

OXYGEN RESISTANCE OF METHANOGENS: AEROBIC RESEARCH TECHNIQUE FOR EXOBIOLGY.

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Introduction: Methanogens are anaerobic organisms in the domain Archea that produce methane through the reduction of CO, CO₂, formate, methanol, methylamines, or acetate [1]. These organisms have been postulated as a model for life on Mars because of their ability to act as the primary producers of a subterranean ecosystem utilizing only CO₂ and H₂ [2]. It is possible that Methanogens, or rather, an organism that utilizes the same metabolic path, could use atmospheric CO₂, and H₂ from the reaction of anaerobic water and basalt. This makes research on this group of organisms important to the continuing search for life on Mars.

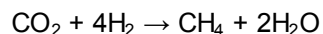
Objectives: One of the primary hindrances to this study has been the extreme sensitivity of Methanogens to oxygen. It has been believed that extremely low concentrations of atmospheric O₂ will kill Methanogens on contact. This makes Methanogens very difficult and expensive to keep because a strictly anaerobic workspace and storage facility are necessary. Work on Methanogens is made tedious by the need to perform all procedures in a glove-box. However, no in-depth study of oxygen resistance in Methanogens has yet been performed. The objectives of this study were as follows:

- To determine if Methanogens can withstand short-term exposure to atmospheric O₂,
- To what level their performance is impaired by said exposure, and
- To develop an aerobic technique for rinsing Methanogen cultures of nutrient media.

Organism	Temperature	Media
<i>M. wolfeii</i>	55°C	MM
<i>M. barkeri</i>	37°C	MS
<i>M. formicicum</i>	37°C	MSF
<i>M. maripaludis</i>	25°C	MSH

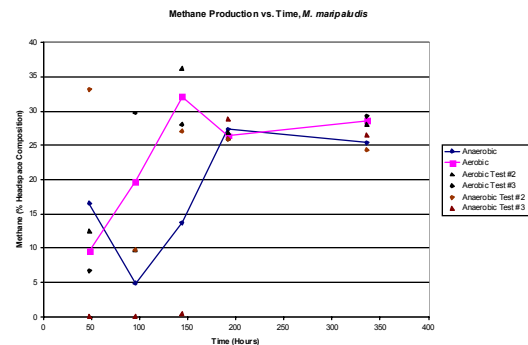
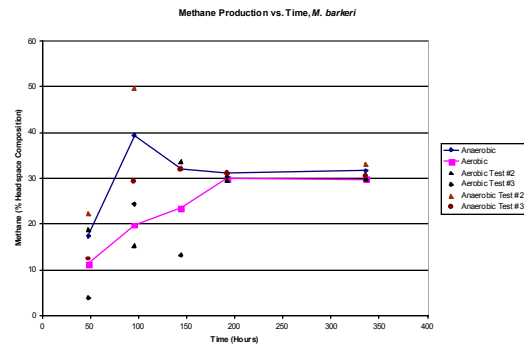
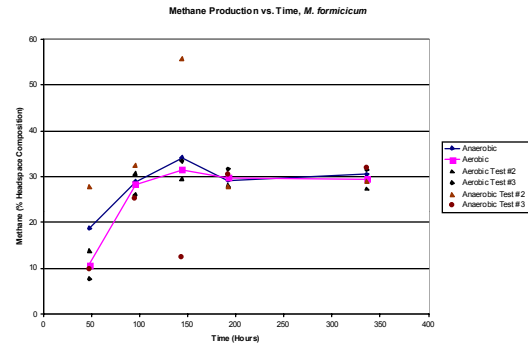
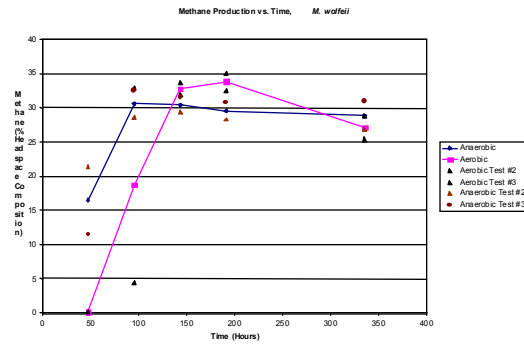
Table 1. Incubation temperatures and media of the samples.

Methods: H₂ + CO₂ serves as the best substrate for most Methanogens [3] by the following process:



Thus, growth of Methanogens can be tracked by measuring methane production against time. Four organisms were used as a broad representative group of the Methanogen family as a whole: *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum*, and *Methanococcus maripaludis*. Each was incubated anaerobically in the standard media and temperature most preferred by each species (see Table 1). Eight cultures, two of each species, were placed in pressurizable test tubes and pressurized to 180 kPa with H₂. As the media was prepared in an anaerobic chamber with a CO₂ atmosphere, the solution was saturated with CO₂ and no more needed to be added. The tubes were then allowed to incubate for five days before being rinsed in NaOH/CO₂ buffer. One tube of each species was rinsed anaerobically in the anaerobic chamber with special care taken to avoid O₂ contamination, whilst the remaining tubes were opened and rinsed in open air. Exposure to oxygen for the aerobic technique was two hours. After the procedure, 1 ml of each culture was used to inoculate new tubes of media which were also pressurized to 180 kPa with H₂ and allowed to incubate at ideal temperatures. Four measurements of the headspace composition were taken with a gas chromatograph at approximately forty-eight hour intervals thereafter, with a fifth test at two weeks.

Results and Conclusions: Though two experiments are not enough to construct a statistically conclusive curve (though the experiment was performed in triplicate, errors forced us to discard the results of the first.), the composite curves shown in Figures -1-4 are provocative to say the least.



Figures 1-4. Composite growth curves of the four test organisms, with blue lines indicating the samples rinsed in the anaerobic fashion, the pink line indicating those rinsed with the new aerobic technique.

Rather than the showing no growth, as the literature would lead one to expect, the aerobically rinsed samples showed virtually identical performance to the samples treated in the traditional, anaerobic manner. Although the experiment should be repeated several more times to gather enough data points for statistically conclusive curves, these results are enough to declare that Methanogens are not, in fact, as sensitive to acute oxygen exposure as has been believed.

Significance: If we accept that Methanogens can easily withstand acute oxygen exposure of up to two hours at atmospheric concentrations, then we realize that many of the operations that would require a glove-box facility to execute anaerobically are not necessary. In the case of the aerobically rinsed samples, the procedure was carried out without once needing an anaerobic workspace—only sealable test tubes and a gas manifold with CO₂ and H₂ gasses available was required. This opens the field of Methanogen research to laboratories that do not have the facilities for purely anaerobic procedures. This is highly

significant as it means that important work regarding Methanogens, including studying their propagation rates and minimal nutrient requirements would be much easier to do. Such work would advance research into the suitability of methanogenic metabolisms for Martian conditions considerably.

Further Study: It would be highly beneficial for this experiment to be repeated several times to obtain statistically accurate performance curves for the organisms tested. Also, varying oxygen concentration and possibly duration to determine the upper limit of oxygen tolerance would be enlightening.

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Resources: [1] Stanley, J. T., (ed.) 1989. *Bergey's manual of systematic bacteriology*, vol. 3 [2] Brink, K. M. 1997. *Hydrogen Consumption by Methanogens: Implications for Mars and Early Earth*. [3] Balch, W. E., *et al.* 1979. Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* **43**.