Can Methanogens Survive on Mars using Insoluble Carbonates as their Energy Source?. B. Leonard[1] and T. Kral[2], [1] Southern University and Agricultural & Mechanical College, Baton Rouge,LA 70813, [2] Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR 72701. BLeonard324@aol.com, tkral@uark.edu.

Introduction: The planet Mars is the fourth planet from the sun, it is one of Earth's closet neighbors and just like the other planets, Mars is approximately 4.6 billion years old. From the different missions to Mars it is believed by some scientists that the planet was at one time the home to a living organism. These thoughts came about as a result of the data obtained from the Mars missions revealing that at one time or another the planet had running water, which is required for any living organism to survive, by the evidence of gullies, channels and valleys.

Some scientists believe that an organism called Methanogens could possibly still live on the planet today. Methanogens are a part of the Archae family and they are chemoautotrophs. They take in hydrogen as their food source and they release methane as their waste, which gives them the name Methanogens. They can be found here on earth in animal and human intestines, swamps, wetlands and permafrost. In order for Methanogens to survive they use an inorganic energy source and they don't require sunlight because it isn't used for their means of producing energy. For the organisms to grow all they need is a water source, a carbon source, hydrogen and a few other nutrients.

This experiment is a continuation of a past experiment that dealt with Methanogens and their growth patterns on insoluble carbonates. The previous research was done in order to see if Methanogens could live on insoluble carbonates, magnesium or calcium carbonate, if the carbon dioxide wasn't able to reach them down beneath the soil. In the previous experiment a mutant form of the species *Methanothermobacter wolfeii* was discovered while running an experiment on the parent strand of the species. The purpose of this research project is to determine if the mutant discovered is able to grow at a higher level on the magnesium or calcium carbonate rather than the parent strand of the species.

Methods: The *Methanothermobacter wolfeii* species was placed in stoppered anaerobic culture tubes and were grown in MM medium in a 55°C incubator. In some tubes an insoluble carbonate, calcium or magnesium, were placed in certain tubes plus other required nutrients. Using a gassing manifold, 100 kPA of hydrogen gas was added to each of the tubes. In some of the tubes another 100 kPa of CO_2 was added. Every week the gas in the tubes was analyzed by gas chromatography for methane and recorded. Another set of tubes were also created a few weeks later using the same species of Methanogens but in this case the pH was changed. The pH was manipulated to see if it had any effects on the growth pattern of the mutant and parent strand of the *Methanthermobacter wolfeii.* In order to change, the pH a small amount of hydrochloric acid was added to two small amounts of solution that contained either magnesium or calcium carbonate. From those two solutions four sets of pH tubes were made: $CaCO_3$+CO_2, $CaCO_3$-CO_2, $MgCO_3$+CO_2 and $MgCO_3$-CO_2. It approximately took one drop of hydrochloric acid to change the pH of the calcium carbonate solution, while for the magnesium carbonate it took about thirty drops of the hydrochloric acid. Again using a gassing manifold 100 kPa of hydrogen was placed into each tube and another 100 kPa of carbon dioxide was placed into certain tubes. Every week, the headspace gas samples were analyzed for methane using gas chromatography and were recorded. For both sets of tubes the readings were plotted as percent methane vs. time.

Results: As stated before the purpose of this experiment was to grow the mutant species of *Methanothermobacter wolfeii* in order to compare the growth rate between the parent species with insoluble carbonates. The data from Figure 1.1 illustrates that the parent with CO_2 grew at a higher level than the others, which is to be expected because of the fact that Methanogens thrive on carbon dioxide. From taking a further look at the data in Figure 1.1, it illustrates that the mutant species didn't grow as well as the parent, but at this point it was very early in the experiment and the mutant species needed more time to grow in order to make a precise analysis. The data in Figure 1.2 shows the growth of the parent and mutant without the pH change after eight weeks of incubation. This data illustrates the fact that the parent strand is growing at a much higher level with CO_2 than the mutant strand. As a result of the slow growth of the mutant strand after eight weeks, it was decided that the mutant strand wasn't going to be used for the next part of the experiment dealing with the pH change. The data from Figure 1.4 shows the growth of the parent strand with the solution having a pH change. This data illustrates that the parent grew extremely well with CO_2 and also with the changed pH. The data also shows that the parent grew at a slightly lower level with the pH change, than with pure CO_2.

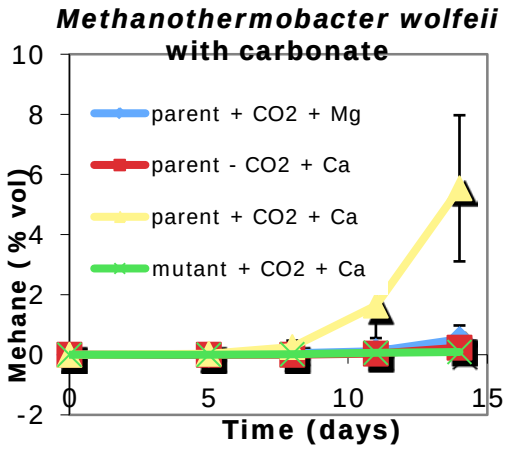

Figure 1.1: This chart shows the methane growth of the *Methanothermobacter wolfeii* parent and mutant species after about two weeks.

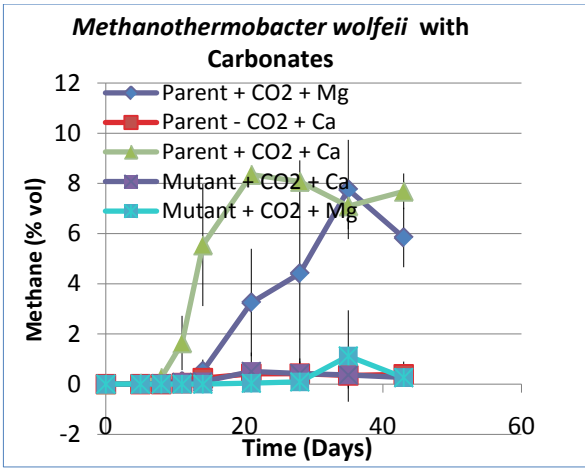

Figure 1.2: This chart shows the methane growth of the parent and the mutant after approximately eight weeks of incubation.

Medium	pH
Mg- CO2	8.2**
Mg+ CO2	6.6
Ca-CO2	7.3
Ca+CO2	6.0

Figure 1.3: This chart shows the levels of pH in the different solutions. Methanogens tend to grow better with a pH between six and seven.

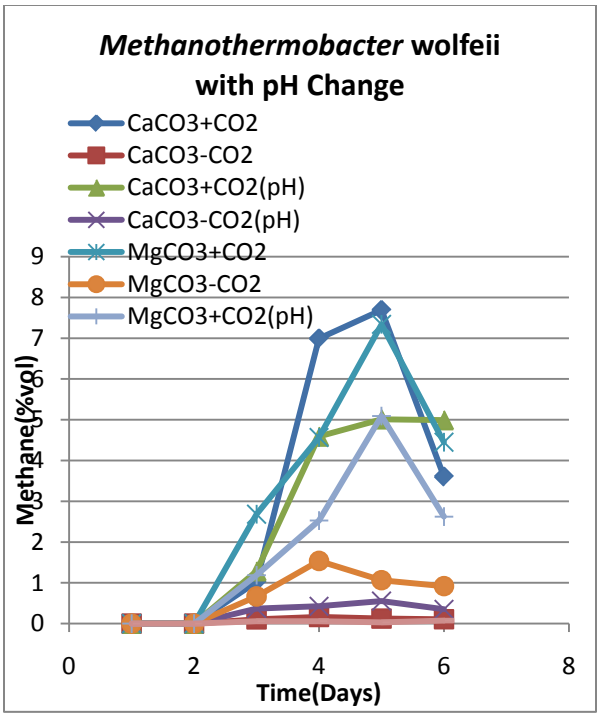

Figure 1.4: This chart shows the growth of the parent strand of the species *Methanothermobacter wolfeii* with the pH change.

Conclusion: As a result of the experiment, it can be concluded that Methanogens grow better with CO_2 than without CO_2. It needs to be taken into consideration that even though the parent strand grew better on CO_2, it still grew at lower levels without CO_2. The mutant species of *Methanothermobacter wolfeii* didn't grow as expected during this experiment, it may have been as a result of the gas equilibrium being off or the mutant species may have needed more time to grow than what was allowed with this experiment.

References:

[1] www.nasa.gov/worldbook/**mars** worldbook.html

[2] www.encyclopedia.com

[3] Bryant Vird.en (2010*) Methanogen Use of Insoluble Carbonates and the Implications for Life on Mars*