METHANOGEN USE OF INSOLUBLE CARBONATES AND THE IMPLICATIONS FOR LIFE ON MARS. B. T. Virden1 and T. A. Kral1,2. 1Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, 2Arkansas Center for Space and Planetary Science, University of Arkansas, Fayetteville, AR 72701.

bvirden@uark.edu, tkral@uark.edu.

Introduction: The surface of Mars may not seem very hospitable to life, but that does not mean that there could not be, or could not have been in the past, life living beneath the reddened surface, theoretically of course. On Earth, there exist certain types of Archaea called methanogens that can be found in a variety of locations such as swamp marshes, animal intestines, and, for the especially hardy ones, in hydrothermal vents beneath the oceans, just to name a few. These are all anaerobic environments, ideal for the strictly anaerobic microbes.

Methanogens are chemoautotrophs, organisms that are able to use inorganic matter for energy, so sunlight is not needed since photosynthesis is not the means of energy production. They just need water, a carbon source, and hydrogen in order to be viable and perhaps a few other nutrients for optimal growth. Methanogens get their name from the fact that they produce methane as a waste product from the consumption of hydrogen and carbon, and its presence is a good indicator of growth and vitality. This gas has been found in fair abundance on the surface of Mars, it’s source perhaps being microorganisms [1]. Hydrogen serves as an energy source with carbon and water simply being necessary in general for any known form of life. A source for hydrogen, and also heat, might be volcanic or hydrothermal activity, or the reaction of basalt and anaerobic water [2]. Liquid water may also exist below the surface, especially if ice has been melted by volcanic activity. The carbon source may be carbon dioxide, a common gas in the Martian atmosphere.

But what if the organisms live so far beneath the surface that the atmospheric CO2 cannot diffuse down to them? Insoluble carbonate has been found on Mars as recently as the Phoenix lander mission and may exist far below the surface [3]. This research project seeks to discover whether or not methanogens can use insoluble carbonates, specifically calcium carbonate (CaCO3) and magnesium carbonate (MgCO3), as their carbon source.

Methods: In stoppered anaerobic culture tubes, four different species of methanogens were grown in their designated liquid media: Methanothermobacter wolfeii in MM medium, Methanosarcina barkeri in MS, Methanobacterium formicicum in MSF, and Methanoococcus maripaludis in MSH. The media contained an insoluble carbonate (calcium or magnesium) plus required nutrients Using a gassing manifold, 100 kPa of hydrogen gas were added to each of the tubes. For each organism, there were tubes with 200 kPa of CO2 gas added and tubes with no added CO2. The different organisms were incubated at their optimal temperature ranging from 55 degrees Celsius to room temperature. Every week, headspace gas samples were analyzed by gas chromatography for methane and carbon dioxide. Data were plotted as percent methane vs. time. Percent carbon dioxide vs. time was also plotted.

Results: In both the calcium carbonate and magnesium carbonate experiments, all four organisms demonstrated substantially higher methane production with CO2 added compared to cultures without. Tubes with no added CO2 did, however, show low but steady methane production over the incubation period.

Calcium carbonate. For M. barkeri, the average methane production for tubes with and without added CO2 showed steady increases throughout the whole of the experiment period (Fig. 1). For M. formicicum, the tubes with no added CO2 showed steady methane production throughout, and the tubes with added CO2 leveled off around day 21 (Fig. 2). For both M. wolfeii and M. maripaludis, all the tubes peaked in methane production around day 21 after which the percent methane gradually decreased for the rest of the experiment.

Magnesium carbonate. M. barkeri showed lower methane production than in the CaCO3 experiment, and percent methane peaked at 42 days. All M. formicicum cultures showed steady methane production throughout as did all tubes of M. maripaludis (Fig. 3). Tubes containing M. wolfeii with added CO2 had peak methane production around day 28. One culture of M. wolfeii with no added CO2 showed low but steady methane production throughout, but another similar culture showed a dramatic increase in percent methane around day 35 (Fig. 4), which may be due to a mutation.

Discussion: It was not surprising that the organisms in tubes with added CO2 produced more methane than the organisms in tubes with no added CO2; higher levels of CO2 typically meant more growth. It must be noted that CO2 levels in the tubes with no added CO2 were low but not zero, probably due to chemical equilibrium.
Figure 1. Methane production by *Methanosarcina barkeri* in the presence of calcium carbonate.

Figure 2. Methane production by *Methanobacterium formicicum* in the presence of calcium carbonate.

Figure 3. Methane production by *Methanococcus maripaludis* in the presence of magnesium carbonate.

Figure 4. Methane production by *Methanothermobacter wolfeii* in the presence of magnesium carbonate. Tube 1 could potentially contain a mutant strain.

The *M. wolfeii* in tube 1 in the magnesium carbonate experiment (Fig. 4) that displayed unexpectedly high levels of methane production could have potentially developed a mutation, but this is not confirmed. Further study of these particular organisms would be a subject for future research.

**Implications for Mars:** It is unknown how deeply the CO\(_2\) in the Martian atmosphere penetrates the red planet’s surface, so it is exciting to know that microbes such as these methanogen species could be viable by using insoluble carbonates as their carbon source. It would be very worthwhile for future Mars missions to have the capability to dig deeply into the crust to search for life.