SENSITIVITY OF DESICCATED AND LIQUID CULTURES OF METHANOGENS TO ULTRAVIOLET RADIATION Navita Sinha[1] and Timothy A. Kral[1, 2]. [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR 72701, [2]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701. nxs017@uark.edu.

Introduction: After the detection of methane in the atmosphere of Mars [1, 2], methanogens have been considered models for putative life on this red planet [3]. They are anaerobic chemoautotrophs, and belong to the domain Archaea. They consume carbon dioxide and molecular hydrogen, and produce methane as a byproduct. They can also be found in extreme places such as permafrost and desert soils. Since Mars is constantly bombarded with UV radiation, mostly between 190 to 300 nm [5], any life forms at or near the surface would have to adapt to radiation over time.

DNA absorbs maximum UV light at 260 nm, which causes the formation of pyrimidine dimers. Massive numbers of dimers in the DNA of a cell can result in serious mutations and/or cell death.

The goal of our study is to determine the sensitivity of desiccated and liquid cultures of some methanogens to UV radiation in an anaerobic condition.

Methods and Materials: The four methanogens tested were *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum*, and *Methanococcus maripaludis*. To make desiccated cells for UV exposure, about 1 ml of each culture was centrifuged. Cell pellets were then placed into the wells of a plate using a syringe. This plate was then placed into a desiccator in a Coy anaerobic chamber. The twenty-four wells of a plate containing desiccated cells were exposed to a UV lamp (254 nm/4 watts) positioned at 10 cm above the samples. They were exposed for different lengths of time (0, 5, 10, and 20 minutes). After exposure they were suspended in anaerobic sterile growth media, pressurized with hydrogen gas and incubated at their respective temperatures.

For liquid culture UV exposure, the liquid cultures of each methanogen were placed on a plate, which had twenty-four wells. They were irradiated with the same UV lamp as mentioned above for varying lengths of time (0, 5, 10, 15, 20, 30, and 60 min) in an anaerobic chamber. The UV lamp was positioned at 10 cm above these samples. After the exposure for specified times they were placed back into their respective growth media and pressurized with hydrogen gas and incubated.

The survivability of these methanogens under both conditions was measured by methane production. Headspace gas of these sample tubes was measured by gas chromatography at different time intervals.

Results: Methane production by *M. wolfeii* and *M. barkeri* (as examples) under these two conditions are shown in Figures 1-4. For desiccated cells following UV exposure, all methanogens demonstrated methane production except *M. formicicum*. *M. barkeri* showed a small percentage of methane production initially but later it showed significant methane production.

For liquid culture UV exposure, three out of the four methanogens demonstrated increasing methane within the first eighteen days while one methanogen showed far more variability in methane production. *M. formicicum* showed little or no methane initially.

Discussion: A couple of factors need to be discussed. First, for liquid culture UV light exposure, UV may not have penetrated all the way through the liquid and the inner cells may not have received UV radiation. Only upper cells were exposed to UV and have protected inner cells from this non-ionizing radiation. However, we have used a thin layer of liquid culture in order to expose them to UV completely. Second, higher numbers of cells are clustering together and providing protection from UV radiation in both conditions.

Previous studies have shown that these methanogens demonstrated significant methane production in anaerobic desiccated conditions [4].

Conclusion: At this point, we can conclude that methanogens can tolerate UV radiation for a few minutes under both desiccated and liquid culture conditions.

Longer UV exposure time (few hours) experiments are in progress, which can provide crucial information about maximum UV exposure survival time of these methanogens.

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[5] Mancinelli and Klovstad (2000) *Planetary and Space Science,* 48, 1093-1097. [6] McAllister S. A. and T. A. Kral (2006) *Astrobiology*, 6 (6).

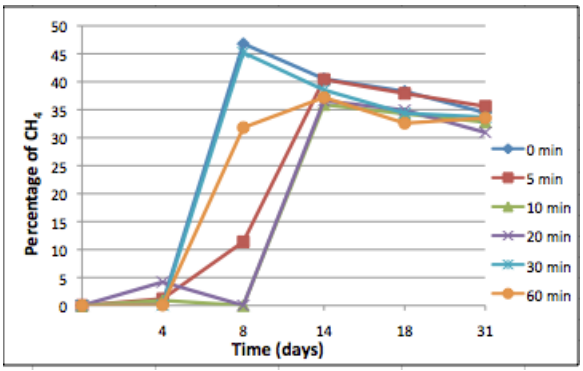

Figure1. Methane production by liquid culture of *Methanothermobacter wolfeii* following various times of exposure to ultraviolet radiation under anaerobic conditions.

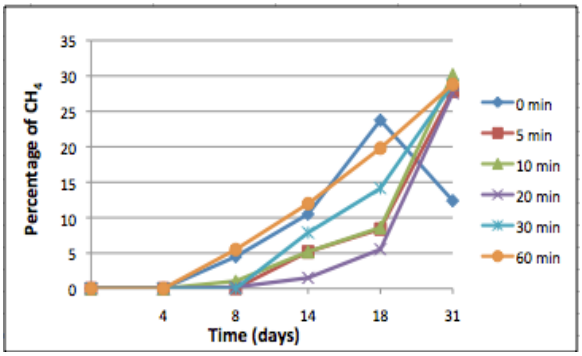

Figure3. Methane production by liquid culture of *Methanosarcina barkeri* following various times of exposure to ultraviolet radiation under anaerobic conditions.

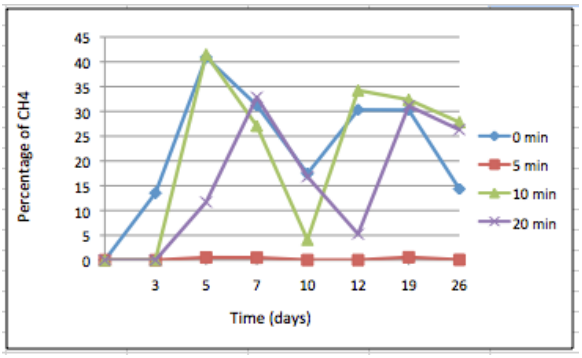

Figure2. Methane production by desiccated *Methanothermobacter wolfeii* following various times of exposure to ultraviolet radiation under anaerobic conditions.

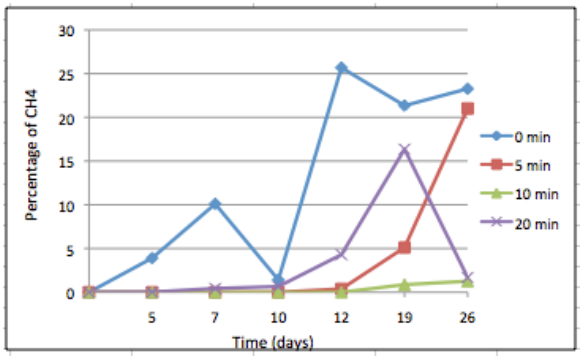

Figure4. Methane production by desiccated *Methanosarcina barkeri* following various times of exposure to ultraviolet radiation under anaerobic conditions.