Effects of Elevated Pressure on Microbial Growth: Using a Model Extremophile to Explore Habitability in Subsurface Ocean Worlds

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Motivation

Extremophiles that thrive in deep-sea hydrothermal vents on Earth serve as analogues for exploring habitability in subsurface ocean worlds throughout the Solar System[1]. All deep-sea vent environments on Earth and all proposed hydrothermal systems on subsurface ocean worlds, like Europa[2] (Figure 1) or Enceladus[3], experience elevated pressure conditions. Here, *Archaeoglobus fulgidus*, a model extremophile was employed to investigate how elevated pressures and subsampling decompression effects microbial growth. *A. fulgidus* is a hyperthermophilic sulfate reducing archaeon that has been found in various shallow[4] and deep-sea hydrothermal vents[4] and has reportedly been able to grow heterotrophically[4] and autotrophically[4]. Exploring the effects of pressure on growth of model extremophiles like *A. fulgidus* is an essential component of understanding habitability of Europa, Enceladus, and other subsurface ocean worlds.

Key questions:

- What pressures can *A. fulgidus* handle for heterotrophic growth (on lactate and sulfate)?
- Does subsampling decompression effect *A. fulgidus* growth?
- Can *A. fulgidus* grow autotrophically (H_2/CO_2+ thiosulfate) at pressure?

Habitability at depth on Europa

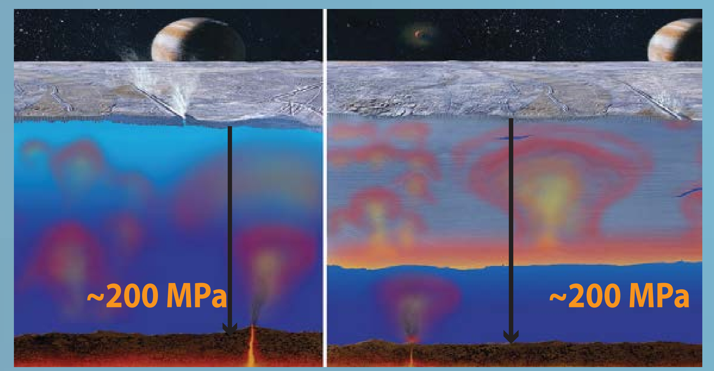


Figure 1: Two proposed cross section profiles of Europa's ocean, all potentially habitable deep-sea environments are at ~200 MPa[2].

Experimental Design

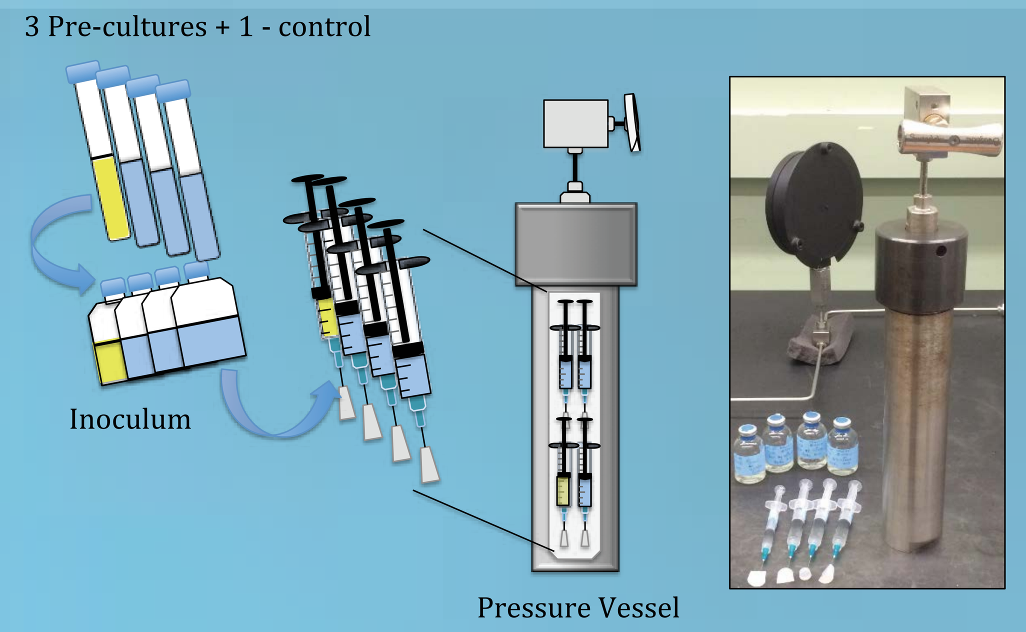


Figure 2: High-pressure batch culture procedure in 5 mL plastic syringes.

Batch cultivation (Figure 2) of *A. fulgidus* was performed at optimum temperature (83°C) for pressures up to 80 MPa. Growth rates and cellular yields were determined from DAPI-stained subsamples for triplicate experiments. Subsampling decompression[5] (and cooling for thermophiles) effects on growth was tested (Figure 3), in four pressure vessels, where cell densities for cultures decompressed multiple times were compared to those that were decompressed once. *A. fulgidus* cultures imaged on a scanning electron microscopy (SEM) were prepared under anaerobic conditions[6]. Sulfide concentrations were tested by the methylene blue method[7].

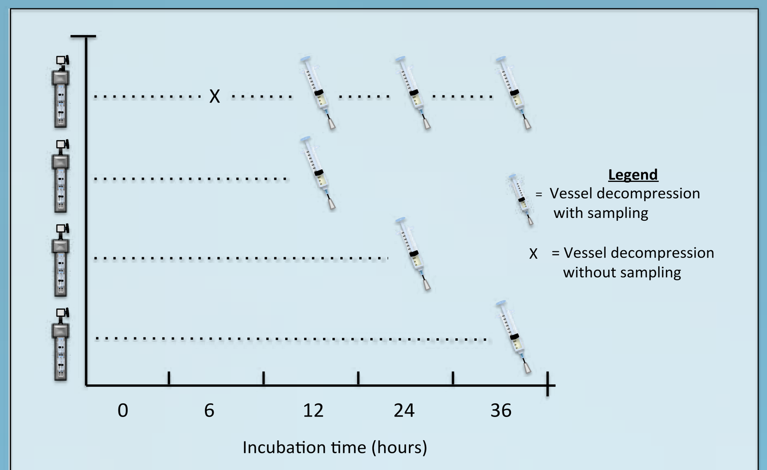
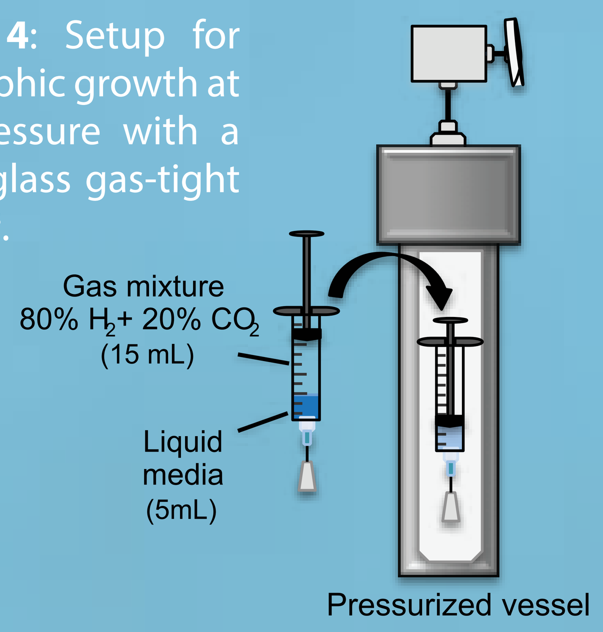


Figure 3: Procedure to test cellular growth after multiple subsampling decompressions.

A. fulgidus was adapted to grow on a H_2/CO_2 + thiosulfate metabolism from a lactate and sulfate metabolism. After adaptation, high-pressure growth was attempted[8] (Figure 4).

Figure 4: Setup for autotrophic growth at high-pressure with a 25 mL glass gas-tight syringe[8].



Results

A. fulgidus growth at elevated pressures

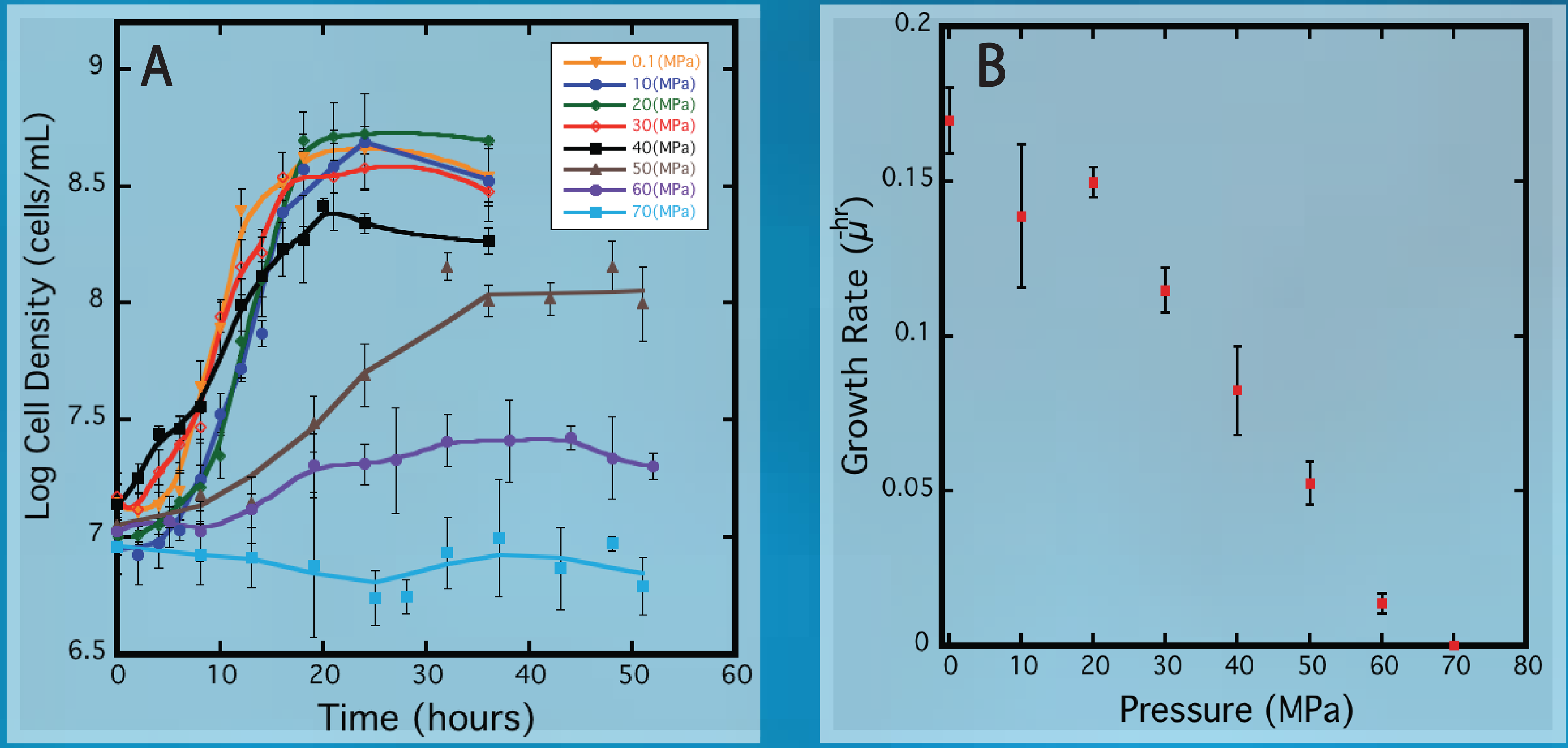


Figure 5: High-pressure growth of *A. fulgidus* from 0.1-70 MPa. (A) Exponential growth curves and (B) growth rates (μ) as a function of pressure.

A. fulgidus growth on H_2/CO_2

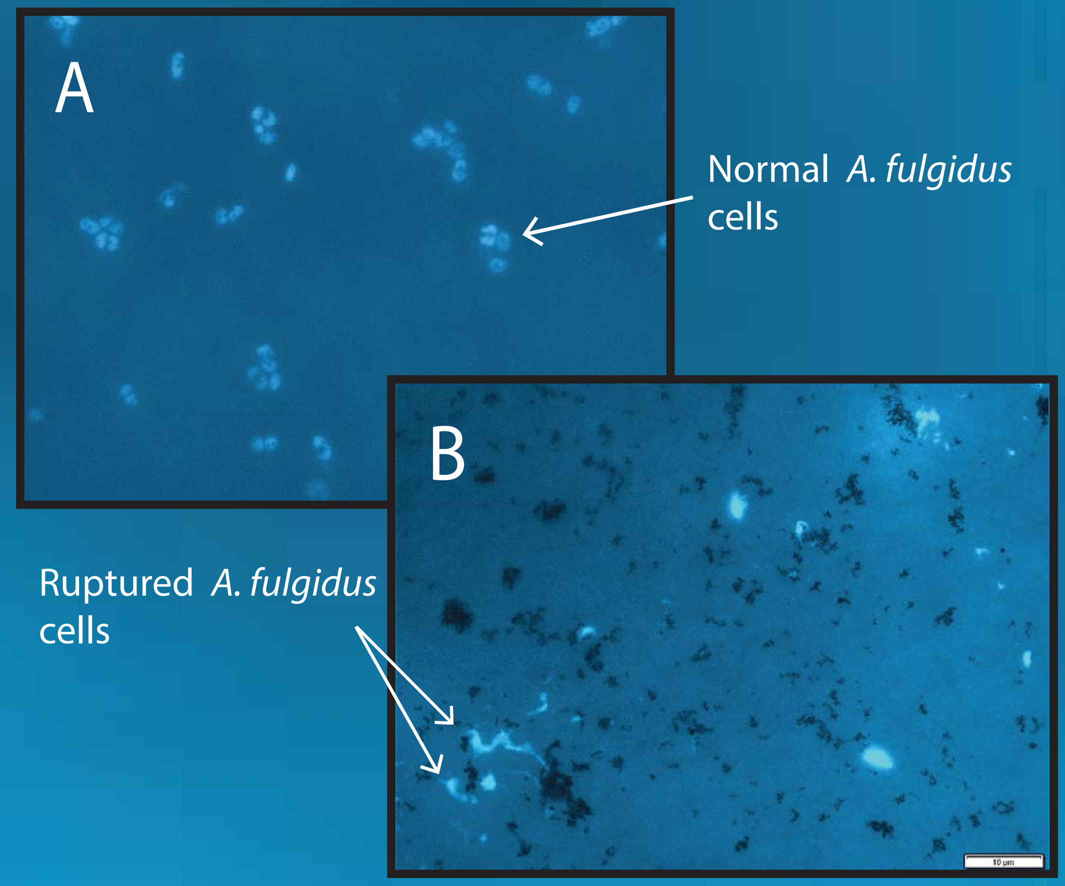
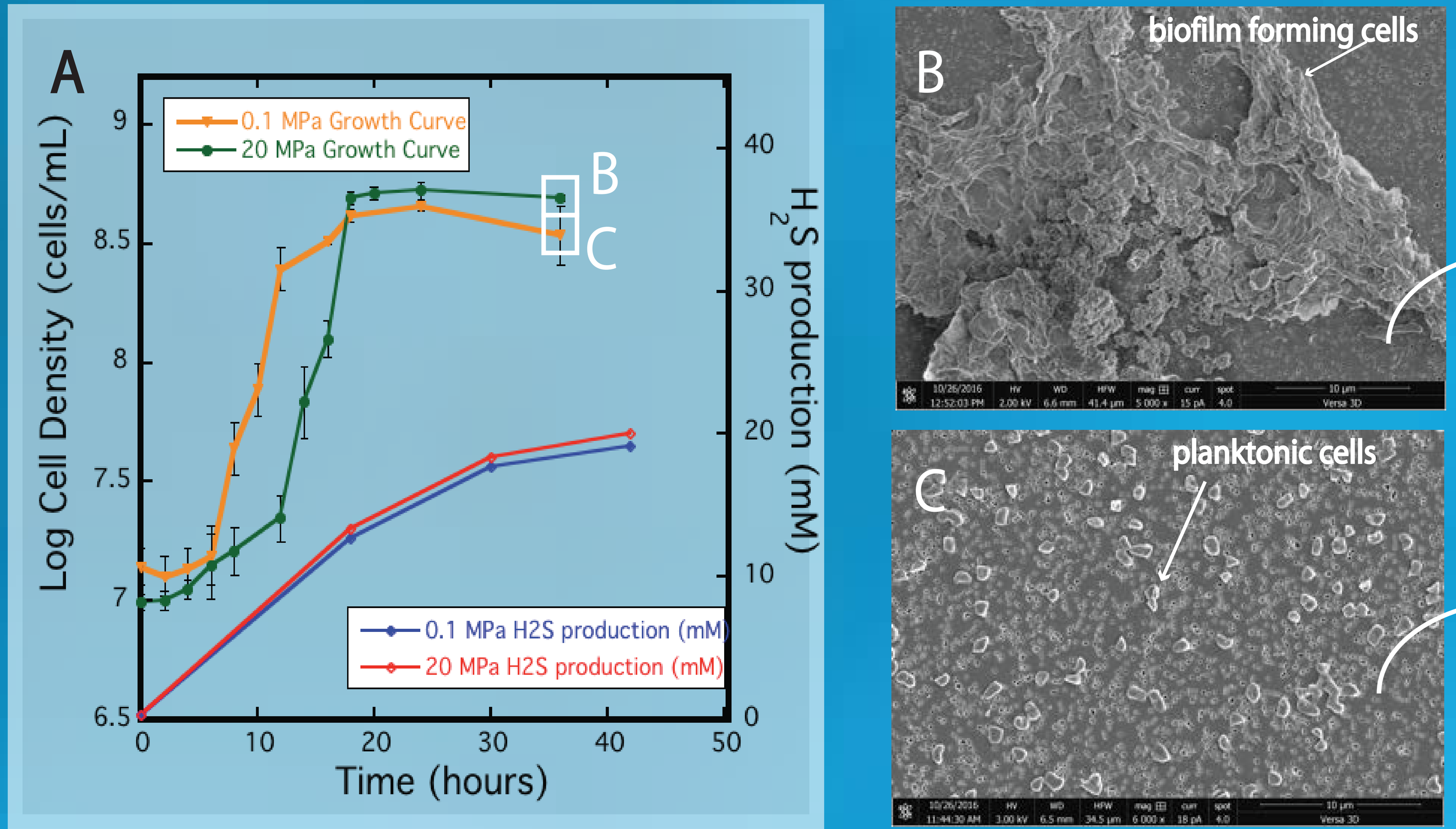


Figure 7: DAPI stained *A. fulgidus* cells grown at 0.1 MPa in a serum bottle (A) and at 20 MPa in a syringe (B, Figure 2). Bar 5 μm.

Biofilm production of A. fulgidus



What causes biofilm production?

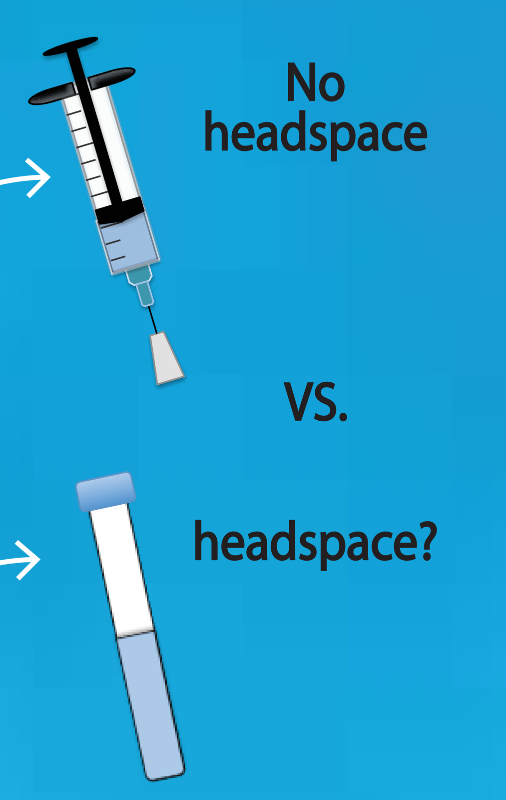


Figure 6: (A) Growth curves and measured sulfide concentrations for *A. fulgidus* cells grown at 0.1 MPa and 20 MPa. (B &C) SEM images of biofilm forming cells (B) grown at 20 MPa and planktonic cells (C) grown at 0.1 MPa. Bar 10 μm.

Decompression tests

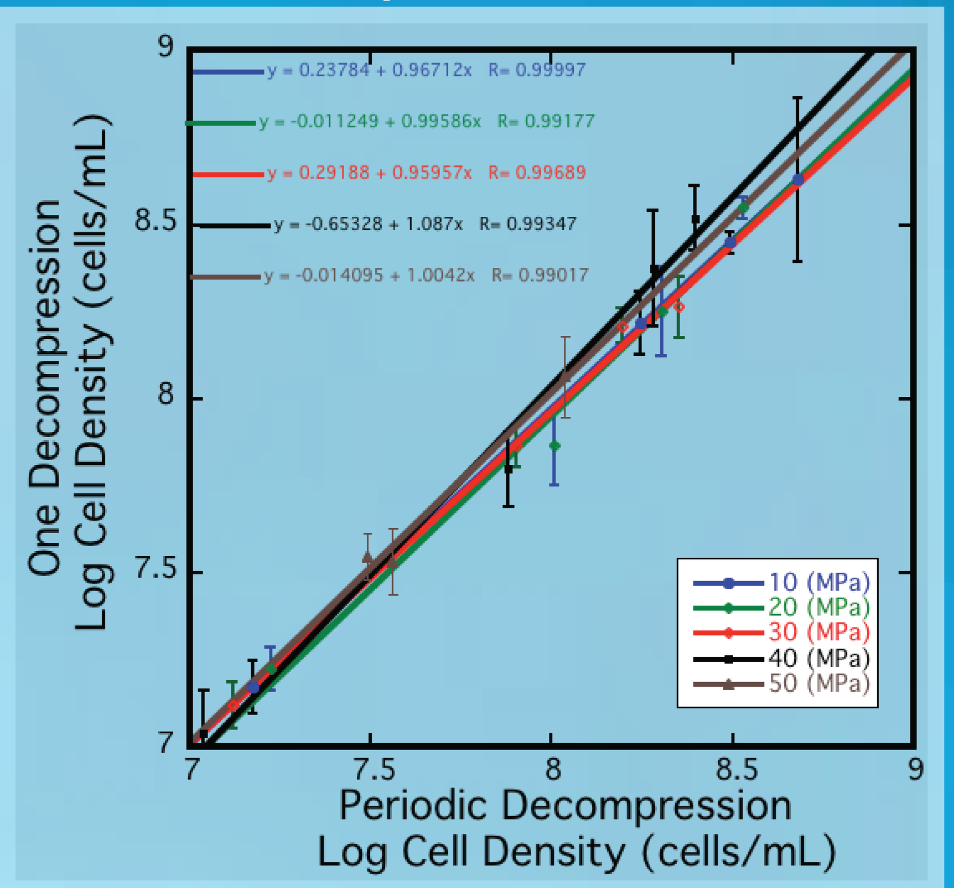


Figure 8: Cell density ratios of cells decompressed once vs. cells decompressed periodically, for *A. fulgidus*, grown from 10-50 MPa.

Discussion

A. fulgidus growth at pressure

A. fulgidus successfully grew at pressures up to 60 MPa (~6000 meters below sea level depths) after multiple decompressions during heterotrophic growth. Biofilm production was perhaps in reaction to toxic concentrations of dissolved sulfide. *A. fulgidus* biofilm production, a common stress response[9], may be an advantageous adaptive strategy and potentially serves as a useful biomarker. Ruptured cells observed after sampling high-pressure growth suggest that *A. fulgidus* might be sensitive to any sample decompression.

Habitability at high-pressure

These results have important implications for future life detection missions to subsurface ocean worlds such as Europa and Enceladus where seafloor pressures are <10 MPa on Enceladus and 100-200 MPa on Europa. Putative high-pressure hydrothermal systems on those moons could realistically support metabolisms similar to those explored here, which depend on CO_2, sulfate, and simple organic compounds (e.g. lactate).

References

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Acknowledgements

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