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Microbe-mineral interaction and biomining on the International Space Station: the BioRock experiment

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Abstract. Microorganisms perform countless tasks on Earth and will be essential for human space exploration. The ESA-supported BioRock experiment studied microbe-mineral interaction on the International Space Station (ISS), with a view to its potential roles for extraterrestrial life support systems, for instance biomining. The experiment was performed in microgravity, simulated Mars gravity and simulated Earth gravity. One of the three bacterial species used in the experiment, *Sphingomonas desiccabilis*, showed no significant variation in final cell concentrations between gravity regimens, and enhanced bioleaching of Rare Earth Elements (REEs) from a basalt rock. These data demonstrate the potential for biomining in space and the efficacy of microbe-mineral interactions for advancing the establishment of a self-sustaining human presence beyond the Earth.

Key words. BioRock - International Space Station (ISS) - Mars gravity - microgravity (μg) - space bioproduction - space microbiology - spaceflight - space biomining

1. Introduction

Microorganisms are ubiquitous in Earth's biosphere and will necessarily follow humanity in its space exploration. Some of these potential uses include manufacturing (Menezes et al. 2015), as building blocks of ecosystems (Gòdia et al. 2002), in soil formation and biomining (Cockell 2011). This latter is an industrial process widely used on Earth, that involves the use of microorganisms to extract economically relevant elements, such as copper, gold and Rare Earth Elements (REEs) (Jerez 2017; Johnson 2014; Massari & Ruberti 2013). These geomicrobiological processes have been recognised to be potentially important for human settlements in space (Cockell 2010, 2011; Montague et al. 2012). Despite the enormous interest in the physiological responses of microorganisms to space conditions, data on the effects of microgravity (Horneck et al. 2010) present discrepancies (Klaus et al. 1997; Zea et al. 2017), while the influence on microbial interactions with minerals and possible applications have been poorly studied. In 2019, we performed the European Space Agency-approved BioRock experiment (Loudon et al. 2018; Santomartino et al. 2020; Cockell et al. 2020) on board the International Space Station (ISS) to investigate microbe-mineral interaction with a natural basaltic surface (an analogue for regolith material on the Moon and Mars) and advance the knowledge on bacterial responses to low gravities. Three bacterial species were used for the experiment: *Sphingomonas desiccabilis* CP1D, *Bacillus subtilis* NCIB 3610 and *Cupriavidus metallidurans* CH34. These were exposed to microgravity (μg), simulated Martian gravity (Mars g) and simulated Terrestrial gravity (Flight 1-g)onboard the ISS, while ground controls were exposed to real Terrestrial gravity (True 1-g). Microorganisms grew in the presence of a basalt slide for 21 days at 20 °C, in 5 mL of 50 % v/v R2A (Reasoner & Geldreich 1985). Here we report the data on final cell concentrations and REEs bioleaching capacity for *S. desiccabilis* under various gravity regimens (Santomartino et al. 2020; Cockell et al. 2020), and discuss the implications of these results for the future of human space exploration.

2. Results

2.1. Final cell concentration and optical density for S. desiccabilis

Cell population after sample retrieval was assessed on the liquid cultures by two methods: direct cell counting and optical density (λ=600 nm) (Santomartino et al. 2020).For *S. desiccabilis* (Figure 1), final cell counts were 0.4-2.7 x 10^9 cell mL^{-1} across all the gravity regimens (Fig. 1*A*), and ANOVA showed no difference between the gravity regimens (F(3, 8) = 1.052, *p*-value = 0.421). ANOVA on optical density measurements confirmed the lack of difference between the three gravity conditions tested in space (Figure 1*B*), while the optical density of True 1-g samples was significantly lower compared to samples subject to μg and Mars g (F(3,8) = 7.148, *p*-value = 0.0119, post-hoc Tukey test *p*-values between μg and True 1-g = 0.016, and between Mars g and True 1-g = 0.018).

2.2. Effects of gravity on S. desiccabilis-mediated REEs bioleaching

The liquid fraction of each sample, including non-biological controls, and cell pellets were analysed by ICP-MS (inductively coupled plasma mass spectrometry) to measure the REEs bioleached into solution Cockell et al. (2020). Statistical analysis comparing *S. desiccabilis* biological samples and the non-biological controls in each gravity condition showed that μg was not significant (ANOVA: F(1,69) = 2.43, p = 0.124), while significant differences were observed in Mars g (ANOVA: F(1,83) = 14.14, $p < 0.0001$), Flight 1-g (ANOVA: F(1,83) = 24.20, $p < 0.0001$) and True 1-g (ANOVA: F(1, 68) = 24.56, $p < 0.001$). The difference between gravity conditions tested on the ISS was not significant (ANOVA: F(2, 123) = 1.60, p = 0.206), however comparison between Flight 1-g and True 1-g showed a significant difference (ANOVA: F(1, 82) = 8.14, p = 0.005). Student's t-tests were used to examine the concentration of individual REEs bioleached compared to non-biological controls, and between gravity conditions. Bioleaching was significantly higher than non-biological controls under Mars g and Flight 1-g for all REEs ($p < 0.05$), except for Pr and Nd in both gravities, and Ce in Mars g, while none was significantly higher in μg. In True 1-g, all REEs showed a statistically significant difference with the non-biological control ($p < 0.05$) apart from Nd, Sm, La, Ce and Eu. Comparison between Mars g and Flight 1-g showed that the concentrations of five elements (La, Sm, Eu, Tb, Ho) were significantly different ($p < 0.05$). All REEs were significantly different ($p < 0.05$) between Flight 1-g and True 1-g, except Er, Tm, Yb and Lu. On the ISS, the highest enhancement was a 429.2 ± 92.0 % increase for Er in Flight 1-g, while the highest enhancement in True 1-g was related to Yb (767.4 ± 482.4 %) (Figure 2). REEs concentrations in cell pellets were less than 5 % of the total REEs in the bulk solution, with a few exceptions (Cockell et al. 2020). Comparison of non-biological control samples between the three gravity conditions on the ISS showed that the gravity condition was not significant (ANOVA: F(2, 109) = 2.91, p = 0.059), while a significant difference was present between the Flight 1-g and True 1-g (ANOVA: F(1, 68) = 6.90, p = 0.011).

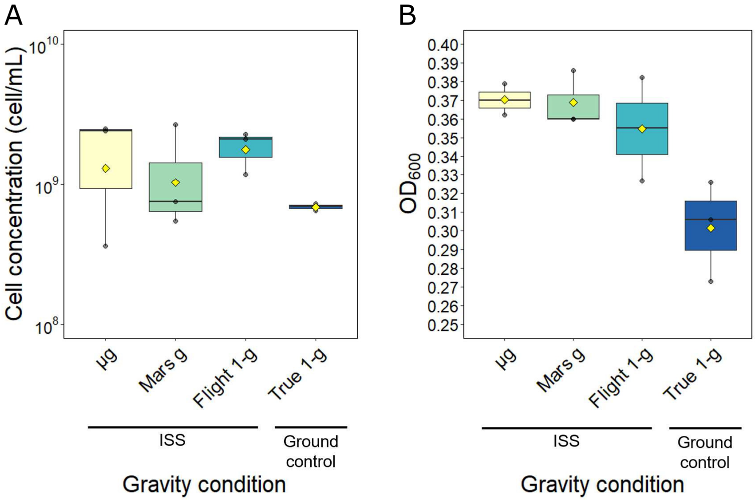

Fig. 1. Final cell concentrations of *S. desiccabilis* obtained after spaceflight. Boxplots of the direct cell count (A) and optical density at a wavelength of 600 nm (B) distributions are shown. Dots represent single measurement (n = 3). Yellow diamonds represent mean values. The horizontal bar indicates the median and boxes represents the 25*th* to 75*th* percentile. ISS indicates samples that were launched on the International Space Station, while ground control refers to samples subjected to real terrestrial gravity.

3. Conclusions

Despite the effects of gravity on micro-scale fluid dynamics (Klaus et al. 1997), we did not observe any significant difference in the final cell concentrations of *S. desiccabilis* between the four gravity conditions tested (μg, Mars g, Flight 1-g and True 1-g) after 21 days of growth. A possible explanation is that all cells reached stationary phase at the end of the experiment, resulting in similar final cell numbers. A significant reduction in optical density was observed in True 1-g when compared to μg and Mars g. However, no difference in optical densities was present between space samples. Interestingly, significant differences in bioleached REEs were observed, when comparing Flight and True 1-g. This may highlight differences between simulation of gravities by centrifugation and real gravities. We also demonstrated the use of *S. desiccabilis* to extract a group of economically important elements (REEs) from basalt rock, a material found on the Moon and Mars, on the ISS. *S. desiccabilis* enhanced mean concentrations of leached REEs in all the gravity conditions investigated, and these were significantly different from non-biological controls in simulated Mars and Earth gravities. Results on μg were not significative in respect to the non-biological controls, however this may be due to the loss of one of the μg non-biology controls. In conclusion, our results demonstrate that biomining is in principle achievable in space under a wide range of gravity conditions, although the scaling up of the system will require several adjustments. Data also suggest that biotechnological applications (bioproduction, bio-manufacture and life support systems) on other planetary bodies with low gravities, such as Moon or Mars, will be possible, as final cell concentrations would not be deleteriously affected by low gravity under similar growth conditions reported here. The experiment thus shows the efficacy of microbe-mineral interactions for advancing the establishment of a self-sustaining permanent human presence beyond the Earth.

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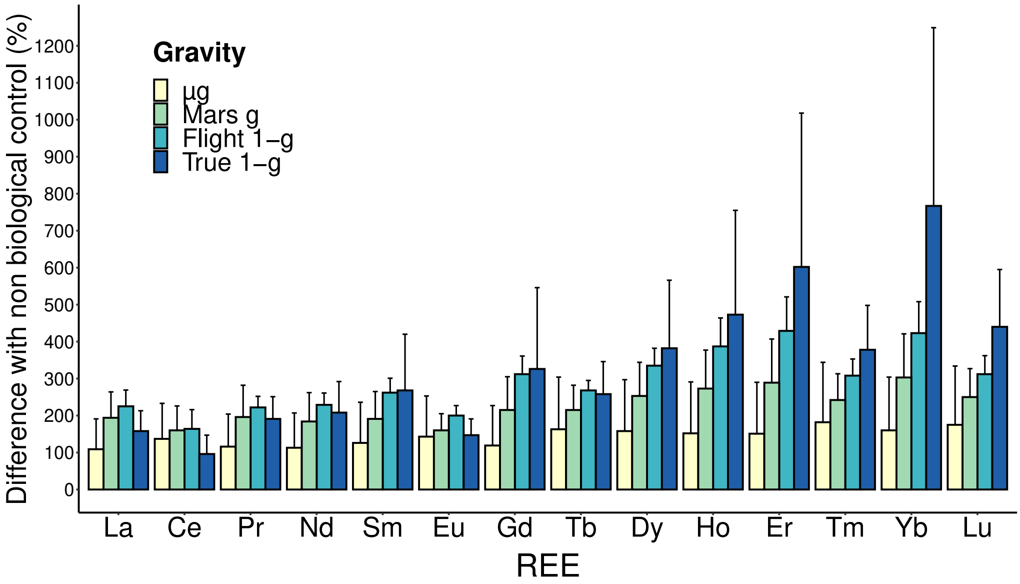

Fig. 2. Effects of *S. desiccabilis* on REEs bioleaching. Relative (percentage) difference in mean concentration of leached REEs in the bulk fluid between biological experiments and non-biological controls, showing microgravity (μg), simulated Mars gravity (Mars g), simulated Earth gravity (Flight 1-g) and real Earth gravity (True 1-g). Error bars represent standard deviations (n=3 for all samples, except for negative controls in μg and True 1-g, where n=2 due to sample contamination).

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