

POSSIBILITY OF LIFE ON ENCELADUS. S. Parashar¹ and T. A. Kral², ¹University of Pennsylvania, Philadelphia, Pennsylvania, parso@sas.upenn.edu ²Department of Biological Sciences, University of Arkansas, Fayetteville, AR, tkral@uark.edu

Introduction: In 2004, NASA launched the spacecraft Cassini with the purpose of exploring the Saturn system. With an initial lifespan of about four years, the mission, renamed the Cassini Equinox Mission, has been extended until September 2010 with the purpose of answering some of the initial questions that were raised during the first four years of the mission. One of the questions concerns the small, icy moon of Saturn, Enceladus.

In 1789, William Herschel discovered the sixth largest moon of Saturn; however, it was not until the Voyager mission of the 1980s that any significant amount of data was known about the moon. Before the mission it was already known that there was a significant amount of water ice on the surface. Among many other characteristics of the moon, the mission flyby revealed that the moon was approximately 500 km in diameter and reflects almost 100% of the light that strikes its surface. The surface of Enceladus has an average temperature of 72 K and has a variety of different terrains. Despite its small size, Enceladus is very geologically active, particularly in the South Pole[1].

In 2005, Cassini witnessed a plume erupt from the “tiger stripe” region of the moon. Using the instruments on board the spacecraft, the plume consisted of largely water vapor, with small amounts of organic molecules too. One molecule of particular interest is methane, whose origin could possibly be biological in nature. The area in which the plume erupted is known to be uncharacteristically warmer compared to the rest of the moon; these hot spots are kept warm due to tidal heating. These hot spots could also give rise to subsurface liquid water aquifers. The hypothetical liquid aquifers could possibly have a number of salts dissolved in it, which would lower the freezing point even further making the chance of liquid water even more plausible[2].

Methods: The methanogens were grown in MS media that contained the appropriate amounts of ammonium chloride and sodium chloride. The media, along with a designated amount of test tubes, were then placed inside an anaerobic chamber and allowed to acclimate to the deoxygenated environment. In each test tube, approximately 10 milliliters of medium were transferred into each tube. After completion of the transfer, the tubes were capped with stoppers, crimped, taken out of the chamber and subsequently autoclaved for about 45 minutes. After sterilizing the tubes, they were shaken and allowed to cool. Once the cooling

process was completed (which normally lasted 24 hours), approximately 12 drops of 2.5 % Na₂S were added to the medium to rid any trace amount of oxygen left in the tubes. Finally 10 drops of either *M.wolfeii* or *M.maripaludis* were inoculated into the test tubes. Since these methanogens use hydrogen as their energy source, all tubes were pressurized with hydrogen and then allowed to grow. *M.wolfeii* is a known thermophile and was kept at an elevated temperature of 55 C, whereas *M.maripaludis* was kept at room temperature[3]. All test tubes were maintained in a horizontal fashion in order to increase the surface area of exposed medium. Methane levels were then recorded by way of a gas chromatograph ever 3 days for 2 weeks and then once a week after the second week.

Results: The purpose of these experiment was to determine the ammonium chloride level would allow for methane production by *M.wolfeii* and *M.maripaludis*. The following graphs show the results of this first experiment:

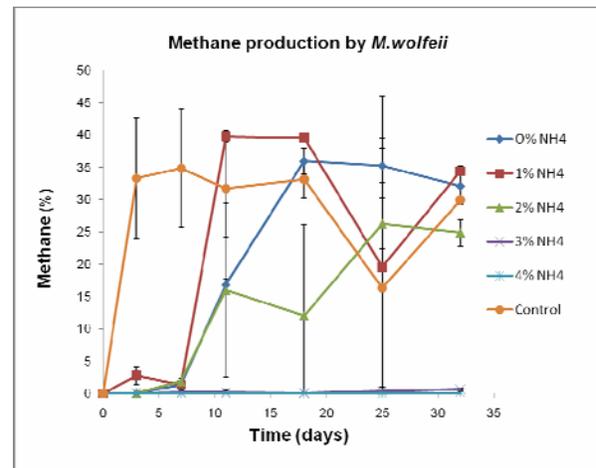


Figure 1: This graph shows that *M.wolfeii* grows at 0%, 1%, and 2% concentrations of ammonium chloride. Over time, the methane production does decrease due to the decreasing amount of hydrogen present to produce methane.

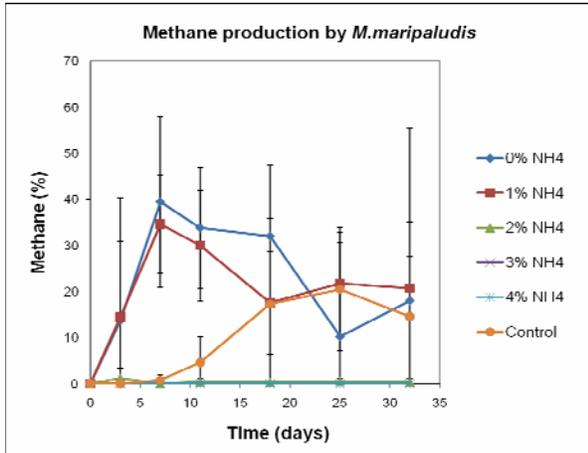


Figure 2: *M.maripaludis* grows well at 0% and 1% ammonium chloride. Due to its halophilic nature, *M.maripaludis* took about 1 week to grow in the control because the medium contains only 0.10% ammonium chloride.

A second experiment was conducted in which *M.wolfeii* from the 2% ammonium chloride tubes was inoculated into 3% and 4% ammonium chloride concentrated test tubes. Samples from the 1% and 2% *M.maripaludis* were also inoculated into 3% and 4% ammonium chloride concentrations. The next two graphs show the data from the second experiment.

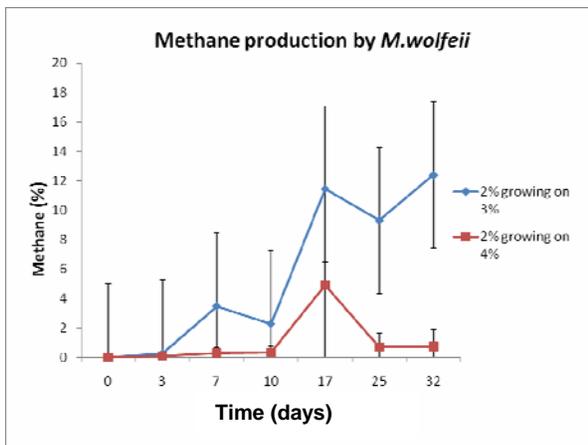


Figure 3: The 2% *M.wolfeii* was able to adapt to the 3% and 4% ammonium chloride concentrations.

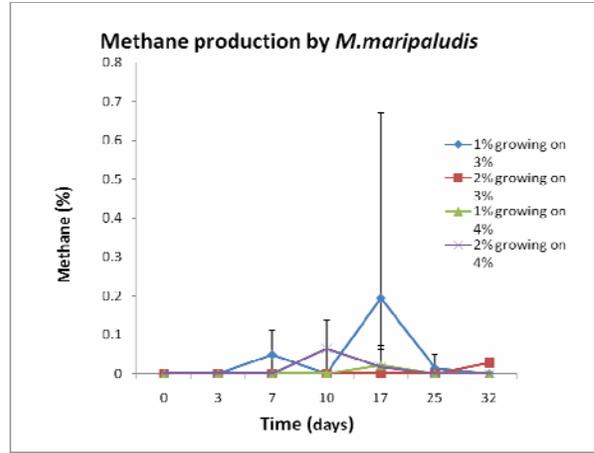


Figure 4: *M.maripaludis* was not very successful in growing at higher concentration of ammonium chloride.

A third experiment was conducted in which the 4% *M.wolfeii* was inoculated into 5% and 6% ammonium chloride concentrations. At this time, there has been no methane production.

Conclusion: From the experiments, *M.wolfeii* grew at 4% ammonium chloride, while *M.maripaludis* grew on 3% ammonium chloride. At this time, 4% ammonium chloride and 3% ammonium chloride for *M.wolfeii* and *M.maripaludis* seem to be the highest concentration level that these methanogens can grow on successfully. Since *M.maripaludis* did not grow at higher concentrations of ammonium chloride, the hypothesis can be partially rejected.

Bibliography:

- [1] <http://saturn.nasa.gov/science/moons/enceladus/>
- [2] Christopher P. McKay et. al. (2008) *Astrobiology VIII*, 909-919.
- [3] Ricardo Cavicchioli. (2007) *Archae: Molecular and Cellular Biology*, 289.