# Perchlorates are compatible with life on Earth – Why not Mars?

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## ABSTRACT

Perchlorates have been found on the surface of Mars. Since they are strongly oxidizing, it is important to discuss how this fact is reflected both on the existence of organic compounds on the surface of Mars and possibly life. We have previously reported that perchlorates, although strongly oxidizing, do not destroy some amino acids, such as glycine and alanine, among others, and also spare other classes of organic compounds. Others have found that perchlorates are utilized by bacteria and Archaea as energy sources. Particularly important are the findings about Archaea, since they show a combination of a biotic and abiotic processing of perchlorates, which implies ancient origins of these pathways, which may have been typical on prebiotic Earth. There are also numerous reports of the presence of organohalogen compounds on Earth which are made by natural sources or living organisms. Such compounds may be simple, such as chloromethane, or very complicated. They are utilized or produced by living organisms on Earth. Significantly, some such compounds are extremely stable to high temperatures, over 400$^{o}$C, which should be taken into account for the chemical analyses on Mars. Finally, organohalogen compounds have been also detected on the meteorites. This combined evidence indicates that eventual finding of the organohalogen compounds on Mars is expected, and that the presence of the strongly oxidizing perchlorates does not rule out life on Mars.

**Keywords:** perchlorates, Mars, Earth, stability of glycine and alanine, bacteria, Archaea, organohalogens, meteorites

## 1. INTRODUCTION

Much of the arguments against life on Mars are based on the extremely oxidizing surface on Mars. Now we know that Mars is covered with perchlorates [1]. The title of a very recent report from Science (April 2013) is informative in this respect: "Pesky perchlorates all over Mars" [1]. Significantly, both the *Phoenix Lander* and *Curiosity* have found perchlorates on Mars. *Curiosity* detected evidence for perchlorates in Rocknest in Mars' Gale Crater suggesting it may be widely distributed over the surface. Analysis of the Martian dust performed in Curiosity's SAM (Sample Analysis at Mars) instrument package has shown chlorine-containing single-carbon compounds as the sample temperature passed through ca. 400$^{o}$C. The experiment was performed by a gradual heating of the sample to 835$^{o}$C. The interpretation of the findings was a confirmation of the presence of perchlorates, since oxygen was also liberated during the experiment. It was stated that the perchlorate would have decomposed to chlorine and oxygen at high temperatures, and that chlorine would have combined with any nearby carbon to form the observed single-carbon compounds [1]. This explanation awaits further experimentation, since it is not complete. What were the nearby carbon atoms which reacted with chlorine? The conclusion of the report was that experiments to date may or may not have detected Martian organic matter, since hot perchlorates react with any kind of carbon [1]. We do not believe that this is the case, as we explain in the next Section. Another assumption, which has been brought up since the times of the Viking Landers, is that the oxidizing conditions on Mars surface would be detrimental to life. We do not believe that this is generally the case, and we present the evidence in Section 3 of this paper. On Feb. 8, 2013 *Curiosity* drilled into a rock in the outcrop designated "John Klein" and detected both chloromethane and dichloromethane, but immediately cautioned that this might be from contaminants on the drill.

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The finding of the chloromethane on Mars was ascribed to a contamination, which we believe is premature. However, no explanation has been given as to how the pre-flight checks and protocols could have allowed a drill, which was to be used in a high sensitivity search for evidence of traces of organic compounds on the Red Planet, could have been flown with such organic contamination as to have critically degraded the major scientific objectivity of this important Mars mission. http://www.weather.com/news/science/space/nasa-life-on-mars-20130312

In the Section 4 we show that the organohalogens are widespread on Earth, where they are made by natural and biological sources, but are also found on the meteorites. It is thus more believable to us that chloromethane on Mars came from such sources than by the contamination of the instrument. In Section 5 we show how organohalogens are utilized or produced by the life on Earth. Similar situations could be on Mars.

## 2. OXIDATION OF ORGANIC MATERIALS WITH PERCHLORATE: RELEVANCE OT THE CHEMISTRY ON THE MARTIAN SURFACE

We published a paper [2] on this subject in 2009 and repeat the key findings here. An analytical chemical study was performed by Martinie and Schilt [3] in which various organic compounds were subjected to hot wet oxidation with perchloric acid. The motivation for the study was to check if such oxidation by perchloric acid destroys all organic compounds, as it was assumed, since such a test is routinely used to liberate various metals which may have been bound to the organics and identify them without interference with the organic materials. The authors [3] evaluated the effectiveness of hot wet perchloric acid oxidations of 85 different organic substances. The samples were subjected to a mixture of perchloric and nitric acids, in a 2:1 ratio, and then heated gradually from 20-120$^{o}$C, at which point the nitric acid began to distil. The nitric acid was removed in the 120-140$^{o}$C range. The temperature then rose quickly to 203$^{o}$C, the boiling point of the perchloric acid-water azeotrope (72.5% $HClO_4$). The samples were maintained at this temperature for 30 min., cooled and analyzed. Screening for organic residues was done by a range of methods, including proton NMR (Nuclear Magnetic Resonance), UV (Ultra Violet) and IR (Infrared) spectroscopy, MS (mass spectrometry) and carbon microanalysis. Surprisingly, many organic compounds survived this treatment!

Many samples were resistant to or only partially oxidized in this procedure. Approximately half of the samples retained measurable carbonaceous matter in solution. We have brought to attention this study, since it is of great astrobiological significance. It is directly relevant to the hospitality of the Martian surface towards various organics and to the possibility of life on Mars. Despite the unchecked assumptions by many that no organics could survive the perchlorates, actual experiments show the opposite. We present here the Table with the results of Martinie and Schilt[3], which is discussed in more detail in our previous paper [2].

**Table 1.** Residual carbon content and the identity of the residues after wet perchloric acid oxidation of selected organic compounds [3]

| **Sample** | **% C** | **Identified Organic Residue** |
|---|---|---|
| ***Amino Acids*** | | |
| Glycine | 20 | Glycine |
| Alanine | 67 | Alanine, acetic acid |
| Serine | 0 | |
| Threonine | 0 | |
| Leucine | 3 | Leucine |
| Phenylalanine | 0 | |
| Tyrosine | 0 | |
| Hydroxyproline | 2 | |
| Proline | 52 | Proline |
| Cystine | 0 | |
| Methionine | 52 | |
| Histidine | 9 | Histidine |
| Tryptophan | 0 | |
| Glutamic acid | 14 | |
| Lysine | 42 | |
| Arginine | 0 | |

| | | |
|---|---|---|
| ***Heterocycles*** | | |
| 2,2'-Bipyridine | 59 | 2,2'-Bipyridine |
| Pyridine | 88 | Pyridine |
| Quinoline | 72 | Quinolinic acid |
| 2,4,6-Trimethylpyridine | 100 | 2,4,6-trimethylpyridine, chloro-dimethylpyridinecarboxylic acid |
| ***Purines and Pyrimidines*** | | |
| Adenine | 3 | |
| Barbital | 14 | Acetic acid |
| Caffeine | 26 | Methylamine |
| Cytosine | 2 | |
| Guanine | 2 | Guanine |
| Uracil | 1 | |
| ***Other Compounds*** | | |
| Anthracene | 0 | |
| Camphor | 4 | Camphor |
| Cholesterol | 0 | |
| Riboflavin | 1 | |

The most interesting finding is that glycine and alanine, which are ubiquitous in meteorites, are very resistant to such an oxidation. In conclusion of this section, we make a case that there should be no surprise if various organic compounds could be found on the Martian surface, despite the harsh oxidizing conditions.

## 3. PERCHLORATE IS COMPATIBLE WITH LIFE

Our understanding of the occurrence of perchlorates on Earth and their biological processing has evolved [4]. Initially, it was believed that they are mostly of anthropogenic origins, but now it is believed that perchlorate and chlorate, termed (per)chlorate, can form from chloride by natural oxidation processes that occur in the atmosphere. (Per)chlorates are usually found at low concentrations, except in the arid environments such as Atacama desert in Chile. It is now believed that the natural concentrations are kept low due to the microbial reduction. (Per)chlorate reducing organisms are found in two domains of life: bacteria (Proteobacteria and Firmicutes phyla), and in hyperthermophilic Archaea (e.g., *Archaeoglobus fulgidus*).

It is instructive to summarize metabolic pathways for (per)chlorate reduction, since they may be remnants of the early evolution of life. Perchlorate and chlorate were probably present on early Earth. Capacity to respire on perchlorate and chlorate many have been an ancient evolutionary development. Such metabolic capability would predate the photosynthesis and respiration on oxygen, and may reflect adaptation of early life to more oxidizing conditions.

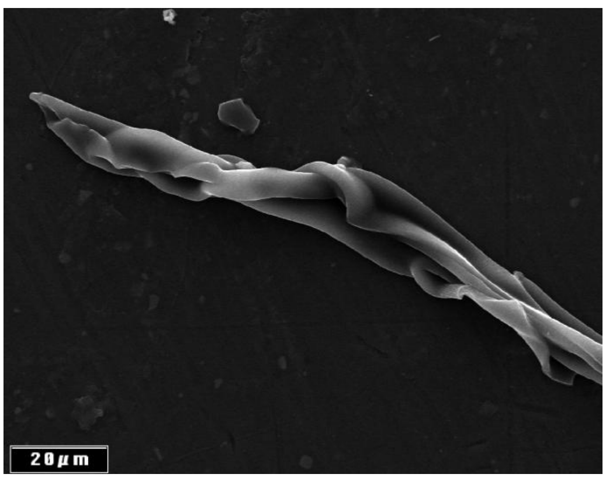
The perchlorate reduction pathway in bacteria utilizes (per)chlorate reductase which reduces perchlorate ($ClO_4^-$) to chlorate ($ClO_3^-$) and further to chlorite ($ClO_2^-$). Then chlorite dismutase transforms toxic chlorite to oxygen and chloride ($Cl^-$). In contrast, *A. fulgidus* does not have a chlorite dismutase. Instead, it detoxifies chlorite through abiotic reduction with sulfide. The latter is either from the environment, or is produced by *A. flugidus* by sulfate reduction. [4]

In conclusion of this section, we can see that perchlorate is not only compatible with life, but is also utilized as energy source by numerous species from bacteria and Archaea. We can imagine similar processes on Mars.

## 4. ORGANOHALOGENS ARE UBIQUITOUS ON EARTH AND ARE PRESENT ON METEORITES

In this section we give selected examples of the presence of the organohalogens on Earth [5-10] and carbonaceous meteorites [11]. In this context, it should be no surprise to find organohalogens on Mars. The literature on organohalogens on Earth is extensive. Abiotic formation of organohalogens during early diagenetic processes was surveyed in 2003 [5]. Naturally produced organohalogens are diverse and numerous. By 2003, more than 3700 organohalogens had been identified [6]. They contain mainly chlorine or bromine, but there are some which also have iodine and fluorine. These are produced by living organisms or are formed during natural abiogenic processes, such as in volcanoes, geothermal processes, forest fires, etc. The Earth's oceans are the largest single source of organohalogens. Plants, fungi, lichen, bacteria, insects, some higher animals as well as humans, also contain organohalogens [7]. The number of organohalogens that are discovered is rapidly growing. Only a dozen was known in 1954, more than 2900 in 1997 [7], 3700 in 2003 [6], and the list is growing to more than 5000 in 2012 [8-10]!

With regard to the possibility of chloromethane and other organohalogens on the surface of Mars, it is possibly very significant that organohalogens have been found in meteorites. [11] Four different carbonaceous meteorites (Cold Bokkeveld, Murray, Murchison and Orgueil) were analyzed and evidence obtained that halogenated organic compounds are present in all of these meteorites. Some of these compounds are relatively labile and can be extracted, but the larger part is polymerically linked in the kerogen which comprises the bulk of the carbonaceous material in these stones. The organohalogen compounds were possibly made by the abiotic natural halogenations but they may have also been produced by cyanobacteria, Archaea or other exotic microorganisms that might have inhabited the parent bodies of the meteorites. A sample of the Orgueil CI1 carbonaceous meteorite provided by the Museum of Natural History in Paris has been found to contain exotic filaments of unknown biological affinity (Fig. 1.a). Energy Dispersive X-Ray Spectroscopy (EDX) data reveals the filament is rich in fluorine, carbon, oxygen and silicon (Fig. 1.b).



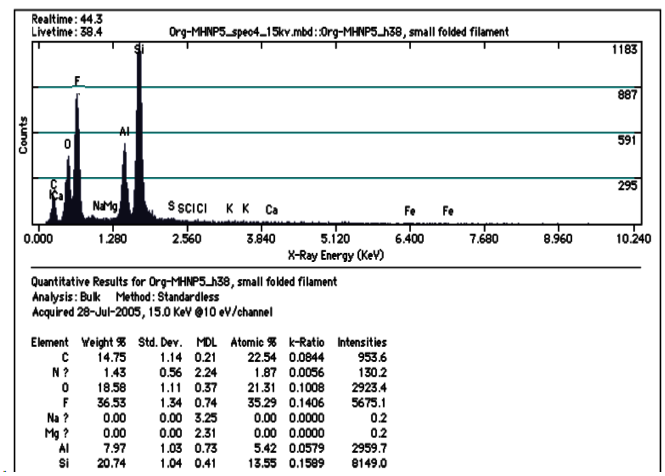




Fig. 1.a. Exotic folded filament found in the Orgueil CI1carbonaceous meteorite. Fig. 1 b**.** EDX data showing the filament is rich in fluorine, carbon, oxygen and silicon.

In light of the above, it would not be surprising to detect methyl chloride or a variety of other organohalogens on Mars. Since for approximately 4.5 Ga, the surface of Mars has been bombarded by carbonaceous meteorites as well as comets and asteroids it would be astonishing if organohalogens were not present on the surface. In addition to carbonaceous meteorites as a source, fungi, bacteria and hyperthermophilic Archaea that might have inhabited ancient Mars when the planet was warmer and wetter than today and when volcanic activity was present. Organohalogens could also be being produced by today by modern cyanobacteria, bacteria and archaea. In the Earth's polar regions many microorganisms thrive in sub-zero brines and other regions that were previously thought to be sterile. Cryptoendolithic microorganisms inhabit thin films of liquid water between ice and sand grains within polar sandstones and cryoconite microbial communities thrive in liquid water pools trapped between dark rocks and ice. [12-15] Microbes grow slowly in ice and supercooled brines and can remain viable for hundreds of thousands to millions of years in permafrost and ice. [13] A new species, *Carnobacterium pleistocenium,* of anaerobic bacteria was isolated and cultured after being entombed for 32,000 years in a frozen pleistocene thermokarst pond in Fox, Alaska [14] and living bacteria have been found deep within the polar ice sheet above Lake Vostok in Antarctica. [15]

## 5. LIFE ON EARTH UTILIZES/PRODUCES ORGANOHALOGENS

We give selected examples of utilization/production of organohalogens in the living organisms on Earth [16-19].
In anaerobic bacteria reductive dechlorination in the energy metabolism is known [16]. Examples include proteobacteria and gram-positive bacteria, for example. Some such bacteria use the energy generated in the dechlorination process for the synthesis of ATP. This is known as "dehalorespiration". Anaerobic bacteria which are capable of reductive dechlorination use various organohalogens. These include chlorinated aromatic compounds, e.g. chlorinated phenols or benzoate, aryl halides, and aliphatic chlorinated compounds, e.g. tetrachloroethane and trichloroethene [16].
Various organisms produce and use methyl chloride [17]. Examples include many species of wood-rotting fungi, which employ methyl chloride in lignin breakdown. Potatoes produce small amounts of methyl chloride when damaged or stored. Methyl bromide is also produced naturally. It is one out of many organobromides produced by marine organisms [16].

Basidiomycetes produce organohalogens [18]. Some of these fungi have the ability to synthesize *de novo* organohalogen metabolites. In the study, 191 fungal strains were tested. About 50% produced adsorbable organic halogen. Some novel chlorinated metabolites were isolated and identified.

Various slime-mould species use a chlorinated signal molecule, Differentiation-Inducing Factor 1 (DIF-1) [19]. Labeling with radioisotope $^{36}Cl$ *in vivo* was used to label DIF-1 and other low molecular weight organohalogens produced during development to study their action. This labeling method appears to be universally applicable for the *in vivo* study of the chlorinated compounds in various other organisms.

It has been shown recently that methyl chloride (chloromethane) is produced by two strains of marine cyanobacteria belonging to the genus *Synechococcus*[20]. This is of possible significance because these small coccoidal cyanobacteria are common in the cryptoendolithic communities that inhabit the interstices between grains in sandstones in the polar regions of Earth. [12, 21] These small cyanobacteria live within thin films of water and brine trapped between the grains. They are able to carry out photosynthesis within the rocks because sunlight can penetrate through the translucent grains of the sandstone. These would represent ideal environments on the surface of Mars, because thin layers of ice could encrust the surface of the rock and allow the saltwater brines to remain liquid within the rock even at temperatures well below $0^{o}C$. Hence the detection of methyl chloride on Mars may have been a solid biosignature of life rather than the result of contamination by the drill. This interpretation is strengthened by the fact that a strong peak of methyl chloride was also detected by Biemann et al. [22]at the Viking 1 Lander site. This peak was also attributed to contamination, but no explanation given as to how the instrument became contaminated by $CH_3Cl$. He also reported detection of methylene chloride (dichloromethane) in the soil samples collected by the Viking 2 Lander, which was also not detected in the flight blanks. The dichloromethane peak was interpreted as having resulted from heating perchlorate and organics to a high temperature, but it may also have resulted from biological activity of thermophilic or halophilic archaea or cyanobacteria. [23] In 2006, Keller et al. published the results of the Odyssey Gamma Ray Spectrometer (GRS) and showed that chlorine is very widely distributed across the equatorial and mid-latitudes of Mars. The mean concentration of 0.49 wt.% indicates a significant enrichment in chlorine in the upper few tens of centimeters of the regolith with respect to SNC meteorites and estimates for the bulk composition of the planet. This is the region of the permafrost in which cryptoendolithic cyanobacteria and other photosynthetic microorganisms would be expected. They would have to be near the surface to carry out photosynthesis but deep enough to be protected from desiccation by frozen water ice crystals between the regolith grains.

In conclusion of this section, life has found a way to interact with or produce organohalogens on Earth. Furthermore, small coccoidal cryptoendolithic cyanobacteria, such as would be best suited to survive within the protective environments of sandstones and the uppermost layers of the regolith of Mars are capable of producing chloromethane and dichloromethane by biological activity. These are the same organohalogens that have been detected by both Viking Landers and by the Curiosity SAM instrument. In these cases these organics have been repeatedly dismissed as instrument contaminants without any explanation as to how the instruments became contaminated. This suggests that the Curiosity data should be re-examined in light of the possibility that they may have detected molecules produced by present day biological activity on Mars.

## 6. CONCLUSIONS

We have presented several lines of evidence which indicate that the presence of perchlorates on Mars should not interfere with the findings of the organic compounds, and it should not exclude the presence of life. We have further provided evidence that organohalogens are ubiquitous on Earth and come from both abiogenic and biogenic sources. Organohalogens are also found in a number of CM2 and CI1 carbonaceous chondites (e.g. Cold Bokkeveld, Murray, Murchison, and Orgueil). Thus, it would not be surprising to find such compounds on Mars. Finally, we have given selected examples of the utilization or production of organohalogens by life on Earth. Thus, the presence of some organohalogens on Mars, such as methyl chloride, could be a sign of life.

## REFERENCES


[1] Kerr, R. A., "Pesky perchlorates all over Mars", Science, 340, 138 (2013).

[2] Kolb, V.M., "Oxidation of organic materials with perchlorates: Relevance to the chemistry on the Martian surface", Proc. SPIE, 7441, 74410E (1-5) (2009).

[3] Martinie, G. D., and Schilt, A. A., "Investigation of the wet oxidation efficiencies of perchloric acid mixtures for various organic substances and the identities of residual matter", Analytical Chem., 48, 70-74 (1976).

[4] Liebensteiner, M. G., Pinske, M. W. H., Schaap, P. J., Stams, A. J. M., and Lomans, B. P., "Archaeal (Per)chlorate reduction at high temperatures: An interplay of biotic and abiotic reactions", Science 340, 85-87 (2013).

[5] Scholer, H. F., and Keppler, F., "Abiotic formation of organohalogens during early diagenetic processes", The Handbook of Environmental Chemistry, Vo. 3, part P, 63-84 (2003).

[6] Gribble, G. W., "The diversity of naturally produced organohalogens", The Handbook of Environmental Chemistry, Vol., 3, Part P, 1-15 (2003).

[7] Gribble, G. W., "Naturally occurring organohalogen compounds", Acc. Chem. Res., 31, 141-152 (1998).

[8] Gribble, G. W., "Recently discovered naturally occurring heterocyclic organohalogen compounds", Heterocycles, 84, 157-207 (2012).

[9] Gribble, G. W., "Occurrence of halogenated alkaloids", The Alkaloids, 71, 1-165 (2012).

[10] Gribble, G. W., [Naturally Occurring Organohalogen Compounds – A Comprehensive Update], Springer-Verlag, Wien, (2010).

[11] Scholer, H. F., Nkusi, G., Niedan, V. W., Muller, G., and Spittoff, B., "Screening of organic halogens and identification of chlorinated benzoic acids in carbonaceous meteorites", Chemosphere, 60, 1505-1512 (2005).

[12] Hoover, R. B. and Pikuta, E. V. "Psychrophilic and Psychrotolerant Microbial Extremophiles in Polar Environments." In [Polar Microbiology: The Ecology, Biodiversity and Bioremediation Potential of Microorganisms in Extremely Cold Environments] (Eds. A. K. Bej, J. Aislabie and R. M. Atlas) CRC Press, pp. 115-157. (2009).

[13] Hoover, R. B. and Gilichinsky, D., "Significance to Astrobiology of Microorganisms in Permafrost and Ice, " In [Permafrost Response on Economic Development, Environmental Security and Natural Resource Potential], NATO-ARW held in NOVOSIBIRSK, SIBERIA, 12-16 Nov. 1998. (R. Paepe, Ed.) Kluwer Publishing, New York, pp. 553-580, (2001).

[14] Pikuta, E. V., Hoover, R. B., Marsic, D., Bej, A., Tang, J. and Krader, P., "*Carnobacterium pleistocenium* sp. nov., a novel psychrotolerant, facultative anaerobe isolated from Fox Tunnel permafrost, Alaska." Int. J. Syst. Evol. Microbiol, **55,** 473-478, (2005).

[15] Abyzov, S. S. Duxbury, N. S., Bobin, N. E., Fukuchi, M., Hoover, R. B., Kanda, H., Mitskevich, I. N., Mulyukin, A. L., Naganuma, T., Poglazova, M. N. and Ivanov, M. V. "Super-long anabiosis of ancient microorganisms in ice and terrestrial models for development of methods to search for life on Mars, Europa and other planetary bodies." Advances in Space Research, Space Life Sciences, 38**,** 1191-1197, (2006).

[16] Holliger, C. H., Wohlfarth, G., and Diekert, G., "Reductive dechlorination in the energy metabolism of anaerobic bacteria", FEMS Microbiology Reviews, 22, 383-398 (1999).

[17] Winterton, N., "A role for methyl chloride in evolution?", Mutation Research, 372, 147-148 (1996).

[18] Verhagen, F.J. M., Field, J. A., Swarts, H. J., and Wijnberg, J. B. P. A., "The natural production of organohalogens by Basidiomycetes", Organohalogen Compounds, 27, 220-224 (1996).
[19] Kay, R. R., Taylor, G. W., Jermyn, K. A., and Traynor, D., "Chlorine-containing compounds produced during *Dictyostelium* development", Biochem. J., 281, 155-161 (1992).
[20] Ambiru, K., Kurihara, M., and Hashimoto, S., "Methyl Chloride production by two marine cyanobacteria: *Synechococcus* sp. (CCMP 1334) and *Synechococcus* sp. (NIES 981). 2012 ASLO Aquatic Sciences Meeting. July 8-13, 2012. http://www.sgmeet.com/aslo/japan2012/viewabstract2.asp?AbstractID=9715
[21] Whitton, Brian (Ed.), [Ecology of Cyanobacteria II: Time Their Diversity in Space and Time], Springer, New York, p. 303. (2012).
[22] Biemann, K., Oro, J., Toulmin III, P., Orgel, L. E., Nier, A. O., Anderson, D. M., Simmonds, P. G., Flory, D., Diaz, A. V., Rushneck, D. R., Biller, J. E. and Lafleur A. L. "The search for organic substances and inorganic volatile compounds in the surface of Mars.", J. Geophys. Res. 82, 4641-4658, (1977).
[23] Abed, R. M. M., Dobretsov, S., Al-Fori, M., Gunasekera, S. P., Sudesh, K., and Paul, V. J. "Quorum sensing inhibitory compounds from extremophilic microorganisms isolated from a hypersaline cyanobacterial mat. " J. Industrial Microbiology and Biotechnology. April, 2013. http://arxiv.org/abs/1304.5324
[24] Keller, J. M. *et al.*, "Equatorial and Midlatitude Distribution of Chlorine Measured by Mars *ODYSSEY* GRS."J. Geophys. Res. Planets E3, 1991-2012, (2006).