et al., 2018; Osinski et al., 2013; Rampe et al., 2017; Sekine et al., 2015; Thomas et al., 2016; Waite et al., 2017; Williams et al., 2013), these environments are the targets of current and future exploration missions, many of which have life-detection as a primary goal. The physico-chemical regimes required to simulate these aqueous environments have been modelled (e.g., Clifford, 1993; Hsu et al., 2015; Jones et al., 2011, Sekine, et al., 2015; Zolotov, 2009) and are shown in Table 1. Although these parameters have not been directly measured there is a good and growing understanding of these values, particular where *in-situ* measurements have been made of Mars' surface (e.g., Squyres et al., 2004; Vaniman et al., 2014) and Enceladus' plumes (e.g., Porco et al., 2006; Spencer et al., 2006; Waite et al., 2006, 2017). The values obtained are constantly being re-evaluated with data from every new mission.

Previous attempts to simulate extraterrestrial environments for microbial experiments (e.g. the sub-surface ocean of Enceladus (Taubner et al., 2018) and the martian sub-surface (for review see Jensen et al., 2008) have used closed-systems that are not truly representative of the natural environment. Although a high-pressure flow-through system has been developed by Foustoukos, et al., (2015) to study sub-surface microbial processes on Earth, the range of operating temperatures is limited (3 to 25 °C) and it is not designed to include the rocks or minerals necessary to fully simulate the natural environment. Until now, the Planetary Environmental Liquid Simulator (PELS) (Martin and Cockell, 2015) has been the only facility that could be used to study microbial processes in a simulated extraterrestrial environment in an open (flow-through) system. However, it can only maintain a maximum pressure of 10^5 Pa, insufficient to study habitability at depth on Mars, or at the ocean floor of the icy moons.

To address this gap in planetary simulation facilities, we have developed a suite of reactors to study: 1) the martian sub-surface (the MSS chamber); and 2) the ocean floors of Europa and Enceladus (the IM chamber). The reactors can be run in dynamic (flow-through) or static mode with variable water-to-rock ratios (high - inferring high permeability, to low - inferring only trapped pore water). The overall set-up of the two systems is shown in Figure 1, and incorporates a high-pressure reactor (Parr Instrument Company, UK; Table 2) connected to a fluid cycling system and gas inlet and housed within a heating jacket.

Before use, the reactor is sterilised by flushing the system with 150 ml of 70 % industrial methylated spirits followed by 200 ml of sterilised ultrapure water. The tubing is then sealed with sterile aluminium covers to maintain sterility and ultra-pure water is added to the reactor. The reactor is then heated to 121 °C for 30 min using the reactor's heating system. After cooling to 90 °C, the water is flushed out of the reactor using N_2 and all tubing is sealed with sterile aluminium covers.

Simulants are added, which have been developed to mimic the composition of silicates in key extraterrestrial locations (for details see Ramkissoon et al., 2019 and Hamp et al., 2019), and fluids with chemistries based on thermochemically-modeled values (e.g., Tosca, et al., 2008; Schwenzer et al., 2016). The required simulant is prepared and sterilised (described in (Ramkissoon et al., 2019) and added to the reaction chamber. For biotic experiments, microbial communities from terrestrial analogue sites are also used (Curtis-Harper et al., 2018; Macey et al., 2019).

The fluid is prepared using sterile, anaerobic techniques, (described in Curtis-Harper et al., 2018) and added to a sterile 10 L acid washed reservoir bottle (Duran®, Germany)). The reservoir fluid is fed into the reactor via a high-performance liquid chromatography (HPLC) pump (MX-Class pump, Scientific Systems Inc, US), which controls the flow rate of solution into the reaction chamber, and tubing composed of: 1) Tygon® (Cole-Parmer, UK); and 2) polyetherketone (PEEK). These tubes are connected by a PTFE luer connector. The HPLC pump is connected to the reactor by stainless steel tubing. The initial pressure of the headspace within the chamber is set using a Druva SS/1G SE(N6) high pressure regulator connected to the reactor by high-pressure flexible hoses. The chamber is initially purged with nitrogen, in order to displace oxygen from the chamber, then purged with the gas mixture in order to displace the nitrogen purge gas. It is then pressurised, as required and an isolation valve between the flexible hose and the reactor closed off to prevent any backflow of gases.

The pressure within the reaction chamber is maintained by a spilt ring clamp fitted with compression bolts and a PTFE O-ring to create an airtight seal; a back-pressure regulator

(BPR) is used to close the sampling tube. The BPR uses an air-articulated diaphragm valve, which only opens when the internal pressure of the reaction chamber is greater than the gas pressure acting to close the valve. Reservoir fluid is continuously pumped into the reaction chamber, the volume of which is maintained by the BPR, set to the required pressure for the experiment (i.e. the specified chamber pressure).

The addition of fluid to the reaction chamber causes the chamber pressure to rise above that of the BRP, resulting in the opening of the diaphragm valve. This allows liquid to flow from the reaction chamber to the sampling vessel *via* the sampling line. This maintains the volume of the liquid within the reaction chamber and enables the experimental to be monitored by collecting fluid sub-samples. We have demonstrated that pressure can be maintained for five weeks.

The headspace within the reaction chamber is prepared using commercial gas mixtures (BOC Ltd), the composition of which is shown on Table 2 and is based on current understanding of the extraterrestrial environments being simulated. For example, carbon dioxide is the main constituent within the martian atmosphere (Carr, 2007), has been measured in Enceladus' plumes (Waite et al., 2017) and is postulated to be present in Europa's ocean (Russell et al., 2017). Hydrogen may be produced by serpentinisation in each of these environments (Holm et al., 2009) but is added because of the slow rate of serpentinisation within the chamber relative to geological processes. Nitrogen is used as an inert gas to regulate the pressure in the reactor has also been detected in small quantities: in the martian atmosphere; in sediments at Gale crater, Mars (Stern et al., 2015; Mahaffy et al., 2013), and nitrogen-bearing organic molecules have been detected in the plumes of Enceladus (Postberg et al., 2018). The composition of the gas can be varied and special mixtures, e.g., containing noble gases, can be fed into the system as desired.

An electrically-heated jacket around the reactor is used to simulate the temperatures associated with the martian sub-surface environment. For the icy moon simulations, a liquid heated jacket is used to provide a means of heating or cooling the reactor, which uses silicone oil thermofluid controlled by a Huber Ministat 125 refrigerated heating circulating bath. The direct contact between the reactor and the jacket allows rapid and uniform heating. A thermocouple is placed within the reaction chamber to monitor internal fluid temperatures. Each temperature control unit is equipped with an oven temperature cut-off, which will switch off the heater if this set temperature is exceeded. Although the reactors can reach >150 °C, for microbiology experiments temperatures are limited to 122 °C, the maximum temperature at which microbial growth has been detected (Takai et al., 2008).

In conclusion, these high-pressure flow-through reactors enable investigations into water-rock interactions in the presence of microorganisms that could occur within potentially habitable environments in the Solar System. Studying these processes is important for understanding the limits of life and identifying potential signatures that could be used as evidence of life in future life detection missions.

Table 1: The estimated environmental parameters for water-rock interactions for Mars, Europa and Enceladus[a].

Depth (km)	**Temperature** (°C)	**Pressure (MPa)**	**Reference**
	Mars sub-surface[b]		
0.01	0-100	0.1	(Clifford 1993; Clifford and Parker, 2001; Jones et al., 2011)
0.1	0-180	1	
1	13-350[c]	10	
10	130-350[c]	100	
	Europa[d]		
70-180	-13 to ~90	100 to 200	(Hand et al., 2004, Kargel et al., 2000, Zolotov, 2009)
	Enceladus[d]		
60-70	0 to ≥90	1 to 8	(Glein et al., 2018; Hsu et al., 2015; Sekine et al., 2015; Thomas et al., 2016 Waite et al., 2017).

[a] Note that for Mars we do not consider volcanic or post-impact hydrothermal systems that move the geothermal gradient towards high temperatures, and we assume a surface temperature of 0 °C. Our system can be used for other geological scenarios, so long as p-T conditions do not exceed the specifications of the pressure vessels.

[b] Temperature is dependent on the salinity of the water.

[c] We give 350 °C as maximum temperature as this is the highest temperature, we are currently able to safely simulate.

[d] Temperature varies between the estimated bulk water and that of the hydrothermal fluids.

Table 2: Standard specification of the Mars sub-surface reactor (MSS) and the Icy moon (IM) reactor. Environmental conditions are those simulated for the water-rock interface of each extraterrestrial environment.

	MSS reactor	**IM reactor**
Specification of the reactor		
Model	4566-T-FMD1(HC)-HC-230-VS.125-3000-4848-A1925E4-CE(PED)	4545-T-FMD1-SS-230-VS.125-WJ-5000-A2110E-CE(PED)
Volume	300 ml	600 ml
Composition	Alloy C276 /316 SS	T316 Stainless Steel
Temperature range	Ambient to 350 °C	-20 to + 150 °C
Pressure range	0 to 6.9 MPa	0 to 34.5 MPa[b]
Gas headspace		
Composition	10% CO_2 40% H_2 50% N_2	10% CO_2 40% H_2 50% N_2
Regulator	Air Products R307	Druva SS/1G SE(N6)
Tubing		
Composition	Polyetherketone /Alloy C276 /316 SS	Polyetherketone/316 SS

Figure 1. Schematic (not to scale) of the experimental setup of reactors to explore water-rock interactions.

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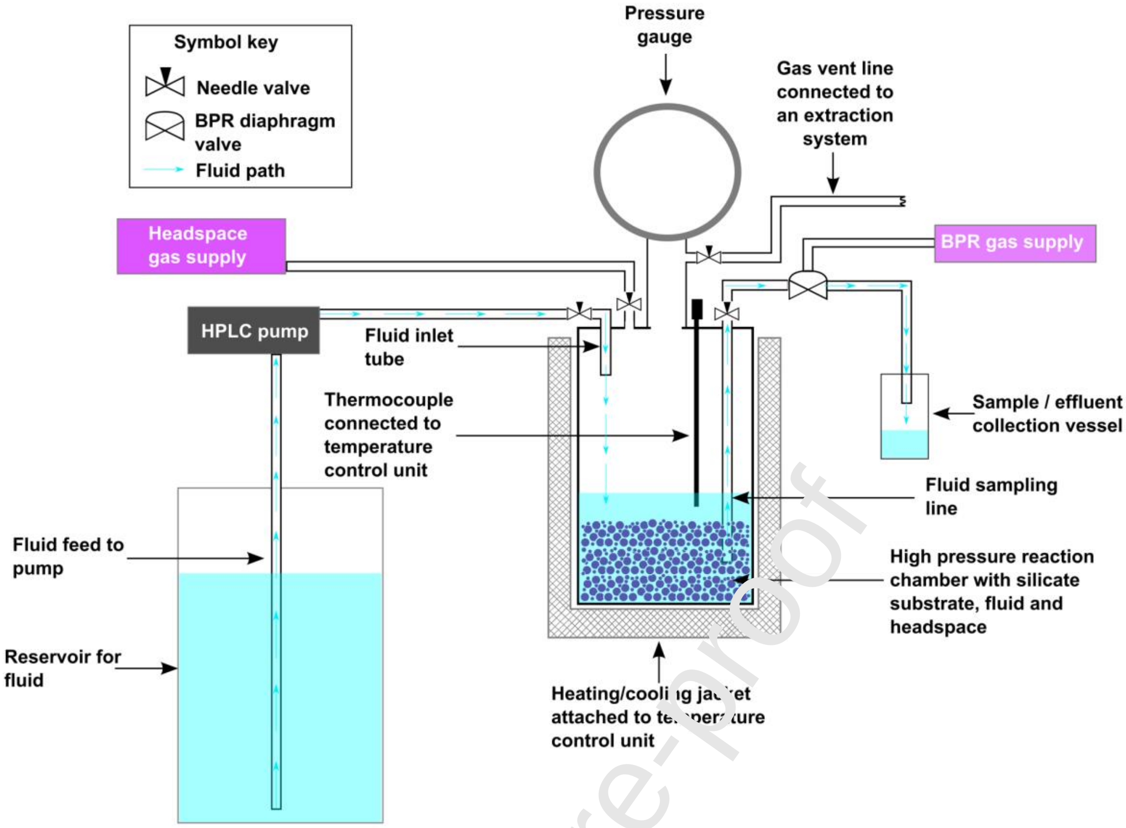

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: