IMPACT SHOCK EFFECTS ON SULFATE-REDUCING BACTERIA IN MARINE SEDIMENTS.

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Introduction:

During the late heavy bombardment (LHB; ~3.9 Ga) the Earth received an impact influx with a delivered mass of 1.8 – 2.2 x 10^{20} kg [1,2]. Disagreement persists on the effect these impacts had on potential pre-existing microbial life [2–4]. Experimental work has demonstrated that sterilization could be avoided if impacts occurred in aqueous environments, as water-saturated impact surfaces are capable of dissipating heat and reestablishing habitable conditions post-impact more quickly than unsaturated surfaces [1]. However, many studies investigating the sterilizing effects of impacts tend to lack substantive constraints on microbial responses [1,3,4]. This work addresses these responses by attempting to provide pressure limits to survival of microbes in their native substrate.

Enriched microbes from marine sediments were subjected to impact shock pressures of ~10 GPa. Marine sediments were collected from Galveston Bay, Texas and aliquots were diluted with growth medium [5] designed to enrich sulfate-reducing bacteria. Sulfate-reducing bacteria (SRB) were selected for investigation because sulfate-reduction has been proposed as one of the earliest metabolic pathways to arise on Earth [6-8], and because spore formers have been identified within SRB taxa [9]. Previous work has demonstrated that spore forming bacteria are more capable of surviving impacts [4] than non-spore formers. Microbial responses to shock pressures were characterized by measuring sulfate reduction rates through ion chromatography, and will be further characterized by 16S gene amplicon sequencing of extracted DNA.

Methods:

Sulfate reducing bacteria were harvested from Galveston Bay, Texas marine sediments. Two sediment cores measuring ~32 inches and ~29 inches, respectively, were removed while submerged in ~24 inches of Galveston Bay seawater. Each core was laid onto an open sterile whirl-pack bag™, separated into thirds (top, middle, and bottom), where the top portion is closest to the sediment-water interface. Samples were stored in individual sterile whirl-pack bags or 50 mL centrifuge tubes. Water present in each core tube was collected in sterile centrifuge tubes (core-associated water). Sediment and core-associated water were stored at 4° C during transportation back to Johnson Space Center (NASA JSC). Three 30 g aliquots were taken from each core section and were supplemented with 60g sterile sulfate-reducing bacteria medium as described in [5]. A fourth set of aliquots was taken from each core section, sterilized in an autoclave at 121° C for 30 minutes and then supplemented with media to provide a sterile control. Cultures (unsterilized samples with medium) and controls were prepared anaerobically as described in [5] and stored in an anaerobic glove bag (by volume: 84.9% N_2; 10.1% H_2; and 5% CO_2) at 32°C. The remaining sediment was stored at -80° C and core-associated water samples were stored at -20° C.

Aliquots of cultures and controls were taken at time zero (the time of media supplementation), 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 168 hours, and weekly thereafter. Aliquots and core-associated water were analyzed for sulfate concentration using a Dionex ICS-2000 Ion Chromatograph. Standards were prepared from Dionex sulfate standard stock solution, as well as from dilutions of sterile medium. Sulfate-reduction rates from all core sections were analyzed to determine: 1) sections with the strongest SRB community; and 2) time of peak exponential growth.

Based on results from the first set of measurements, a fresh set of cultures and controls were prepared from the selected core sections (table 1) and incubated for 8 days before lyophilization. The samples were stored at -80°C until preparation for impact studies.

Samples were loaded into stainless-steel containers and then stored at 4°C for ~8 hrs. The containers were then mounted in the flat-plate accelerator at NASA JSC and impacted at 7.92 km/s (experiment sample) and 7.52 km/s (control; 9.26 and 8.73 GPa, respectively). [Future experiments will investigate higher shock pressures; 30 and 45 GPa. Pressures were chosen based on survival events recorded in cyanobacteria mounted in dry sandstone (10 GPa), bacterial spores in wet sandstone (30 GPa), and lichen and bacterial spores encased in dunnite (45 GPa)[4].] After impact, samples were collected and massed. A portion of the sample was saved for DNA analysis, and the remainder of the sample was supplemented with fresh media, incubated, and sampled at the same time intervals as the original aliquots. IC analyses were performed.

Table 1: Impact Experiment Sample Names

Sample Name	Treatment	Sample Purpose
Experimental Sample	Unsterilized Shocked	Test effect of shock pressure on microbial activity
Positive Control	Unsterilized Unshocked	Ensure that sample handling did not kill microbes & characterize microbial changes to sulfate under normal-lab conditions
Negative Control	Sterilized Shocked	Characterize abiotic changes due to impact
Process Control	Sterilized Unshocked	Ensure that sample handling did not introduce new microbes

Results:

Initial incubations of sediment SRB were found to reach exponential growth after 8 days. New incubations were prepared and subjected to a flat-plate shock pressure of ~10 GPa, the results of which are depicted in table 2 and figure 1.

Discussion

The impacted samples, both sterilized (negative control) and unsterilized (experiment sample), showed increases in sulfate concentration within 48 hours of incubation. The negative control reached peak sulfate concentration after 12 hours, and the experiment sample after 48 hours. This initial increase in sulfate concentration in both samples suggests an abiotic process (e.g. desorption) may be responsible. The 36-hour delay

Sulfate Concentration (mg/L)				
Time (Hrs)	Positive Control	Experiment Sample	Process Control	Negative Control
0	3903.8	3888.2	3842.1	3859.6
6	3744.0	3757.3	4035.3	3882.5
12	4218.0	3848.0	4121.1	4185.8
24	4093.4	3928.5	4148.2	4022.8
48	3463.6	4110.4	4082.8	3566.6
72	3927.5	3801.9	3534.6	3659.7
336	3841.7	3976.5	3914.3	3739.0

Table 2: Sulfate concentration (mg/L) change over time in samples containing Galveston Bay, Texas sediment, growth medium, and 1) unsterilized active native microbial communities or 2) sterilized prior to incubation. Samples were either kept at lab conditions (positive and process controls) or subjected to impact with a flyer-plate at a pressure of ~10 GPa (experiment sample and negative control).

in peak-concentration times may be due to microbial sulfate-reduction offsetting the sulfate desorption in the experiment sample. The delay in peak concentration may also be a result of sulfate interaction with shot-lysed cells or pre-shot sulfate-reduction metabolic products (e.g. sulfite, elemental sulfur) freed from shot-lysed cells. After peak concentration was achieved, both samples show a similar trend; sharp sulfate concentration decreases followed by gradual sulfate concentration increases. The presence of these trends in both the experiment sample and the negative control indicates an abiotic process, such as absorbance of sulfate back into the remaining sample sediment and desorption into the sample liquid-phase.

The process control increased in sulfate concentration initially, while the positive control sample initially decreased in sulfate concentration, which was followed by an increase in sulfate concentration to greater levels than the process control. The immediate increase in sulfate concentration in the process control suggests desorption of sulfate from sediment sulfate into the medium/water-liquid phase. The initial decrease in sulfate concentration in the positive control may be due to microbial sulfate reduction, but this would require initial microbial sulfate-reduction rates to overcome rates of sulfate desorption. Then, either sulfate desorption rates increase, overwhelming microbial sulfate reduction, or microbial sulfate reduction rates decrease, falling beneath desorption rates. This would then have to be followed by microbial sulfate reduction returning to a rate greater than sulfate desorption.

After reaching peak sulfate concentrations, the sulfate levels in both the positive and process controls decreased in concentration. The trend of sulfate-concentration increasing and then sharply decreasing, in all samples, suggests an abiotic mechanism. The positive control's increase in sulfate-concentration after 48 hours may be due to microbial activity (e.g. sulfate oxidation), but the observation of similar trends in the sterilized controls suggests otherwise.

Future work will include characterization of sulfate-concentration from sample aliquots retrieved prior to impact, providing a comparison between pre- and post-impact microbial sulfate reduction. Culture aliquots taken pre- and post-impact will also be characterized through 16S gene amplicon sequencing.

Conclusion

Sediment cores were retrieved with indigenous microbial communities from Galveston Bay, Texas. These were prepared and provided medium to select for sulfate-reducing bacteria. Bacterial growth was characterized by measuring aqueous sulfate concentration with an Ion Chromatograph. Core segments with the greatest decreases in initial sulfate concentration were identified, and duplicate incubations were subjected to shot impact with a flat-plate accelerator at 8.76-9.23 GPa. Sulfate-concentration changes in experimental samples and all controls were similar, with varied lag-times. It is unclear whether the tested microbes survived the sample handling and impact process. Additional experiments will attempt to clarify the issue.

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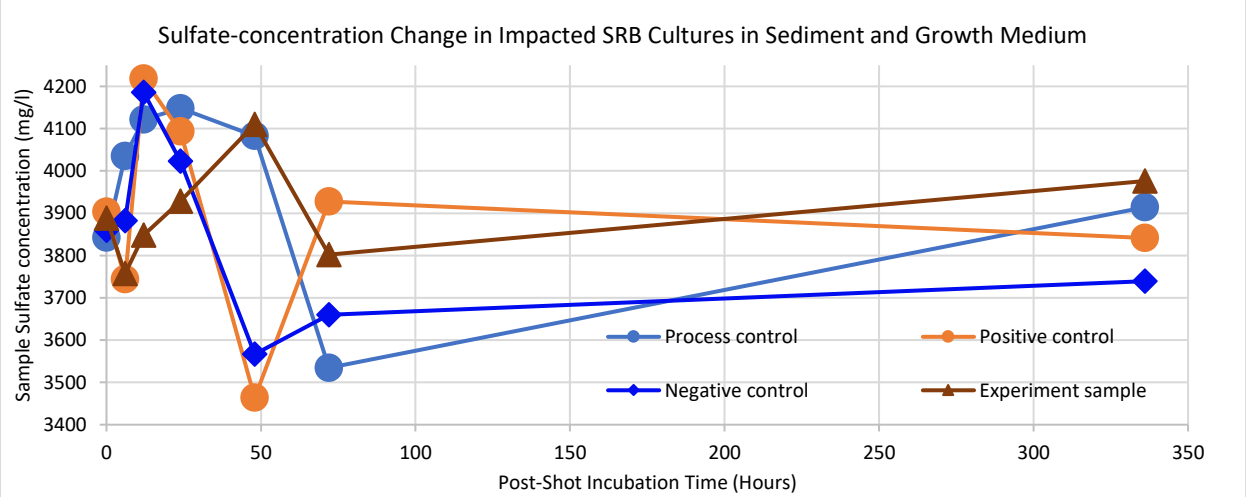

Figure 1: Sulfate concentration (mg/L) change over time in samples containing Galveston Bay, Texas sediment, growth medium, and unsterilized active native microbial communities or sterilized prior to incubation. Samples were either kept at lab conditions (positive and process controls) or subjected to impact with a flyer-plate at a pressure of ~10 GPa (experiment sample and negative control).