MANGANESE-BASED BACTERIAL METHANOGENESIS IN SIMULATED MARTIAN CONDITIONS.

M.V. Tarasashvili[1] and S. Z. Barjadze[2]; [1]Javakhishvili Tbilisi State University; 1, Ilia Chavchavadze Avenue, 0179 Tbilisi, Georgia; e-mail: mariam.tarasashvili@ens.tsu.edu.ge, [2]Institute of Zoology, Ilia State University, 3 Giorgi Tsereteli street, 0162, Tbilisi, Georgia; e-mail: shalva.barjadze@iliauni.edu.ge

Introduction: Through the decades, data from the various sources have shown the strong evidence of the seasonally variable methane plumes on Mars [1,2]. On Earth, almost all methane sources are of biological origin, however on Mars, with no obvious proof of the past or present life, methanogenesis is more likely linked to the geo-chemical processes. Although, recently conducted experiments demonstrated that several species of methanogenic bacteria can thrive under the simulated Martian conditions for several months [3], and based on these results, survival of the archaea - artificially or naturally transferred fro Earth to Mars - is still not entirely out of the question.

Caves of the manganese ores are similar to the Martian conditions mainly by two environmental components: content of the manganese oxides and hypoxic conditions; most importantly, these are the sites where intense biological methanogenesis takes place, thus could partially shed the light on the origin of Martian methane plumes.

The purpose of this work was to investigate possible inter-dependence of the presence of manganese oxides in the environment and stimulation of the biological methane-production, in relation to the various temperatures and low-pressure CO_2 of Martian atmosphere.

Materials and Methods: The samples have been obtained from the manganese ore caves of Chiatura, Georgia (42°17′52″N 43°17′56″E), in the layers of manganese oxide (42%), peroxide (19%) and carbonate (39%) at the depth of 4 meters, where high concentration of the methane is present (figure 1).

Fig. 1. Collecting samples of the manganese deposits at Shvilobisa cave, Chiatura, Georgia.

The mines of the manganese ore are deprived of water; intense outbursts of the methane plumes are due to the presence of several types of Methanogenic archaea. Manganese ore extends down to 16-18 meters underground.

Grind samples of the ore (figure 2) have been mixed with the artificial Martian ground - AMG (GEO PAT 11-234 (2015)) initially containing 0.4 % of Manganese dioxide and 4%-manganese oxide (by mass) [4]. 250 ml gas sampling quartz jars with 100g of ground mixture have been moisturized with bi-distilled water, filled with CO_2 and sealed. Pressure in the containers has been reduced with syringes.

***Fig. 2**. Sample of the manganese ore - microbiological analysis revealed several species of the genus Methanosarcina, Thermococcus celer and others.*

Control samples have been inoculated in the pure sterilized Zeolit mixed with silicagel without manganese content. The jars have been placed in laboratory and simulated Mars conditions in Mars Climate Simulation Chamber - MCSC (GEO PAT 12 522/01) **[5]** for 8 weeks.

Methane production measurements have been conducted with Vernier IR CO_2 gas sensor and LabQuest software (provides 100/1 ppm/ml measurement), and produced CH_4 has been calculated from the accumulated CO_2, assuming archaean species present in the sample were not using hydrogen source (from peroxides) for their metabolism.

Temperature measurements: activation energies of archaean growth were determined from the slope of a semi-logarithmic plot of the growth rates *vs* reciprocal t˚ according to the logarithmic form of the Arrhenius equation [6].

Temperature dependence of the methane production in parallel samples has been investigated at -25˚C, -5˚C, +5˚C, +25˚C and +45˚C for the simulation of various locations and depths, where Martian methanogens can theoretically thrive – polar and equatorial regions, as well as underground hot springs. [7].

Results and Discussion: The results of the experiments have shown that the intensity of the methanogenesis was considerably higher in the samples with manganese. Presumably, with the CO_2 as the sole carbon source, manganese oxides served as the terminal electron acceptor. By the end of each week amount of accumulated CO_2 gas in the jars with manganese oxides was about 10-15 ppm higher than in control, resulting however in the faster exhaustion of the sealed ecosystems.

Low temperatures did not inhibit the rate of methane production as expected from calculations, however, increase (of t˚) has significantly intensified methane production. Assuming many different species of the archaea have been present inside the samples, would be reasonable to imagine that at different temperatures, individual species of the methanogenes are prevailing, thus, dramatically increasing sustainability of the colonies.

From our observations in simulated Martian conditions we have also obtained indirect evidence that the rate of biological methanogenesis could somehow correlate with the level of ultraviolet radiation, both duration and intensity. Although the details of this phenomenon have not yet been investigated.

Conclusions: Experiments have been conducted under pure CO_2 atmosphere with the scarce hydrogen sources (hydrogen peroxide in the original sample from the manganese ore was present). Thus, with its present hydrogen content [8, 9] methanogens on Mars could produce the methane using other alternatives of the CO_2 fixation, such as methylation or symbiotic iron/sulfate uptake [10]. Therefore, if biological methanogenesis is present on Mars, future investigations will reveal an even higher diversity of the living methanogenic extremophiles, compared to the experimental samples.

As the described process is notably temperature-dependent, the question of whether the methane on Mars is of geological or biological origin, remains open. Reliability of either may rise as soon as more data from the "InSight" equipments measuring temperature of Martian crust becomes available.

Acknowledgments: We thank the Department of Optics and Cybernetics at Georgian Technical University for providing the modern tools for the measurement of the Radiation and Electromagnetic Sources; we would like to express the gratitude to the Department of Geology and Minerals at the Iv. Javakhishvili Tbilisi State University for their valuable materials and expert advice.

References: [1] Krasnopolsky, V. A. et al. (2004) *Icarus 172*, 537–547. [2] Mumma, M. et al. (2009) *Sci., 323*, 1041–1045. [3] Tarasashvili M. V. et al. (2010) *LPI AbSciCon*, Abstract#5229. [4] Mukbaniani O. et al, (2016) *IJA*, *15(2)*, 155-160. [5] Tarasashvili et al. (2013) *IJA, 12(2)*, 161-170. [6] Conrad R. et al. (1983) *FEMS Microb.*, *62,* 285-294. [7] Ruff S.W. et al. (2019) *Astrob. 19(12)*, 1-25. [8] Feldman, W. C., et al. (2004) *J. Geophys. Res.*, 109, E09006. [9] Weiss, B. P. et al. (2004) *Proc. NASUSA, 97,4*, 1395-9. [10] Schirmack, Janosch et al. (2015) *Front. Microb.* 6(210).