5-2024

Growth Of Methanogens on Kaolinite, A Clay That Has Been Identified on Mars

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Growth Of Methanogens on Kaolinite, A Clay That Has Been Identified on Mars

An Honors Thesis submitted in partial fulfillment of the requirements for Honors Studies in Biology

By

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Spring 2024

Biology

Fulbright College of Arts and Sciences

The University of Arkansas

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I. Abstract

Methanogens have been studied as a model for life on Mars for 28 years now in the Kral lab. The discovery of methane in the Martian atmosphere by ground-based and orbital observations as well as Curiosity Rover (Formisano, V. et al., Krasnopolsky, V.A. et al., Mumma, M.J. et al.) has added relevance to these types of studies. Methanogens were chosen due to their ability to live in harsh environments, very similar to the Martian terrain. In addition to methane in the atmosphere, phyllosilicate clays have also been identified. One of those clays is kaolinite. Kaolinite has been found to not be toxic to methanogens. In the research reported here, the methanogens were placed in a bicarbonate buffer containing kaolinite along with molecular hydrogen as an energy source and carbon dioxide as a carbon source, to determine if the kaolinite could support the growth of the methanogens.

The methanogens tested were *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanococcus maripaludis*. Organisms were inoculated into their respective media followed by incubation at each organism's growth temperature. Following two weeks of growth, cells from each culture were centrifuged and washed with sterile buffer three times. Cell pellets were suspended in sterile buffer, then added to anaerobic tubes containing sterile kaolinite (0.5g per tube). Sterile buffer was added to each tube to reach a final volume of 5 mL. Each tube was pressurized with 2 atm of molecular hydrogen followed by incubation at each organism's ideal growth temperature. Following six weeks of incubation, 0.5 mL of each culture was transferred to a fresh, sterile tube containing 0.5g kaolinite. Again, volumes were increased to 5 mL with sterile buffer. The purpose of the transfers was to dilute out any residual nutrients from the original stock cultures. Methane production, commonly used

to measure methanogen growth, was measured by gas chromatography of headspace samples at regular time intervals.

Three of the four methanogens tested, *M. wolfeii*, *M. formicicum*, and *M. barkeri*, showed measurable methane following incubation. The amounts were far less than found in the control tubes which contained growth media, which is expected. *M. maripaludis* did not show any methane production, most likely because it is a halophile, and no salts were added. The methane production eventually tapered off, and the methanogens were unable to sustain methane production. It is unclear if these microorganisms could survive an extended period of time using kaolinite as a main substrate.

II. Introduction

There have been several possible biosignatures detected on Mars, and one such biosignature is methane (CH_4). CH_4 is a common greenhouse gas found here on Earth, and most is of biogenic origin (Yung, Y. L. et al.), which alludes to the idea that Mars (and other worlds with detectable CH_4) could potentially harbor life. Mars also appears to have a regular cycling of CH_4 that is not currently understood given current knowledge of geochemical processes that occur on the Red Planet (Yung, Y. L. et al.). Methanogens are the only known living organisms that produce CH_4 as a byproduct of their metabolism. Hence, the focus of this project is the growth of certain methanogen species.

Every living organism on Earth requires water to live. The hunt for substantial water on Mars has been confined to subsurface exploration. There is no significant amount of liquid water on the surface of Mars due to its atmospheric pressure being much lower than what is required of liquid water. There are several indications, however, of liquid water that persisted in its past for

significant amounts of time on the Martian surface, evidenced heavily by a potential ancient streambed in Gale Crater (Nazari-Sharabian, M. et al.). Phyllosilicates are a group of clay minerals that play a crucial role when deciphering certain aqueous processes. Kaolinite is one such phyllosilicate and has been previously identified on Mars (Cuadros, J., et al.). Current data on methanogen metabolism and substrate preference is often incomplete (Jabłoński, S., et al.). Given that water appears to be a requirement for life and is necessary for the formation of phyllosilicates, kaolinite could be a potential substrate for the growth and survivability of methanogens on Mars.

If kaolinite can be shown to support the growth of methanogens, further investigations could be focused towards searching for extant or extinct life in these potentially desirable places. The NASEM Decadal Survey for Planetary Science and Astrobiology 2023 – 2032 has identified 12 priority questions under three significant themes: origins, worlds and processes, and life and habitability. These questions and current understandings were curated and selected by several panels and hundreds of scientists to help maximize the advancement of space sciences for the next decade. This paper focuses on the work towards answering such questions from the decadal survey: Where are or were the solar system's past or present habitable environments? Is or was there life elsewhere in the solar system?

The research reported here was designed to determine if selected methanogens could grow (produce methane) on kaolinite along with water, carbon, dioxide, molecular hydrogen and sodium sulfide.

III. Methods and Materials

Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum and Methanococcus maripaludis were inoculated into their respective media and grown at each of their growth temperatures. Once the organisms underwent two weeks of growth, cells of each culture were centrifuged and washed with sterile buffer to remove residual nutrients from the media. Each methanogen was then placed into three anaerobic tubes that contained 0.5g of sterile kaolinite and 5mL of sterile bicarbonate buffer (sterilized using an autoclave). The bicarbonate buffer, the buffer used to make the growth media, was prepared by dissolving 4 g of sodium hydroxide in 1 liter of deionized water. This buffer was then saturated with carbon dioxide using a gassing manifold. The buffer serves as the carbon source for the methanogens. Prior to inoculation, 0.10 mL of a sterile sodium sulfide solution (2.5%) was added to each tube to remove residual molecular oxygen. Methanogens are strict anaerobes and will not grow in its presence. These tubes were also pressurized with hydrogen gas (200 kpa) to provide an energy source (Kendrick, M. G., et al.).

All cultures were incubated at growth temperatures for the individual organisms, 55^{o}C for *M. wolfeii*, 37^{o}C for *M. barkeri* and *M. formicicum*, and room temperature (approximately 22^{o}C) for *M. maripaludis*. Headspace gas samples were injected into a gas chromatograph (Varian model 4900 MicroGC) for methane analysis every two weeks. Methane production is a proxy for methanogen growth. Aliquots (0.5 ml) of cultures demonstrating methane production after six weeks were transferred to identical tubes containing sterile kaolinite in buffer. This transfer procedure was repeated whenever methane production occurred following six weeks of incubation.

IV. Results and Discussion

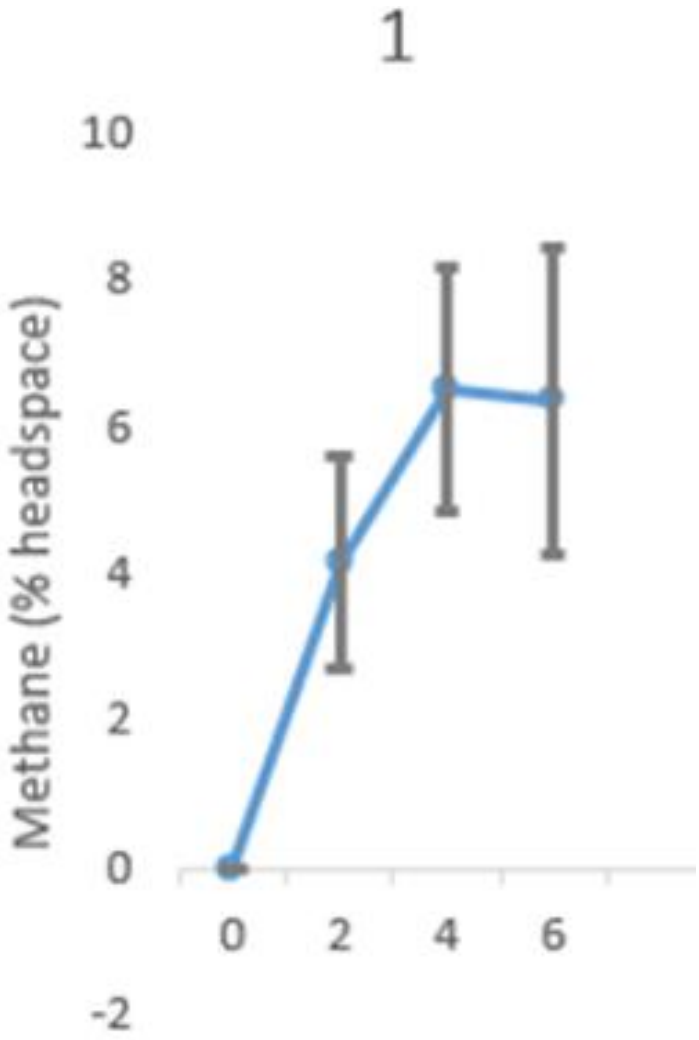

Figure 1. Methane production by *Methanobacterium formicicum* on kaolinite following transfer of cells from a stock culture.

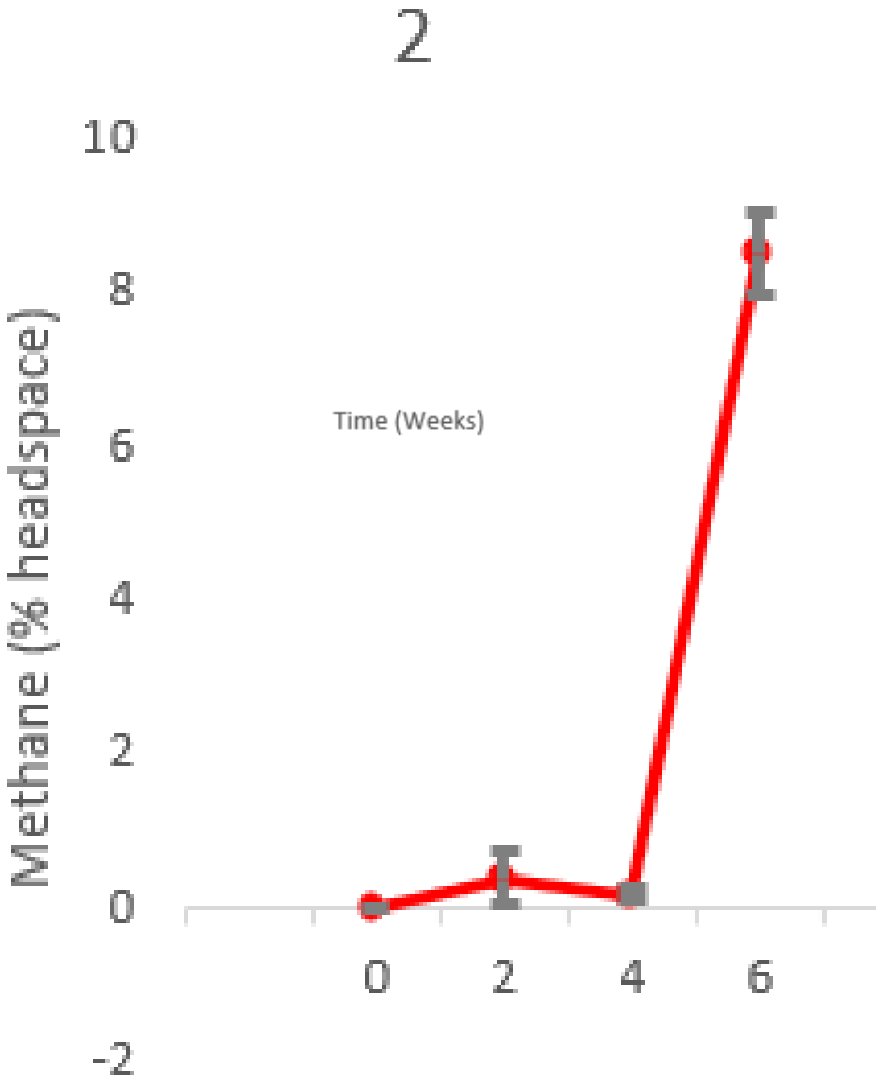

Figure 2. Methane production by *Methanobacterium formicicum* on kaolinite following transfer of cells from first-transfer cultures.

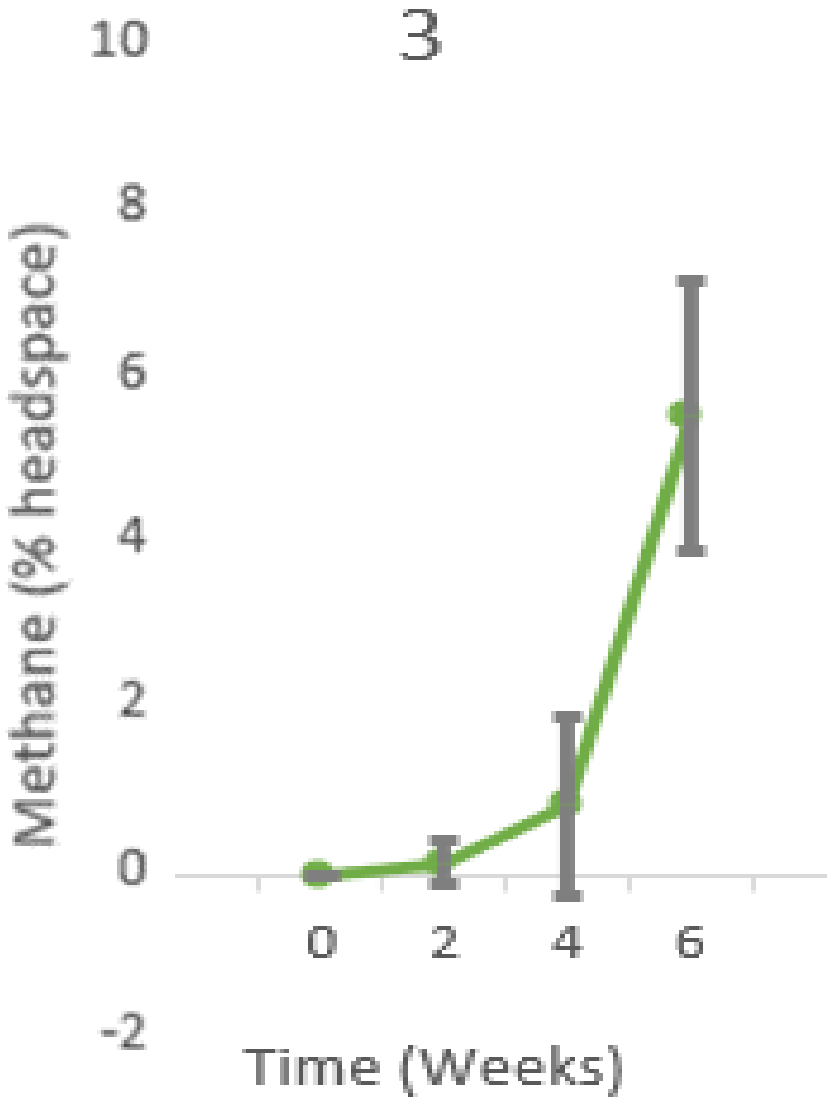

Figure 3. Methane production by *Methanobacterium formicicum* on kaolinite following transfer of cells from second-transfer cultures.

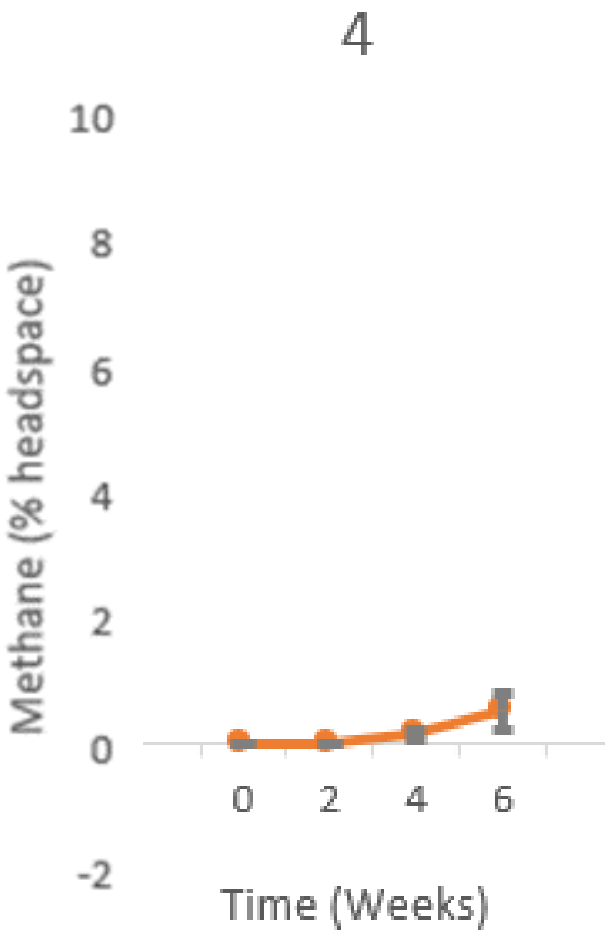

Figure 4. Methane production by *Methanobacterium formicicum* on kaolinite following transfer of cells from third-transfer cultures.

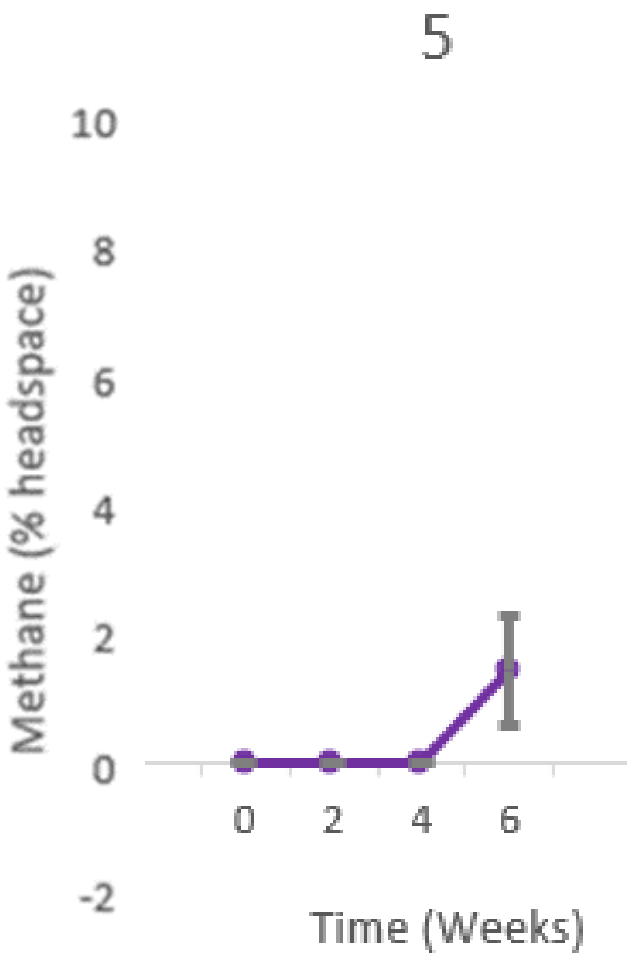

Figure 5. Methane production by *Methanobacterium formicicum* on kaolinite following transfer of cells from fourth-transfer cultures.

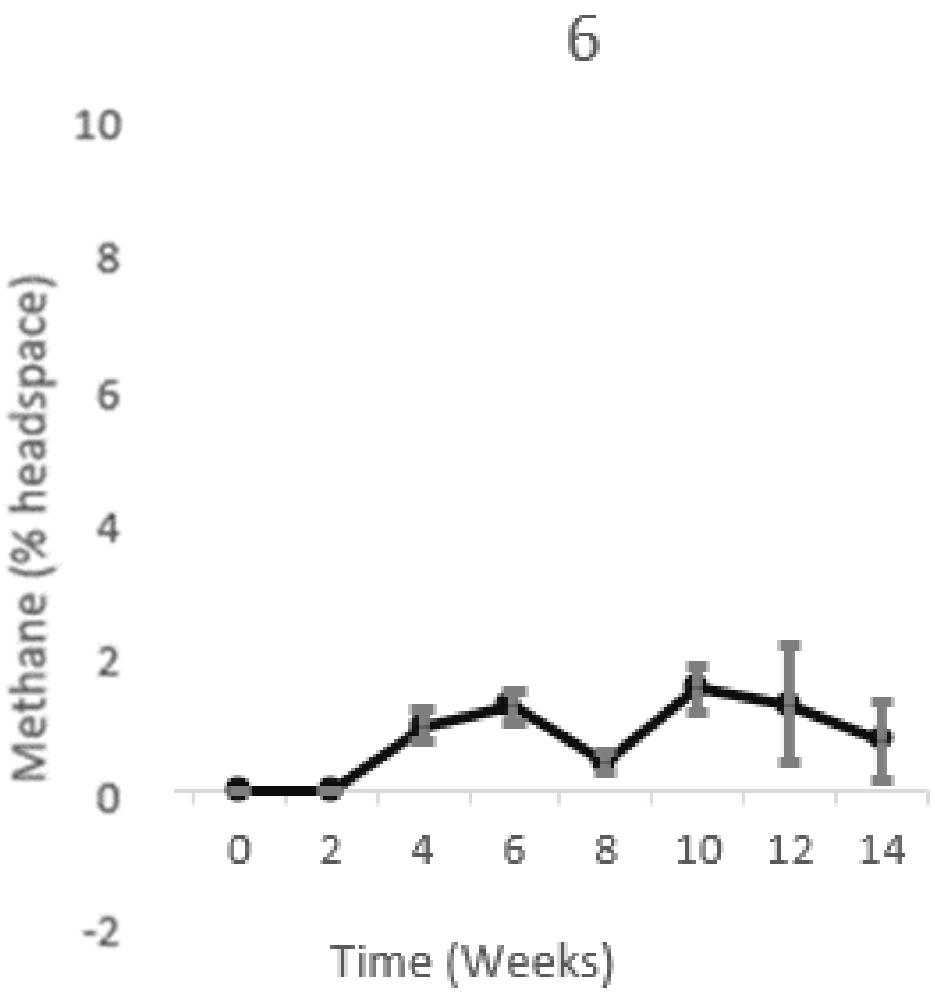

Figure 6. Methane production by *Methanobacterium formicicum* on kaolinite following transfer of cells from fifth-transfer cultures.

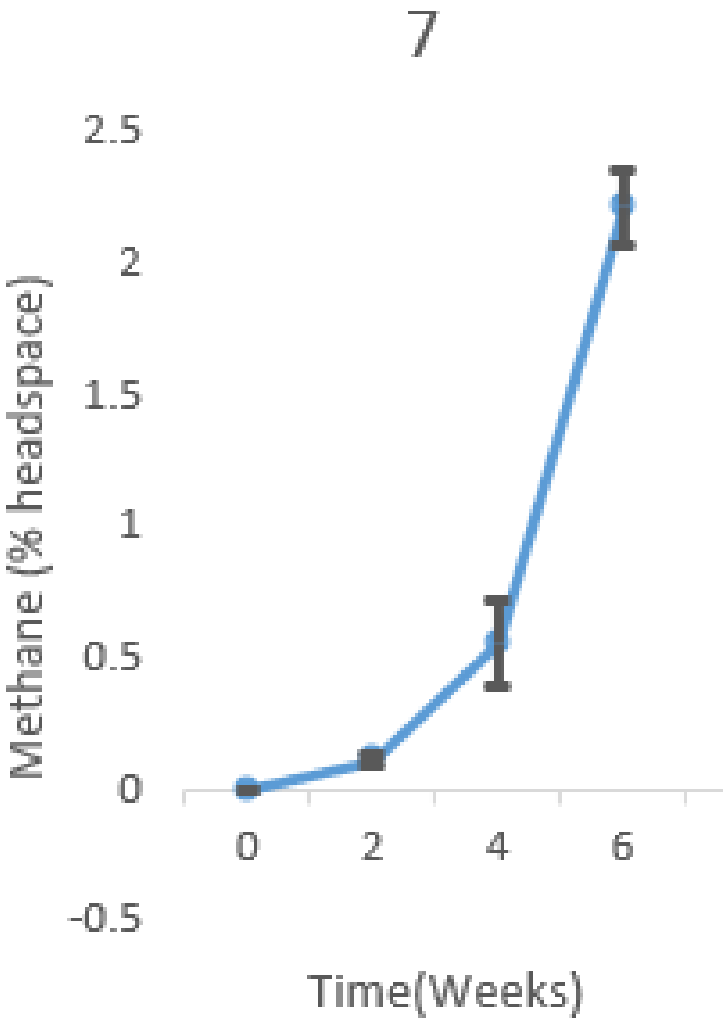

Figure 7. Methane production by *Methanosarcina barkeria* on kaolinite following transfer of cells from a stock culture.

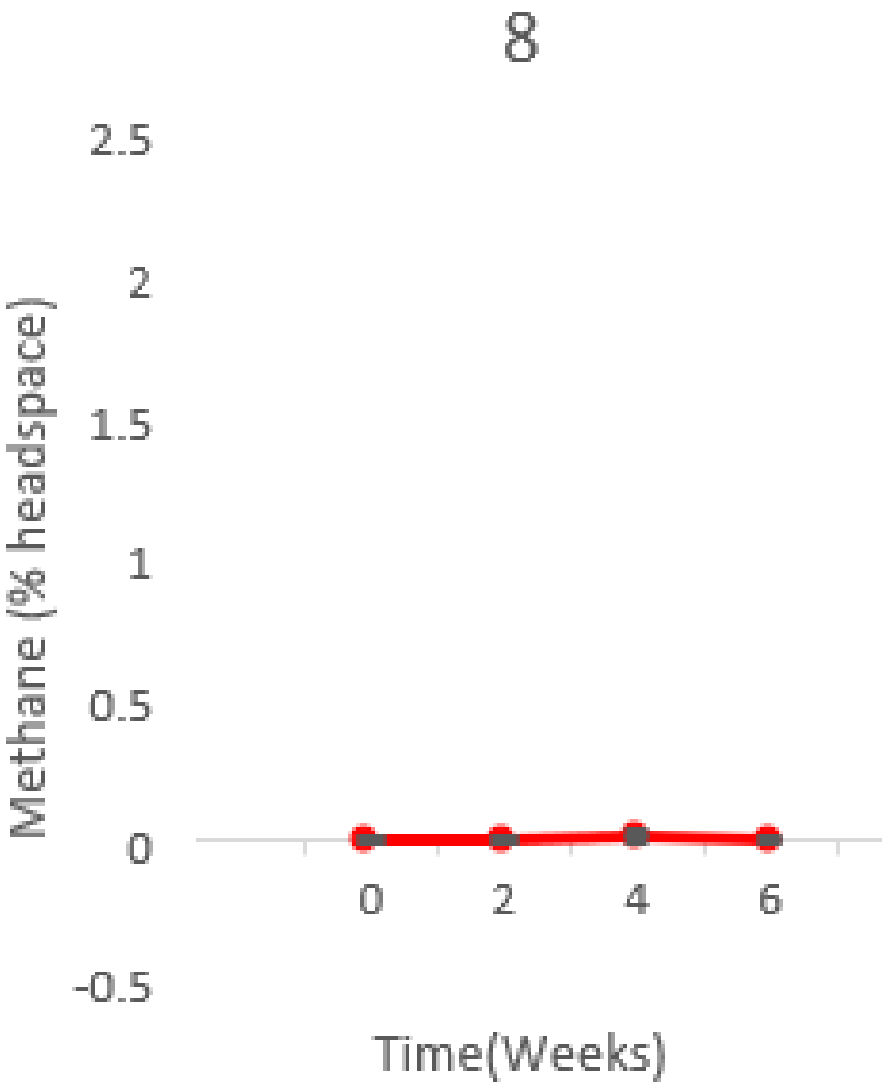

Figure 8. Methane production by *Methanosarcina barkeria* on kaolinite following transfer of cells from first-transfer cultures.

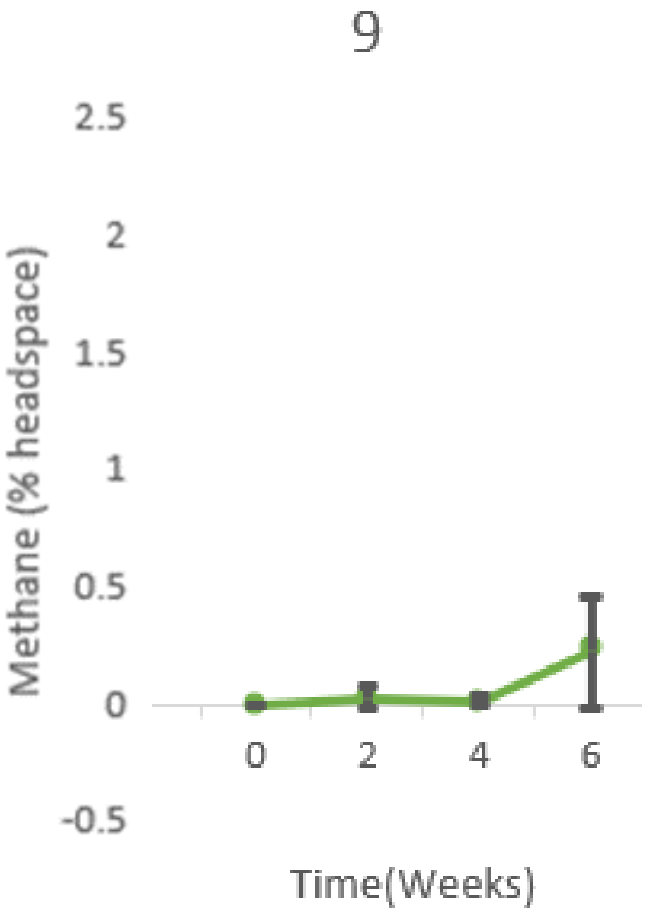

Figure 9. Methane production by *Methanosarcina barkeria* on kaolinite following transfer of cells from second-transfer cultures.

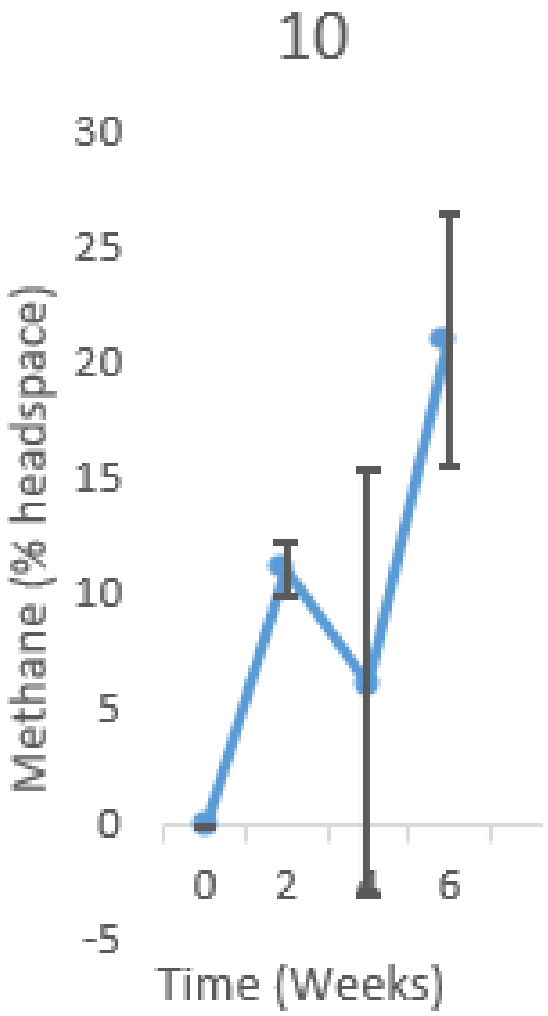

Figure 10. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells from a stock culture.

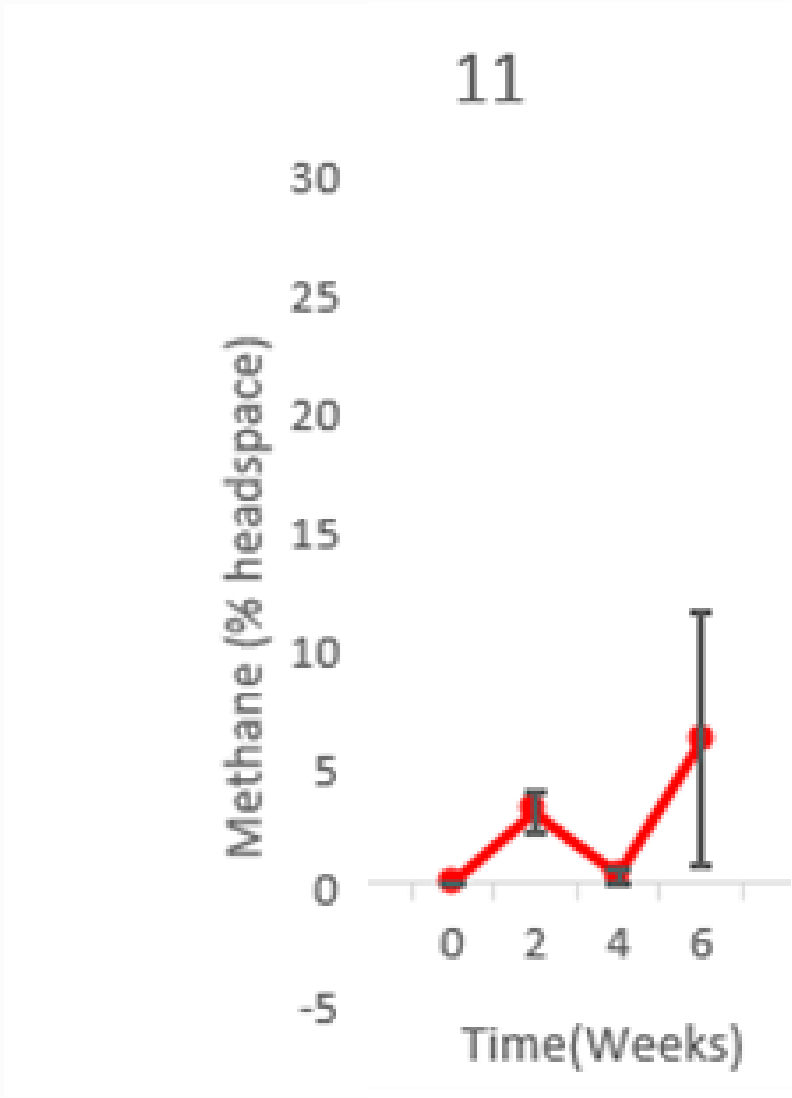

Figure 11. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells from first-transfer cultures.

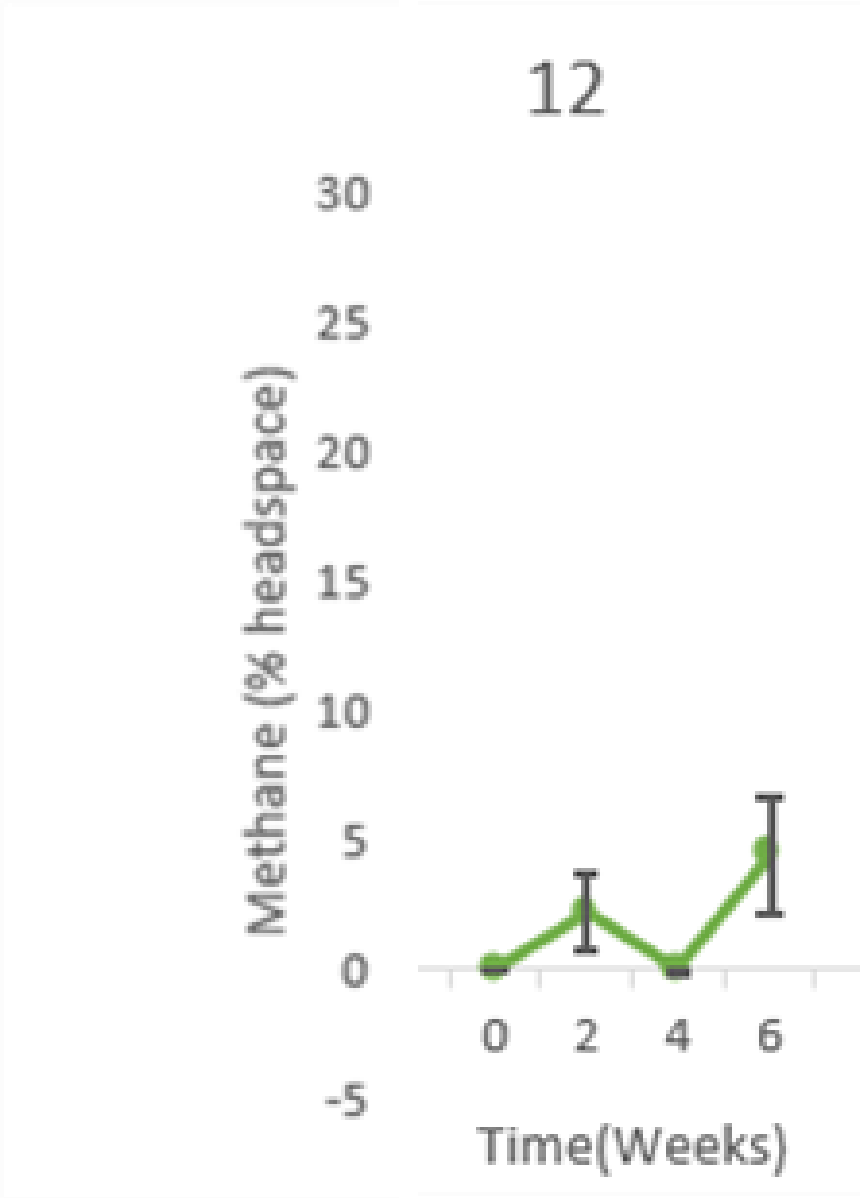

Figure 12. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells from second-transfer cultures.

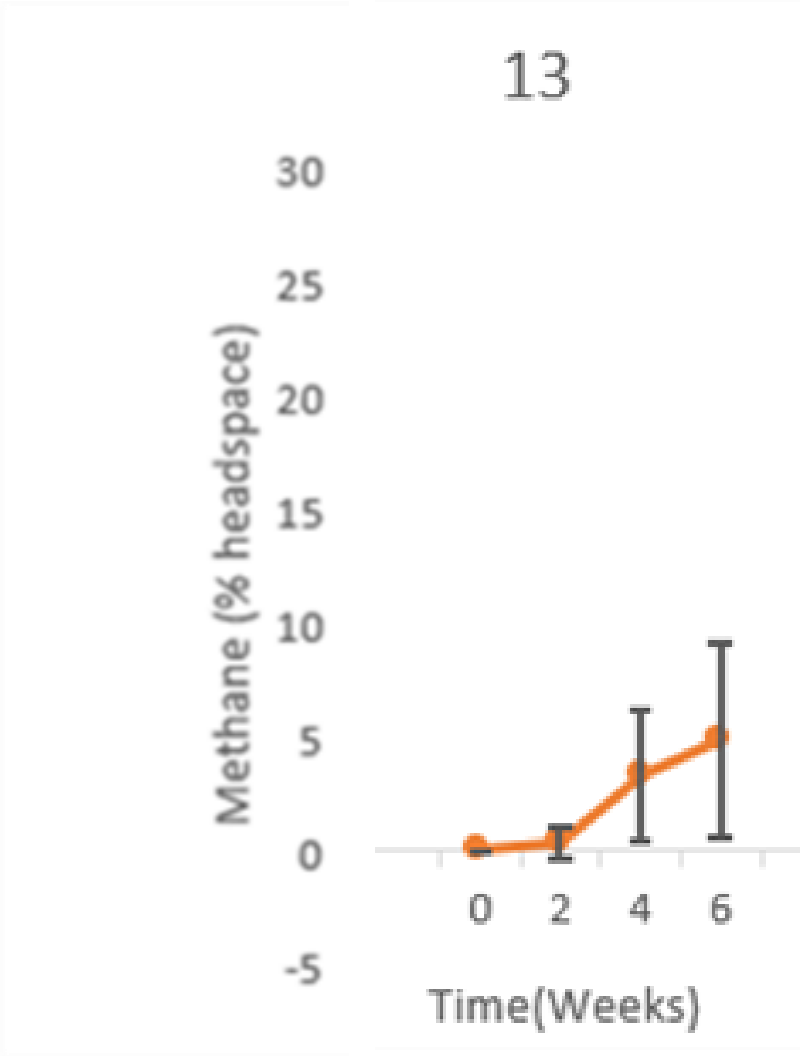

Figure 13. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells third-transfer cultures.

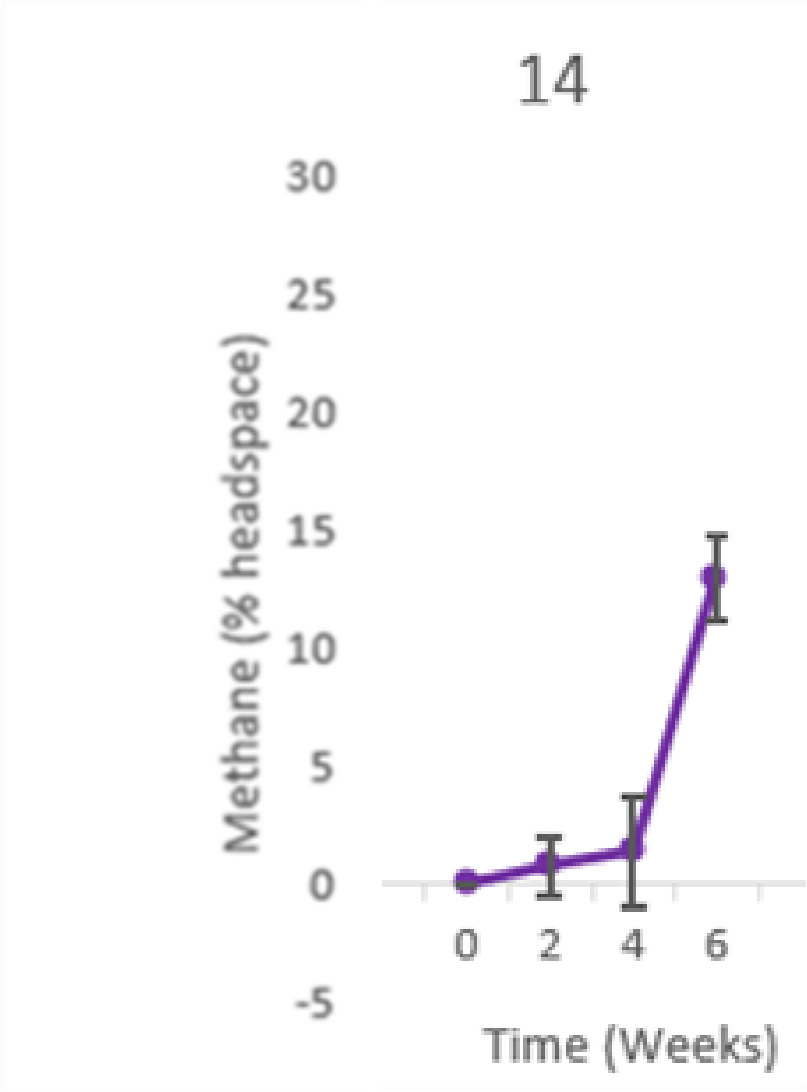

Figure 14. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells from fourth-transfer cultures.

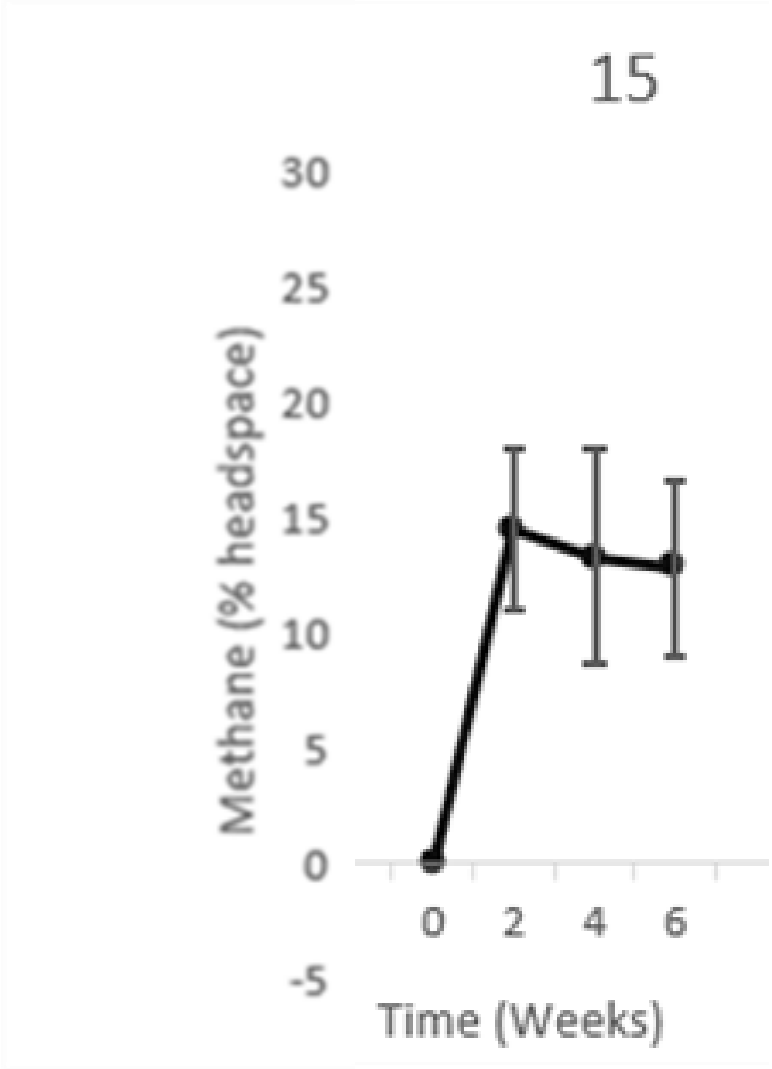

Figure 15. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells from fifth-transfer cultures.

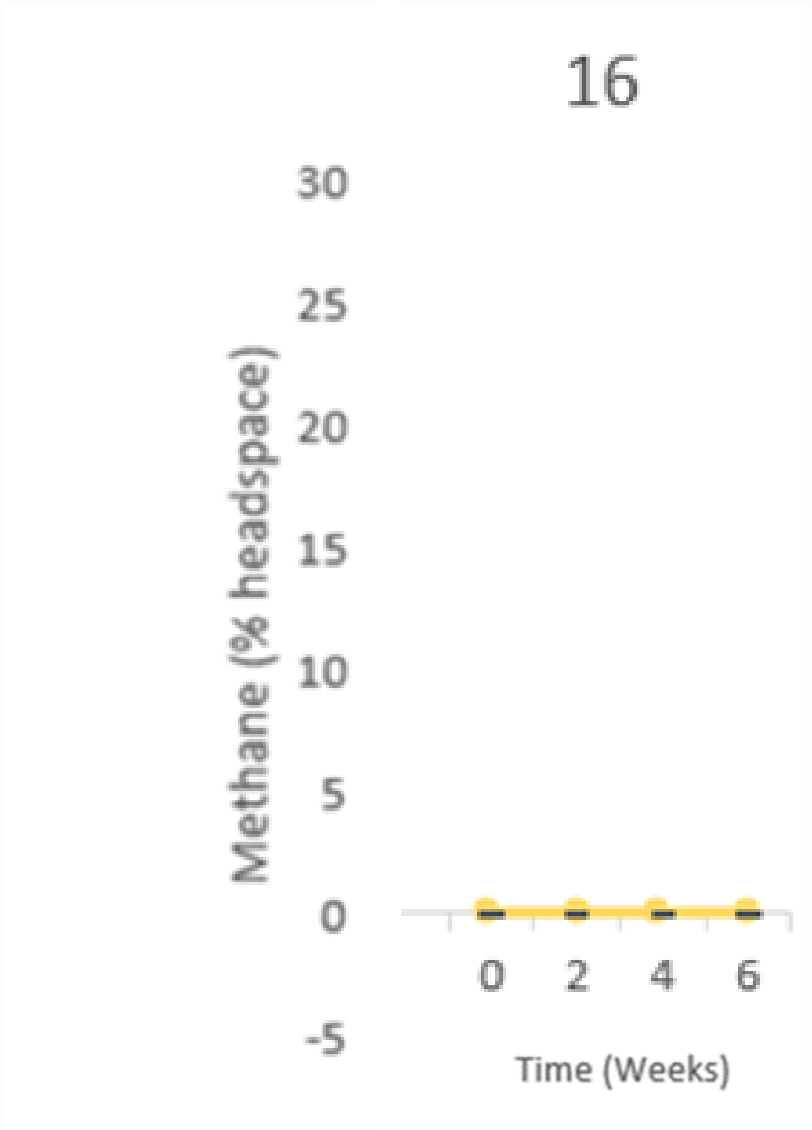

Figure 16. Methane production by *Methanothermobacter wolfeii* on kaolinite following transfer of cells from sixth-transfer cultures.

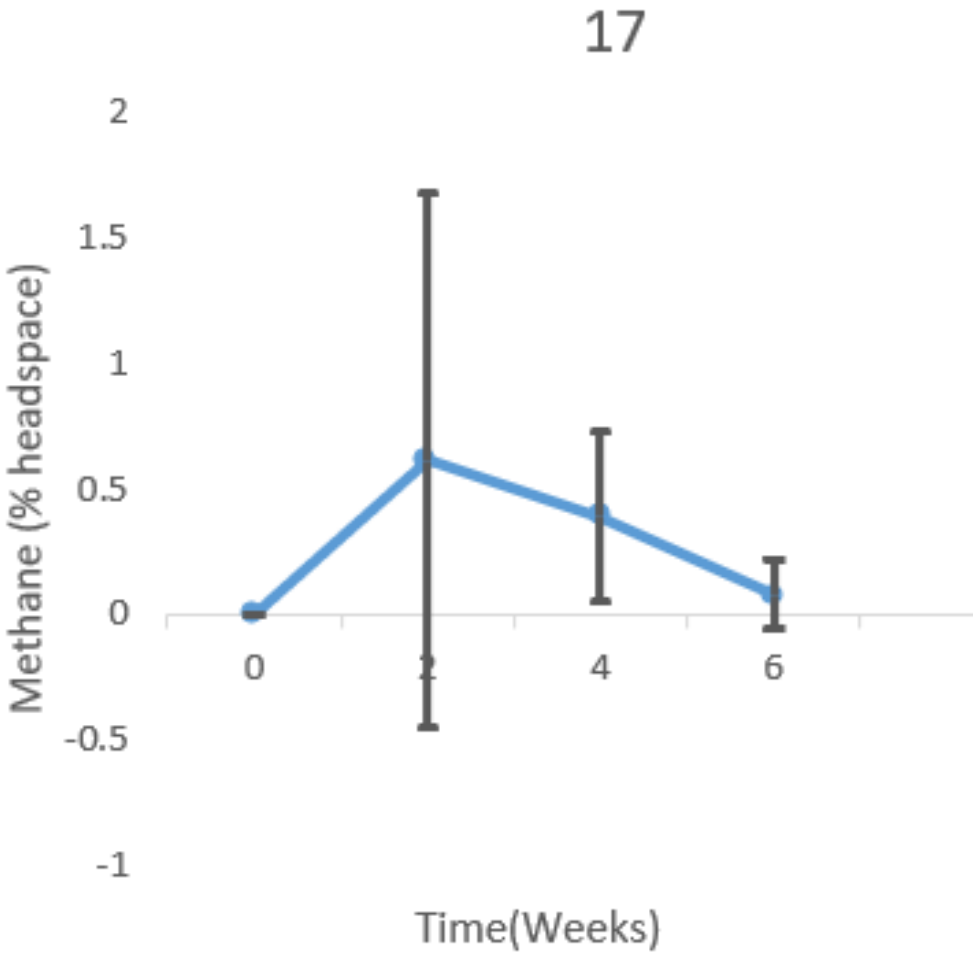

Figure 17. Methane production by *Methanococcus maripaludis* on kaolinite following transfer of cells from a stock culture.

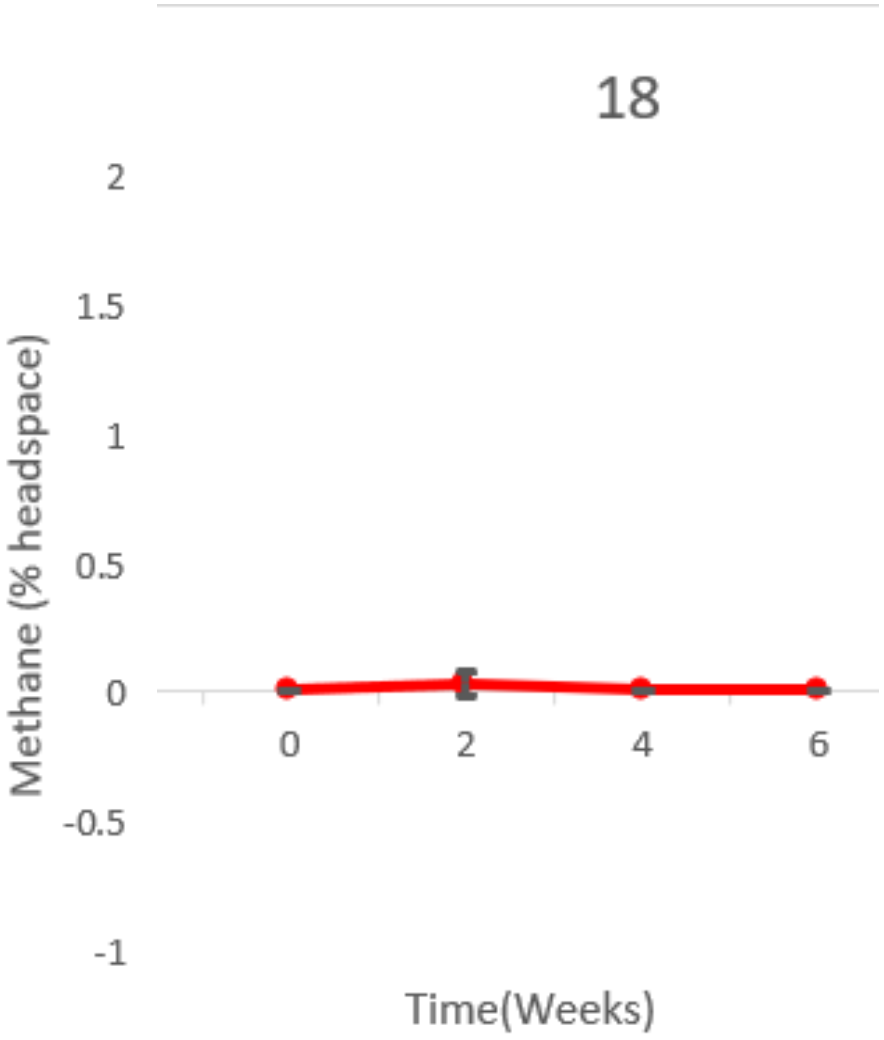

Figure 18. Methane production by *Methanococcus maripaludis* on kaolinite following transfer of cells from first-transfer cultures.

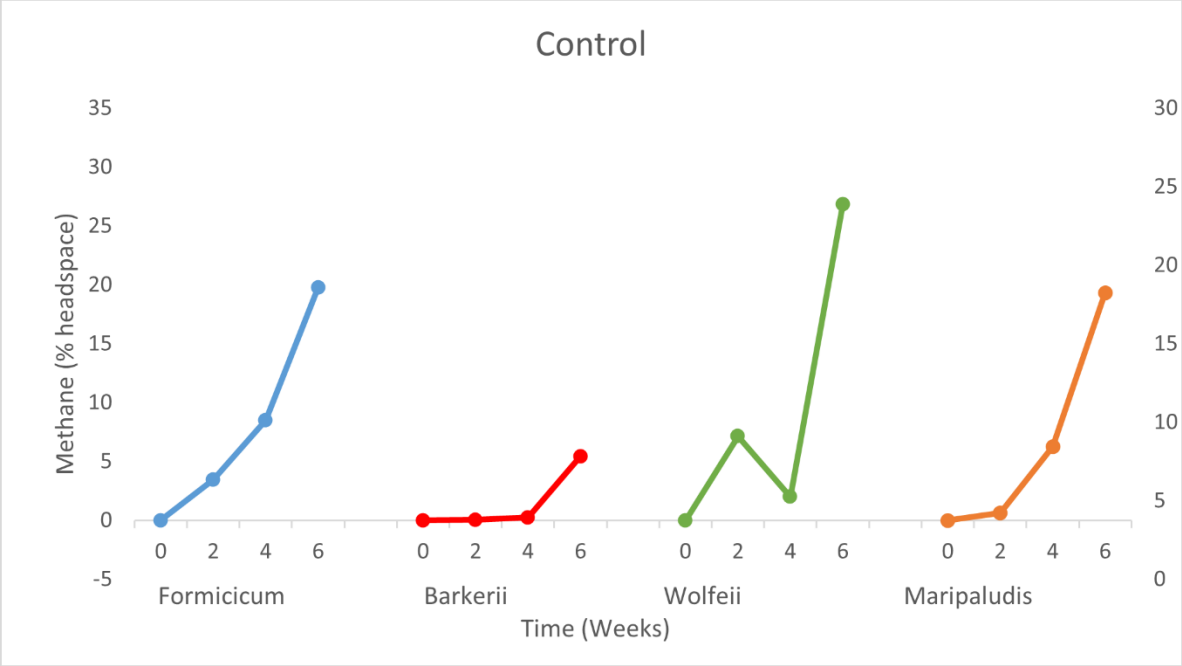

Figure 19 (Control). Methane production by *Methanococcus maripaludis, Methanothermobacter wolfeii, Methanosarcina barkeria, Methanobacterium formicicum* in their respective growth media and temperatures.

Results found in Figures 17 and 18 show that *M. maripaludis* had no significant growth, likely because *M. maripaludis* is a halophile, requiring higher concentrations of salt to grow, and no salts were added. Figures 7 through 9, 10 through 16, and 1 through 6 show that *M. barkeri*, *M. wolfeii*, and *M. formicicum* (respectively) could sustain growth for at least one transfer from stock cultures that had been centrifuged and washed. Further transfers for *M. barkeri* yielded no significant methane production. *M. formicicum* was able to sustain significant methane production for six transfers. The sixth transfer (Figure 6) was monitored for longer than six weeks to potentially see greater amounts of methane, but there was instead a decrease. This is common. Because carbon dioxide is more soluble in water than methane, as headspace gas samples are removed, more carbon dioxide than methane comes out of solution, thus the methane concentrations are reduced. There appears to be some sort of growth limitation or threshold preventing the methanogens from growth for longer periods. Further testing of certain methanogens is warranted to gain a better understanding of this phenomenon. Although *M. wolfeii* showed no significant growth past six weeks for the seventh transfer, it is possible that the organisms have simply slowed growth, which could be multifactorial. Long term studies of the growth patterns of these methanogens would certainly paint a clearer picture of the usefulness of phyllosilicates in their pathway.

Although it is unclear what exactly prevented further growth of these organisms, it is interesting that both *M. formicicum* and *M. wolfeii* were able to show significant growth through several transfers. Kaolinite may have the potential to sustain methanogen growth, but there are several other clays and phyllosilicates that could act as a main substrate for these methanogens. Given a relatively incomplete database of how different species of methanogens react and grow using different substrates, further studies can shed light on certain pathways that these

methanogens can use. If a conclusion can be reached about what substrates can be usable by methanogens, then it can further guide the search and necessary instrumentation for detecting extant/extinct life on Mars and other planets.

IV. References

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