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Biological exploration of Mars: Survival and metabolic activity testing of extremophiles exposed to simulated Martian soil and atmosphere, using a Mars environment simulation chamber

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Abstract

The importance of studying Mars and its environment lies in the fact that one could indicate a lot about its past and the possibility of a future colonization. Here, we studied its atmosphere in terms of its past history but also its present environment searching for microbial life able to tolerate it.

In the first part of this thesis, an investigation of the origin of the Martian atmosphere was made based on the outgassing and collision scenario. The results indicate a possible favorable planet for about 0.8 Ga to 1.5 Ga from the begging of its history.

In the second part, the study focused on its present potentiality to be hospitable for bacteria found on the extreme environment of Atacama desert. The experiments operated in conditions of perchlorate presence, low atmospheric pressure, anaerobic and carbon dioxide dominated atmosphere, 17% relative humidity, UV exposures and the using of analog soil simulants. The results showed that some of them were able to tolerate some or all of the testing parameters.

In the third and last part, metabolic activity measurements proceed to detect possible residual gases coming from the bacteria exposed to anaerobic and nitrogen atmosphere with 15% of relative humidity in both room and 4°C temperature. The results showed carbon dioxide emission in both conditions, with a decrease in the production compared to regular conditions especially for the lower temperature exposure.

Acknowledgements

The accomplishment of the present thesis project would not have been fulfilled without the valuable help and guidance of my mentors, the mechanical workshop and all of the academic staff of the University of Copenhagen.

I would like to thank all of those who continuously helped me with my project. Firstly, the University of Copenhagen and especially the Niehls Bohr Institute which gave me the opportunity to meet and collaborate with all of those excellent professionals. Secondly, my supervisors but mostly my mentors: Uffe Gråe Jørgensen, Morten Bo Madsen and Anders Priemé who were always there to guide me with patience and provide me with their valuable knowledge. Then, to all of those people in Microbiology department who accepted me and let me work in their environment and also the mechanical workshop and Jannis Bouchikas who helped me carry out many reconstructions with the Jens Martin Martian Chamber. Then, my co-partners in this project, Cecillie Patricia Knudsen and Poul Kari Madsen for an excellent collaboration, and also my microbiology laboratory partner Miruška Gužíková who was always there providing me with suggestions and advice. Also, the grant that supported the project. We acknowledge support from the Novo Nordisk Foundation Interdisciplinary Synergy Program grant no. NNF19OC0057374; Effects of bacteria on atmospheres of Earth, Mars, and exoplanets".

And last but not least, I would like to thank my family for always being there for me emotionally and financially supporting my dreams.

Motivation for the selected project

Starting my school years as a curious person for our cosmos Astrophysics driven me to chose my bachelor to be in Physics with a specialty in Astrophysics. Soon after I realised that my curiosity and passion was focused in the field of Astrophysics that was related to biology exploration beyond Earth.

During my research for the right Master studies soon I came across the program provided by Niehls Bohr Institute and the great academic staff that were focused in the field of my interest. Gaining knowledge from all of the courses I choose to follow in the first year of studies the selection of my supervisors was the most accurate combination that would provided me with the best guidance in the multidisciplinary field of Astrobiology.

Knowing only a little about life and possibilities of life beyond Earth the nearest physical laboratory for testing and exploring is our neighbour planet, Mars. The proximity and the history of the planet along with the various surface and atmospheric exploration through the last decades have provided us with a range of data that are valuable for further exploration of the planet including the scenario of its past habitability and maybe its future colonisation. In order to explore the potential habitability of Mars the gain of knowledge from different fields of studies is necessary for a better understanding.
The project was started with the Microbiology department providing us with soil from the extreme environment of Atacama desert, and alongside with the newly reconstructed Martian chamber we were be able to test if microbial life coming from these regions is able to cooperate in the extreme simulated Martian conditions in the laboratory. Also, using instruments like the Quadrupole Mass Spectrometer and Gas Chromatographer our goal was the detection of microbial exhausted gases in the Martian analogue atmosphere. A project coming from this idea was an extreme opportunity for me to explore and learn to combine concepts and theories from the field of f Astrophysics, Experimental physics and Microbiology that would come along and complete each other.

Working background and limitations

In order to complete the project and achieves its goals the functionality of the Martian chamber was crucial for various of our experiments. A team consisted of Morten Bo Madsen, Cecillie Knudsen, Poul Kari Madsen and myself along with the help providing from the mechanical workshop was constantly trying to improve the performance of the chamber. While figuring out the necessary improvements the chamber was in need to, a five months absence from the laboratory occurred due to reconstructions caused by a leak on the ceiling in the laboratory room and following the isolation of us from the University facilities due to COVID-19 restrictions leaving behind our first attempt for

exposing bacteria using the chamber.
Taking advantage of the isolation, Cecillie Knudsen and I decided to do a research for the origin of the Martian atmosphere in order to estimate for how long time the Martian atmosphere was able to keep conditions favorable for life. Starting together from the two origin theory scenarios of outgassing and collision, Cecillie managed to find that due to our model something interrupted the cohesion of the Martian atmosphere while I did a research based on previous studies that the strength of the solar wind was a key factor that influenced the atmosphere of the planets. Also, together we wrote the first introductory chapter of our projects as both of us focused on the same concepts regarding life on planet Mars.
For the preparation of the bacteria cultures and the various of the parameters we wanted to expose them, Cecillie, Poul and myself worked together constantly exchanging ideas and learning Microbiology concepts from the biologist, Poul. Due to the restrictions we managed to take permission for a limited time in the laboratories to prepare our experiments while the colonies counting performed by Poul at his home and some at the laboratory.
With the new opening of the University facilities, I was able to have permission again to work in the laboratories. Gaining theoretical and experimental knowledge from the previous experiments, I was able to structure my own experiment focusing in the detection of exhausted gases coming from bacteria that were exposed to Martian conditions. The reconstruction of the new experimental set-up in the chamber that would be able to measure the residual gases as a result from their potential metabolic activity was in need for new improved equipment components. Some of them was easier to find in time and some others took us several months until they were available due to unpredicted delay in delivery. For this reason, until the moment where the chamber will be completely functional and able to measure the small fraction of these gases, the Gas chromatographer placed in the Biology department used to analysed the samples.

Contents

1 Background to understand Thesis and its experiments

Are we alone? Three small words, that imply an enormous question. A question that has sparked interest and baffled people throughout time. What if we are? What if we are not? The questions are many and the answers will most likely vary. In the last decades the search for clues has increased extremely in an attempt to try and answer the question "Are we alone?". Both ground and space based telescopes, rovers and satellites are trying to find clues to resolve this question. At the moment, one of the best suggestions for finding signs of life, either past or present, in our own solar system is Mars. In 1976 the first successful landing of a lander on the Martian surface took place, with Viking 1 and Viking 2 [Freedman et al., 2016]. From 1976 and up until today the examination of the planet is still ongoing. Though not every mission focuses of finding sings of life, other missions are still providing key information about the planet and its past.

The possibility of finding life or for life surviving on Mars is the main focus for the following sections. Before heading to the red planet, there is a short introduction going through the creation of the Universe. From the Big Bang to the creation of the elements, and from there to the creation of planetary systems (Section 1.1). This serves as a short introduction to the building blocks for the creation of everything, life included, ending with the creation of solar systems. The next section (Section 1.2) focus more in-depth on the Martian environment and the evolution of the planets through time. Focusing on the geology, the magnetic field, the atmosphere as well as the possibility of water both past and present on the surface, concluding with a section on the search for life on Mars. This leading to the third part (Section 1.3), a short introduction to biology and life with focus on bacteria. The last section (Section 1.4) takes a closer look at the possibility of terraforming Mars, as well as some of the ethical and possibly moral problems when it comes to space exploration.

1.1 From the Big Bang to the building blocks of life

In order to understand the physical processes in the Universe, including the physical principles that govern the rules of life we have to go back in time and see how the cosmos began.

1.1.1 The origin of elements

From a cosmic point of view, all of the observed matter was created during the first few minutes in the history of the Universe, in the Big Bang, 13.8 billion years ago [Ade et al., 2016]. During the first minutes, the Universe expanded extremely rapidly, with this phase being known as the inflationary epoch. After this time the Universe continued to expand with a slower expansion rate than previously, despite still being in a very high density and temperature state.
The Big Bang nucleosynthesis -primordial nucleosynthesis- took place during the first few minutes. This was while the temperature was very high and it refers to the production of hydrogen, helium, small amounts of deuterium, lithium and beryllium. At this point the

universe had a temperature low enough for deuterium to survive, but also high enough for fusion reactions to occur since there were still free neutrons available.

Biological life requires heavier elements, than the primordial, in order to be formed. The formation of these elements came by the result of thermonuclear reactions in the core of stars, and also during supernova and kilonova explosions. When a star's core runs out of hydrogen and expands into a red giant, it begins to produce carbon atoms by fusing helium atoms. Elements heavier than carbon are more complex to produce because the various reactions can lead to many nucleus in risk of being destroyed due to high temperatures. Stars with higher masses continue the nuclear burning. The elements formed in these stages range from oxygen through to iron. It has been noticed that nuclei with atomic mass multiple of 4 are more abundant (^{12}C, ^{16}O, ^{20}Ne, ^{24}Mg, ^{28}Si, ^{32}S, ^{36}Ar, ^{40}Ca, ^{56}Fe). In total 98% of elements in the universe are hydrogen and helium and three quarters of the rest are made up of carbon and oxygen. Also, an overabundance for the elements around iron is observed, the reason is the strong binding energy of nuclei with a mass close to iron.

During the later phases of red giants, supernova or kilonova, the star releases great amounts of energy that leads to the formation of elements heavier than iron, such as uranium and gold. In these type of explosions, all the elements produced during the previous stages of life of the star are ejected into space.

The energy from these nuclear reactions is the high energy and it emits in the x-ray/gamma rays part of the spectrum. In the end, it is the nuclear reaction that drives the light emitted from the stars. And it is this light that heats the surface of the planet. This energy is responsible for warming the planet, influencing the weather and providing energy for life.

"The cosmos is within us. We are made of star-stuff. We are a way for the universe to know itself." — Carl Sagan

1.1.2 Interstellar Medium

The matter and radiation that exists in the space between the stellar systems in a galaxy, the interstellar medium or ISM, is an area where further reactions can occur and form more complex molecules. The interstellar medium consists of gas and dust. The gas phase dominates the medium (99% by mass [Boulanger et al., 2000]) with about 90% hydrogen, 10% helium (by number), and trace amounts of other elements, especially oxygen, carbon, and nitrogen [Ferriere, 2001]. The dust part consists of dust grains varying from millimeter to sub-millimeter size and having a carbonaceous or silicate-based core surrounded by an icy mantle of water (H_2O), carbon dioxide (CO_2), or ammonia (NH_3) [Srama et al., 2004]. The first diatomic molecules were discovered in the ISM almost a century ago. Today, more than one hundred species have been detected in the ISM [McGuire, 2018]. In this list of molecules, there are some related to the essential molecules of life, like glycine [Jiménez-Serra et al., 2014].
These molecules have been detected using atomic and molecular lines as tracers (e.g.

CO, HI 21cm, Hα). Analyzing the spectra observations makes it possible to estimate the different parameters (like temperature, abundance of elements, column density) that characterize such environments [Stahler and Palla, 2008]. Studying the densest regions of ISM, where stars are born and eventually planetary systems form, can tell a lot about the chemistry that takes place during star formation. This can lead to a better understanding of the origin of life and possibly elsewhere.

1.1.3 Planetary systems

Now knowing how these elements necessary for life were formed, the next step is to understand how they ended up in planetary systems. The standard theory that describes the way that solar systems formed, was first explained in 1796 by Pierre-Simon Laplace. This theory explains how rotating gas cloud collapse due its own gravity and as a result form a disk (Fig. 1). The solar system formed about 4.5 billion years ago from a dense cloud of interstellar gas and dust. The cloud collapsed, possibly due to a shockwave. The central parts of the cloud collapsed to form the Sun, and the remainder of the material created a flattened disk, the protoplanetary disk [Boss and Ciesla, 2014].

At the center of the disk, gravity accreted most of the surrounding material and the pressure in the core becomes so high that hydrogen atoms began to fuse and form helium. The mechanisms that lead to the formation of planets are still being a current topic in research across the world. One of the leading theories is that they formed due to accretion. After the dust particles had grown large enough, the Sun's gravity dragged them towards the mid-plane of the nebula. The dust began to collide and then grew until it reached a planet-sized body. As these planetesimals (solid objects exist in the protoplanetary disk) build up, some clumps were able to collide and build the final planets. More elliptical orbits would lead to collision of planetesimals and building up the planets.

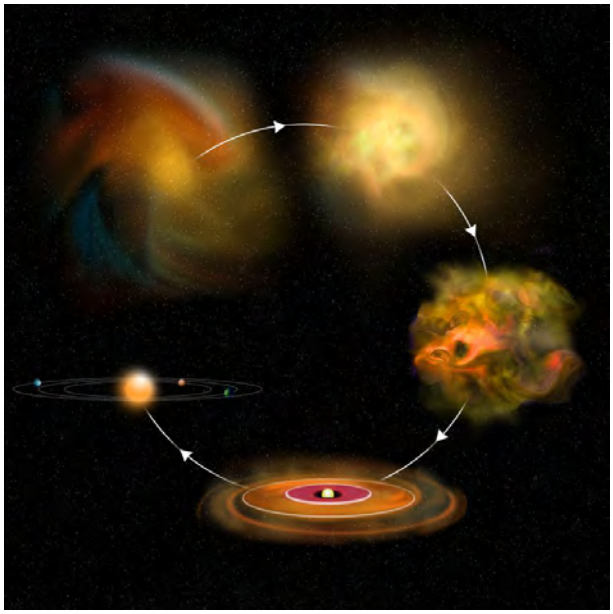

Figure 1: *Illustration of the planetary formation theory - image source Bill Saxton, NRAO/AUI/NSF.*

The theory for the solar systems is that the Sun and the planets formed around the same time, however there is no direct evidence supporting this. One can determine the age of a young cluster and then get an idea of the age of the Sun, but nothing about the age of the planets. The age of meteorites in the solar system having an estimated age of 4.5 Ga supports this age theory [Hedman, 2008]. Further support to the theory comes from looking at the direction of the orbits of the planets around the sun. If they had not been created from the disk at the early stages in the solar system, but rather been accreted from elsewhere in the Universe, we would see a large variety in the directions of the orbits.

Studying regions in which stars are born (like the famous M16 region), and running creating and examining simulations of the creation of solar systems could help to answer an elementary question. Is the solar system unique and different, or is it simply one out of many other solar systems created under the same initial conditions.

1.1.4 The snow-line and the water paradox

Early in the life of the solar system, before the star had fully accreted the surrounding materials and planetesimals were still accreting in the disk. Elements condense at different temperatures and some elements can only condense further out where the temperatures are sufficiently low. This leads to the planets growing bigger and when they reach approximately ten times the size of Earth, they tend to attract hydrogen and helium gases of the disk. If these gases are cold enough, then they can condense onto the planet. Further in, closer to the Sun, elements which can condense at higher temperatures form the inner planets.

There is a specific distance from the Sun, the so-called snowline, which is estimated to be at approximately 2.7AU to 3.2AU[1], depending on the model used to calculate it [Hayashi, 1981, Martin and Livio, 2012, Podolak and Zucker, 2004]. At this distance it is cold enough for water molecules to be bound into a solid state. The lower temperature in the nebula beyond the snow-line makes a large number of solid grains available for accretion into planetesimals and eventually planets. The snow-line separates the terrestrial planets from the gas planets [Kaufmann, 1987].

Due to low pressure in space water can not exist in its liquid state. As a result water can only exist in the form of gaseous and solid state. The interesting thing with the solar system is that the terrestrial planets are closest to the Sun (within the snow-line) and the gas giants are further out (beyond the snow-line). When observing exoplanetary systems gas giants are usually found much closer to the host stars than in the Solar system. These gas giants are thought to have formed outside the snow-line and later migrated inwards to their current positions [Chambers, 2007] [D'Angelo et al., 2010]. The fact that Jupiter did not migrate closer to the Sun than it is today, may play a role in the existence of life and of Earth as known today. The planets beyond this line will have a core mainly of water. A remarkable point is that while water can only condense outside of the snow-line, all known life exists within the snowline and relies heavily on water. The origin of this

[1] Astronomical units $1.5 \cdot 10^8$ km

water on the inner planets remains unknown. [Jørgensen, 2019].

Bearing in mind how all these mechanisms contribute to the creation of the planets and life as we know it, it would be strange if we were the only life form in the Universe.

1.2 Mars - Earth's smaller neighbour

Having gone briefly through the evolution of the universe, the Big Bang, the creation of elements, stars and planets the focus is now shifted onto one specific planet - Mars. Mars is the fourth planet from the Sun and is now one of the top candidates for the search for life within the Solar system. At the time of this thesis it is not known if life exists on Mars or if it ever has. But when looking at the possibility of life on a planet, the planet itself must first be understood. The following sections (Section 1.2.1-1.2.5) takes a deep dive into the red planet, the geology and its evolution through time. As well as taking a closer look at different planetary factors in order to understand the environment with the possibility of life in mind. A closer look at the magnetic field and atmosphere, the evolution of those and the possibility of liquid water both now but also in the past. The section ends with a short look at the investigations for life and habitability, from the past, the present and future.

1.2.1 The geological periods of Mars

Mars is believed to not always have been the dry wasteland that it is today. But to be a planet, where the environment and geological features have changed over time [Carr and Head III, 2009], much like seen on Earth. The geological history of Mars can roughly be separated in to three geological periods, the Noachian, the Hesperian and the Amazonian, each of them named after larger areas on the Martian surface [Carr and Head III, 2009]. The span of each period varies and the exact time for when one period changes to another are not completely defined. Throughout each period, the geological features as well as the environment of Mars has changed. Since the exact time of change from one period to the next is not entirely defined, the age might vary depending on where one looks up the information. For the age and primary source of information on the geological periods, the paper by [Carr and Head III, 2009] is used. For any location references on Mars see Fig. 2.

One of the reasons why the areas can be hard to date is due to the lack of rock samples. When an area on the Martian surface is dated it can be done by crater count. An area or surface of a planet is initially barren (crater less), during time more and more craters will form on such surface. Simplified, a given number of craters on a surface therefore equals a specific age of the given area or surface. The number of craters forming will vary depending on the planet or moon (due to size, gravity, atmosphere etc.). It should however be noted that an age based on a crater count will vary depending on the model used. And also that it is just a model age until the age of actual rock samples can be determined and compared with the age from the model. This has been done with collected rock samples from the Lunar surface giving a more precise model age. At the moment since no Martian rock samples with a known origin has found they way to Earth, the

models used for determining the age of the Martian surface and areas are made from extrapolated data from the Moon. This means that in the case of Mars (and any other extraterrestrial surface except for the Moon) the age found by using one of the models, will only be an estimate or a model age and will again vary depending on which model is chosen.

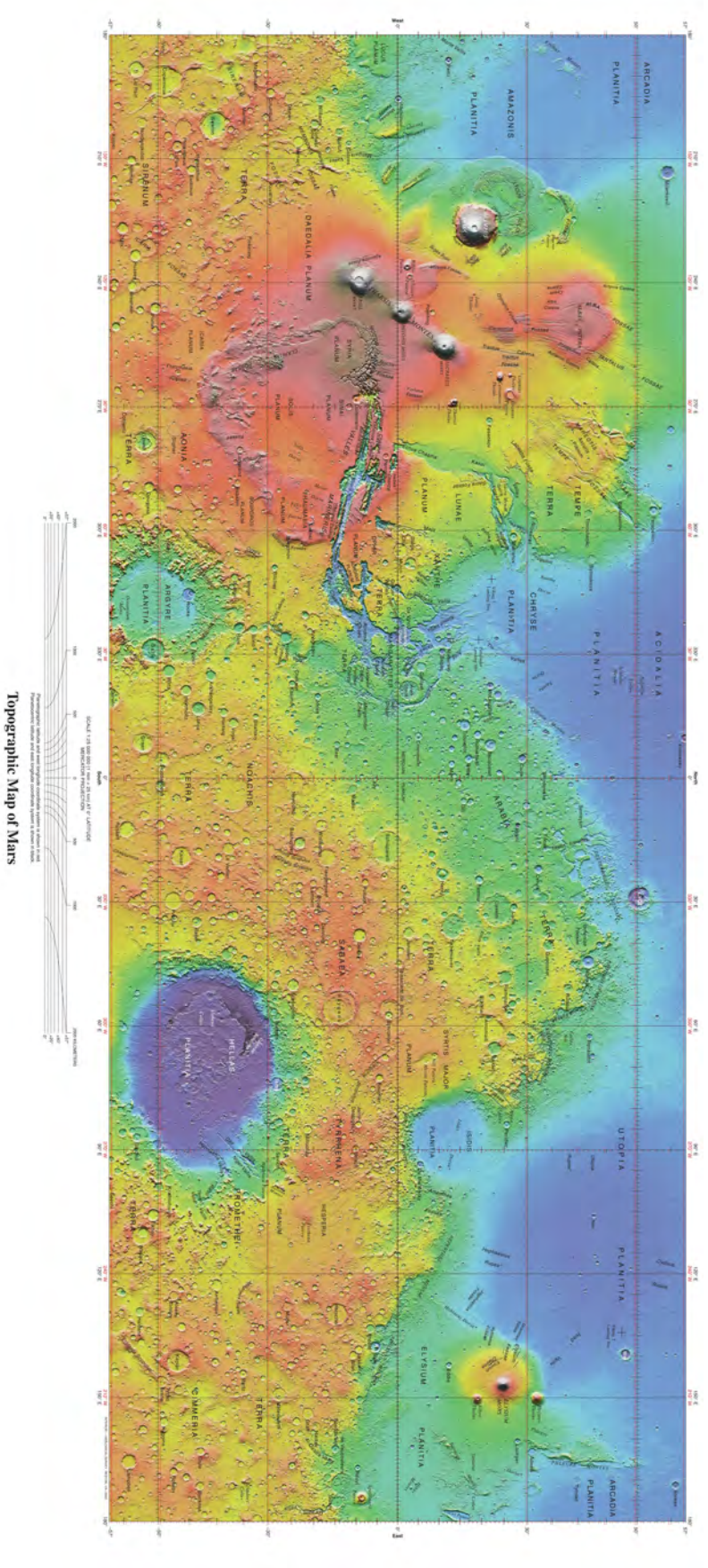

Figure 2: *Topographical Map of Mars [USGS, 2020].*

The Noachian period

The Noachian period is the first of the three periods, though a Pre-Noachian period is believed to have existed as well [Carr and Head III, 2009]. The Pre-Noachian period is believed to be from 4.5 – 4.1 Ga ago and not much is known about it, due to nearly non geological record. Periodically large basins and craters was created and as a result this, had a huge effect on the environment. During the Pre-Noachian period the planet also had a magnetic field [Carr and Head III, 2009].

The Noachian period is believed to stretch from 4.1 Ga ago till around 3.7 Ga ago [Carr and Head III, 2009] (overlapping with the Late Heavy Bombardment (LHB) stretching from around 4.1 - 3.8 Ga years ago [Freedman et al., 2016]). The Noachian period is named after the area found on the southern highlands, the Noachis Terra/region [Carr and Head III, 2009], located just west of the Hellas basin.

The period is recognized for its high number of valley formations and its large crater formation rate as well as erosion [Carr and Head III, 2009]. As mentioned the Noachian period overlaps with the LHB, and about 40 % of the Martian surface dates to the period. The largest impact craters also dates to the Noachian period [Solomon et al., 2005]. With the creation of Hellas marking the beginning of the Noachian period [Carr and Head III, 2009].

Volcanic activity was present on the Martian surface during the period, through most of it, likely took place in the Tharsis area [Carr and Head III, 2009]. When observing from planetary orbit (with a resolution of 100 meters) the Noachian landscape is, even though volcanic activity was present, influenced by impacts. [Carr and Head III, 2009].

Evidence suggest the possibility of having shorter periods of time with warmer conditions as well as rainfall during the Noachian period. There are however uncertainties as to if this phenomena was local or on a global scale due to the difficulties in maintaining the conditions that would be required. [Carr and Head III, 2009]. How these warmer conditions, during the Noachian period, came to be is still unknown. It is possible that it was due to impact or volcanic eruptions [Carr and Head III, 2009]. Parts of the valley systems are however believed to have been formed by water, indicating that water must have been present in some quantities, during the period [Jørgensen, 2019].

The end of the Noachian period overlaps with the earliest evidence of the first microbial cells on Earth, which might be as early as between 3.9 and 3.8 Ga ago. [Madigan et al., 2015]. The conditions on the Martian surface might have been similar to Earth and might have been present long enough, for the time it took life to emerge on Earth. The conditions on Mars might even have lasted longer, since the planet is smaller and as a result potentially cooled down to a suitable temperature before Earth [Jørgensen, 2019].

The Hesperian period

The Hesperian period is the second of the three periods and begins after the Noachian 3.7 Ga ago and stretched to 3 Ga ago. The period has gotten its name from the Hesperia Planum [Carr and Head III, 2009], which is located just north-east of the impact basin Hellas Planitia. The erosion, weather and the formation of valleys slowed down and were much lower than in the Noachian period. Despite this, there were still volcanic activity taking place throughout the period [Carr and Head III, 2009].

During the Hesperian period the valleys formation rate dropped, but did not stop completely. Episodically and locally the circumstances for formation of small valleys was present [Carr and Head III, 2009]. Despite the low rate of valley formation, especially in the later parts of the period, huge floods of water would occasionally form. In the northern lowlands bigger bodies of water might have been created as a result [Carr and Head III, 2009].

Sulfates are found on the Martian surface, though primarily detected in the western hemisphere and near the north pole. Observation has been made from both orbit and from landing sites, detecting deposits of sulfates from orbit and concentrations in the soil at different landing sites [Carr and Head III, 2009]. In some of the valley networks sulfates are present, suggesting that some water activity was still present during the period [Carr and Head III, 2009].

The Amazonian period

Following the Hesperian period, is the most recent and present period, the Amazonian. The period is named after the area Amazonia Planum [Carr and Head III, 2009] located in the northern part of Mars. The Amazonian period stretches from the end of the Hesperian at 3 Ga ago up until today and is the longest of the three periods [Carr and Head III, 2009].

During the Amazonian period any larger scale geological changes were mostly limited compared to the two previous periods [Carr and Head III, 2009]. Just like in the previous period volcanic activity was still present, though primarily in the Elysium and Tharsis area. The activity was also significantly lower (by a factor of ten) than previously. One, if not the most, notable mark for the period, might be the change in the landscape due to ice. Wind might also have played an important role [Carr and Head III, 2009].

Locally areas being covered by ice might have happened, in a number of places and small amount of floods might also have appeared occasionally. [Carr and Head III, 2009]. Another change due to water are gullies, most of those found on steep slopes was formed late in the Amazonian period [Carr and Head III, 2009].

1.2.2 The Martian magnetic field

The magnetic fields are commonly believed to be generated by the dynamo effect created inside the planetary core. The magnetic field arises as a result of the core alloys movement. In the solar system the terrestrial planets Mercury and Earth have magnetic fields but Venus and Mars do not. The presence and lack of magnetic fields for the planets are believed to depend on the composition of the alloys in the core and how quickly it cools down[2] [Ehlmann et al., 2016]. Even though Mars does not have a global magnetic field today, [Solomon et al., 2005] the planet is believed to once have had one, much like Earth has today. [Sakata et al., 2020]. Signs of remnants of a magnetic field are found in the Martian crust [Acuña et al., 1998][Solomon et al., 2005]. Magnetic remnants can appear when a magnetizable object has been exposed to a magnetic field, leaving the object slightly magnetized when the external field is removed [McElhinny and McFadden, 2000]. Weaker magnetic anomalies are found in some areas in the northern lowlands and stronger magnetic anomalies are found near the southern highlands/uplands [Solomon et al., 2005].

Data today indicate the Martian magnetic field disappeared (due to the halting of the dynamo) at around 3.9 Ga to 4.1 Ga ago [Ehlmann et al., 2016]. One of the arguments for this is the lack of magnetic anomalies found in the large impact basins (Argyre, Hellas, Isidis and Utopia) [Solomon et al., 2005]. The deficiency of magnetic anomalies in the impact basins could indicate that the dynamo had already stopped when the impact took place. [Ehlmann et al., 2016]. Hellas, is the oldest of the four impact basins, as mentioned above, and is dated to be somewhere between 3.9 Ga and 4.1 Ga old (estimated from crater counts) [Ehlmann et al., 2016]. Looking at basins with similar age, but where magnetic anomalies are found, and comparing these with the four basins mentioned above, suggest that the cease of the Martian dynamo might have been very sudden [Ehlmann et al., 2016].

As mentioned the Martian magnetic field is believed to have stopped somewhere between 3.9 and 4.1 Ga years ago. This is an estimate and the actual age for the ceasing of the magnetic field cannot be precisely determined until rock samples can be tested. The exact age of these samples also needs to be determined in order to get an age [Ehlmann et al., 2016].

It is thought that with the ceasing of the Martian magnetic field that the atmosphere was more vulnerable, allowing the solar winds and the Martian atmosphere to interact leading to the atmosphere being stripped away. The lack of the magnetic field not only led to the possible stripping of the atmosphere, but may also have led to a high number of charged particles (cosmic rays) reaching the Martian surface. The presence of charged particles can damage near surface materials chemical bonds, which in turn can create different oxidants (like perchlorates) [Ehlmann et al., 2016].

Though commonly believed that the magnetic field will protect against the interaction between the solar wind and the planetary atmosphere [Ehlmann et al., 2016], in some cases a weak magnetic field might lead to a greater loss of atmosphere [Sakata et al., 2020]. Some simulations made of the Martian magnetic field show that a weak magnetic field

[2]This however, does not seem to be only depending on the rotational rate of the planets, since both Earth and Mars are rotating faster. Nor does it seem to be only depending on size, since both Earth and Venus are approximately the same size [Ehlmann et al., 2016]

might add to atmospheric loss. Which in turn leads to a greater loss of ions like O_2^+ when a weak magnetic field is present compared to no field at all [Sakata et al., 2020].

1.2.3 The Martian atmosphere

The study of the planetary atmospheres, the origin as well as the physical and chemical processes that occur and the mechanisms that determine their evolution, is crucial to determine a planet's habitability. The study of the terrestrial planets in the Solar system can lead one step closer to understanding if life could exist presently or previously. This could also help improve the expectations and conclusions in planets' surveys beyond the solar systems, the exoplanets.

The exact mechanisms that caused the formation of the atmospheres which is seen today is still unknown and being studied. From proto planetary conditions and the proto atmosphere,[3] to the secondary atmospheres that evolve to the one seen today, the atmospheric conditions of the planets have been changed throughout their history. Two main scenarios for the origin of Earth's atmosphere, may also applied for other terrestrial planets. The first scenario is due to outgassing and the second for the creation of the atmosphere is due to accumulation by meteorite impacts events. For the outgassing scenario, volatiles [4] may have been produced from within the interior of the planet (volcanic activity). The impact scenario describes the possibility that meteorites may carry elements which in turn could generate an atmosphere.

At 4.1 Ga ago, the atmosphere of Venus, Earth and Mars would most likely have been a greenhouse atmosphere combined with liquid water surface areas. In the next million years a primitive form of life could maybe have be found on Earth. Along with potentially life-friendly environments on the other two planets (Venus and Mars) with atmospheres composed of carbon dioxide, nitrogen, and water [Baines et al., 2013].

During the next years, the planets' atmosphere most likely changed. These changes was a result of multiple potential factors. The solar flux, in terms of light and charged particle radiation, and the possibility of occasional bombardment by the asteroids and comets may have contributed to these changes. Explicitly for Mars, the effect of solar winds evolution was an important factor that affected the atmospheric loss of the planet, especially when the magnetic field was absent and therefore offered no protection from Sun's charged particles. All of the above reasons have left the planet with an atmospheric loss rate of ∼2-3 kg/s [Jakosky et al., 2018]. The Martian atmospheric loss is currently being studied through two missions, from The Indian Space Research Organisation with Mangalyaan (2013) [Sundararajan, 2013] and from NASA MAVEN mission (2013) [Jakosky et al., 2015b].

The mass loss evolution of the Sun-like stars in the main sequence decreases exponentially with time (Eq.1.1) [Wood et al., 2002]. The same study suggests that the strength of the

[3]Proto atmosphere or early atmosphere of a planet is the one that was formed due to accretion of gaseous matter of the proto planetary disc.

[4]Organic compounds with low boiling points and high vapour pressure at room temperature.

solar wind must have been one thousand times stronger than it is today, when the Sun was at the first stages of its evolution [Wood et al., 2002].

$$\dot{M} \propto t^{-2\pm0.5} \tag{1.1}$$

For the case of Mars, it's climate differs from Earth's primarily because of its thin, dry atmosphere, combined with the greater distance from the Sun [Leovy, 2001].The last years a series of orbit and also surface explorers have taken place. The purpose of these missions are to explore the physical and chemical properties that contribute to the planet's climate, atmospheric composition, geology as well as to see if there are any signs of ancient water and any biologically related material.

One great instrument that provided information related to the martian atmosphere is the SMA (Sample Analysis at Mars) instrument. This set up, including the SAM Quadrupole Mass Spectrometer (QMS) of the Mars Science Laboratory (MSL) Curiosity rover, arrived on Mars in August 2012 [Mahaffy et al., 2012]. These instruments are equipped to analyze the Martian atmospheric gases and volatiles, studying the chemical and isotopic composition of the Martian atmosphere. The results were: carbon dioxide 0.96%, argon 0.02%, nitrogen 0.02%, oxygen $1.73 \cdot 10^{-3}$ % and carbon monoxide $7.94 \cdot 10^{-4}$ % [Franz et al., 2017].
An important gas, that is a potential bio-marker on planets is methane. This is based on the fact that 95% of Earth's methane is biologically derived [Formisano et al., 2004]. The Martian methane has been measured from satellite telescopes, Earth based telescopes and also in-situ from Curiosity rover. The measurements from ExoMars Trace Gas Orbiter (TGO) showed a maximum value of 0.05ppbv [Korablev et al., 2019]. The Canada–France–Hawaii ground based telescope measurements up to tens of ppbv [Krasnopolsky et al., 2004] and the Curiosity analysis at Gale crater values up to 7ppbv [Webster et al., 2015]. As seen from measurements, the menthane concentration has variations and depends on where one look on Mars and is equally dependant on time.
However, the origin of methane on Mars is still controversial and being researched. Some potential sources are related to biological, geological and volcanic processes.

The temperature on Mars can fluctuate, depending on the time of the day, seasonality and location, from 183K to 268K according to the measurements made by Viking lander on the martian surface [Kieffer, 1976]. Amongst others, more recent studies based on the Phoenix mission show a temperature range from 181K to 253K in polar altitudes [Smith et al., 2004]. The seasonality is analog to Earth's due to the rotational axis to the orbital plane of Mars (25.2°) is very close to that on Earth (23.45°). But since the Martian year is longer than one Earth year (∼1.9 of Earth's year), seasons are longer. Also, Mars' perihelion is 31 sols (sol = martian solar day) combined with the eccentricity of the martian orbit, this means that the southern winter is longer than the northern winter [Simon et al., 1994]

The Martian atmosphere always has some airborne dust resulting in the optical depth, to stay at τ ∼0.1-0.2 [Kahn et al., 1992]. The atmosphere is influenced by dust storms initiated by strong winds. These storms hit approximately 100 μm diameter particles

from the ground along the surface, this is called saltation [Greeley and Iversen, 1987]. The dust, also, absorbs sunlight and can reduce the daily temperature with up to 5K [Pollack, 1979].

1.2.4 Water on Mars - Past and present

Past Water on Mars
Parts of the Martian surface are covered by what appears to be a network of channels or valleys. This evidence along with the fact that the majority of the northern hemisphere, compared to the southern hemisphere is lower in altitude, strongly indicates the presence of liquid water in the Martian past. Looking at the number of impact craters and crater statistics, the northern hemisphere is also younger than the southern. The lack of craters in this area could indicate that there once was an ocean covering a larger part of the Martian surface [Jørgensen, 2019]. Signs of ancient water, along with clues to the evolution of the surface water, is found on the Martian surface today [Villanueva et al., 2015]/ [Williams et al., 2013].

During the Noachian period the erosion rates were much higher compared to later times, this indicating both the possibility of water and wind activity [Ehlmann et al., 2016]. There is also evidence pointing to water interaction with the surface [Solomon et al., 2005]. A large number of the networks of valleys (and smaller offshoots) created by rainfall or groundwater dates to this period as well [Solomon et al., 2005]. Branching valley networks became limited during the late Hesperian period and/or the early Amazonian period [Ehlmann et al., 2016]. Despite this, there were still, locally and occasionally, Paleolakes and other associated landforms created by water [Ehlmann et al., 2016]. In the Amazonian period water-created landforms can still be found, though they are limited. This could be features like channels of water on glaciers or weak branching valley networks [Ehlmann et al., 2016]. More present features include gullies and recurring slope lineae, that could indicate water [Ehlmann et al., 2016].

The possibility of water on Mars today
Regardless of the amount of water present on Mars in the planet's past, water is found there today. Though mostly found in its solid state [Jørgensen, 2019] or as water vapor in the atmosphere [Whiteway et al., 2009], some evidence suggest the presence of liquid water on the Martian surface today [Martín-Torres et al., 2015]. Due to the low atmospheric pressure on Mars today, water cannot naturally exist in its liquid state. Because the atmospheric pressure is below the triple point of water, water ice will not "melt" but directly change to its gaseous state, water vapor. Today a large amount of water ice is found on the Martian poles and in permafrost [Jørgensen, 2019]. Even though water on Mars cannot naturally exist in its liquid form, there are observations that indicated liquid water might actually be found on the Martian surface anyway.

From both on-site and remote examinations, a number of different salts have been found on the Martian surface. [Ojha et al., 2015]. The salt might play an important role in finding liquid water as they can form brines. Different salts can in fact contribute to the lowering of water's freezing point, even by as much as 80 K [Ojha et al., 2015]. Beside the possibility of lowering the freezing point of water, the salt are also hygroscopic which

means that they among other things attract and absorb moisture found in the atmosphere [Ojha et al., 2015].

As already hinted in the above section, it might be possible to find liquid water on the Martian surface. One possible sign of liquid water on the surface is what is referred to as "recurring slope lineae" or RSL for short. Sometimes they are referred to as "Seasonal flows on warm slopes" as well [Vincendon et al., 2019]. They are found on slopes on the Martian surface, and are lines "traveling" downward [Ojha et al., 2015]. The lines are relatively narrow ranging from 0.5-5 m in width and have a low albedo [Stillman et al., 2019]. The RSL are observed on warm slopes, areas where the temperature of the slope normally surpasses a temperature of 250 K and more often 273 K [Ojha et al., 2015]. They have been observed in both the northern and southern mid latitudes as well as areas around the equator [Schaefer et al., 2018]. They are fading when inactive and are re-appearing over multiple Martian years [Ojha et al., 2015].

If recurring slope lineae are due to liquid water movement, this could be very interesting. Water is a crucial part for all life we know of today, [Ojha et al., 2015] which is also one of the reasons why the possibility of liquid water on the planet today is so interesting. But whether or not liquid water can be found on Mars, has not been determined yet. As well as the origin or origins of RSL which remains a mystery. [Schaefer et al., 2018]. One possible explanation is, as mentioned above, the presence of liquid water due to the salt concentration in the Martian soil. But another possible explanation is that the RSL are created due to dust movement and are of dry origin [Vincendon et al., 2019].

1.2.5 The search for life on Mars - Past, present and future

Are we alone? A question that has been asked through time and one that has still not been answered. This is not for the lack of trying, a number of missions and scientific studies have been taking place through a number of years in search for an answer. But space is big and there is more than one place to search. One of the closest places to search is Mars and the surface of the red planets has been investigated through time. The first successful lander to reach the surface was the Viking landers, (Viking 1 and Viking 2) [Freedman et al., 2016].

The Viking landers were made to test for life and one of the experiments could monitor for metabolism [Levin and Straat, 2016]. Both Viking lander 1 and Viking lander 2, which tested approximately 6440 km (4000 miles) apart had similar outcomes for the experiment, the results seamed to be positive. The results have however been questioned since and the conclusion is still debated today, believing that it might have been something non-biological the experiment detected [Levin and Straat, 2016]. One argument for why it was not biological, was that it could be a form of oxidants present in the soil, along with the Martian environment being too hostile for life [Levin and Straat, 2016]. With both arguments for and against detection of life on Mars, an answer to the question is still in the future.

At this moment no similar experiments have been carried out on Mars in the search for life [Levin and Straat, 2016]. But the exploration of Mars and search for sings of

life continued. A number of missions has been launched since, some with the focus on investigating the Martian environment and others in the search for potential life, but all with the goal of better understanding Mars.

Another mission which focuses on searching for habitability is the still operating Curiosity rover which launched in 2011. The main focus for the Curiosity rover was to investigate if Mars had ever been habitable or able to support microbial life [Curiosity, 2020]. The Curiosity rover landed in the Gale crater. A second mission is the more recently launched Perseverance rover (2020 Mission) which has been successfully landed on Mars on February 2021. The main focus for the Perseverance rover is to search for signs of ancient life on the red planet while also collecting rock samples (with the possibility of return them to Earth in the future). The landing site for the Perseverance rover is Jezero crater [Perseverance, 2020].

1.3 Understanding life

But why is it important to understand the Martian environment. As well as the evolution of the planet's surface, the magnetic field, the atmosphere along with the possibility of water, either past or present. The previous section 1.2, partially answers that question. Each of the sections go through radical environmental changes, leading to the barren planet seen today. The interest in regards to the the evolutionary changes is to better understand the environment and by extension the possibility of life. Not necessarily life as known from everyday life here on Earth, but life in more extreme environments or under more extreme conditions. However, to search for life in extreme conditions one does not have to go far, microbial life are found all over Earth and some even in places too extreme for macroorganisms [Madigan et al., 2015]. This is exactly the reason for interest in the Martian environment. Even though Mars is significantly different from Earth, areas on Earth similar to Mars are found. Places with very low temperature or high UV radiation or anaerobic systems. If life can be found in the most extreme places on Earth, then maybe life can be found elsewhere to.

1.3.1 Requirements for life

All life known here on Earth is made up of cells, some are made of only a single cell and others of trillions. To better understand the fundamental of the cell, what goes on inside the cell must be understood. Inside the cell a number of processes take place, these are referred to as the metabolism. In order for the cell to work properly it needs two things, energy and material to work with. Life on Earth is said to be carbon based, the structure of the cell and the way it works is due to carbon. This is why the two important sources that the metabolism needs can be specified to an energy source and a carbon source [Bennett and Shostak, 2012].

The carbon source and the energy source can come from a range of different places. The carbon source serves as building blocks for the organic molecules in the cells. One way for the cells to get carbon is from eating something organic, something that contains carbon. Other cells can get carbon straight from there surroundings, for instance in the form of CO_2 from the atmosphere. But the cells also need a way of getting energy, an energy

source. One way some cells are getting energy, which is much like the way of getting a carbon source, is by eating. In this case the energy comes from chemical reactions which release the energy. Other cells gets their energy from there environment, this could be in the form of sunlight (using photosynthesis) or from chemicals which do not contain any carbon [Bennett and Shostak, 2012].

For humans both our carbon source and our energy source come from the food we eat. Some molecules, from the food that we eat, are used for cellular construction other molecules will instead go through chemical reactions which in turn releases energy. But as mentioned above, this is not the case for every organism, the sources can come from different places [Bennett and Shostak, 2012].

One last, but very essential thing required for any life form on Earth, is liquid water. Water is essential for all cell activity. Liquid water therefore plays a key role for life, and life on Earth, can only exist in places where liquid water can be present. There are some organisms that can become dormant when no liquid water is present, but they cannot survive that way forever [Bennett and Shostak, 2012].

1.3.2 Life in extremes

When talking about the limits of life we are referring to the known boundaries of life on Earth, where life as we know it can survive. These boundaries are referring to the physical and chemical conditions including radiation, temperature, water availability and toxicity of materials in the environment. On Earth all known organisms have different requirements to function. On regular basis, a human being needs a certain amount of water in order to maintain his or her functionality. But, there are some microorganisms that they can survive longer periods without water. These organisms can be extremely dry and as a result become dormant.

As already mentioned life comes in many forms and can be found in almost every corner of Earth. But not all organisms are as the life encountered in everyday life. And not all places on the Earth are suited for all lifeforms. Though humans are quite resilient, we are also very fragile when it comes to environmental differences. Things like changing the temperature either up or down or changing the concentration of different elements (like oxygen) in the atmosphere will have an extreme effect, and depending on how big the changes are, humans would only be able to survive it in a short period of time. This limits us in different places on Earth, but will also limit us in future exploration on other planets and moons, for instance a planet like Mars. But life comes in many forms and though humans would not be able to survive in these extreme environments, some organisms might be able to.

But are there any already existing organisms on Earth that could survive the Martian environment? The best candidates to survive such conditions would most likely be extremophiles. These kind of organisms can live on the physical, chemical and biological boundaries, at least as we define boundaries. For the extremely low temperatures on the martian surface there might be extremophiles like the psychrophiles that could survive [De Maayer et al., 2014]. These organisms have an optimal range of possible growth that

lies in the lower temperatures [Madigan et al., 2015].

1.3.3 The Martian Environment

Atmosphere
The environmental conditions on the surface of Mars make it impossible for humans to survive there, at least without any form of protection gear. The atmospheric pressure ranges between 0,7% to 1% of that on Earth [Martínez et al., 2017] and is also considered lethal for organisms that need oxygen for surviving, due to the atmospheric composition. But some bacteria and archaea have found ways to substitute oxygen with an alternative source.
Most of the nutrients required for life on Earth have been found on Mars, like iron in the form of ferrous iron, manganese, sulfur, carbon, calcium, magnisium, phosphorus, potesium and sodium [Foley et al., 2003, Nachon et al., 2014]. These are some elements the cell needs in order to function. There are presently known bacteria that utilize carbon monoxide and dioxide on Earth [Bartholomew and Alexander, 1979].
As already hinted above, the surface pressure on Mars is very low, which in turn courses the boiling point of water to be extremely low as well. This does however contribute to relative humidity (RH) in the atmosphere with levels of (RH) been measured at 17-23%, sometimes reaching as high as 70% [Fischer et al., 2019].

Radiation
The low atmospheric pressure, the thin atmosphere and the absence of a magnetic field [Acuña et al., 1998], could lead DNA to be damaged by UV radiation [de Gruijl et al., 2001]. The UV spectrum consists of three sub-parts, UVA (315nm - 400nm), UVB (280nm-315nm) and UVC (200nm-280nm). With the third type (UVC) being the most harmfull for organisms [Cockell et al., 2008]. The daily measurements of radiation on Mars, have been found to vary from zero at night and can reach up to 20 W/m^2 at the day time [Gómez-Elvira et al., 2014]. There have been found organisms like *Bacillus subtilis Strain MW01* which were tested at the International Space Station and they show no preference to growing in UV exposure or not, vaccum space and Martian atmosphere [Wassmann et al., 2012].

Temperature
The fact that the temperature lies mostly in lower ranges (Section1.2.3) makes the Martian surface a cold environment and many organisms would have difficulty surviving. Also, because of the low temperatures, surface water will be in the state of ice, making it unavailable for organisms. But some organisms like it cold, and one of them is *Planococcus halocryophilus*. A specific strain of *Planococcus halocryophilus*, isolated from Artic region, has shown growth and metabolic activity at 258K and 248K respectively [Mykytczuk et al., 2013]. Today, the lowest temperature in which bacteria (*Sporosarcina*,*Chryseobacterium*) have shown metabolic activity is at temperature 240K [Bakermans and Skidmore, 2011].

Perchlorates
Another interesting factor when discussing the qualities and differences between Earth

and Mars is the soil composition. And one compound is especially interesting, namely the perchlorates, which plays a key role for the experiments in this thesis. The following lines will take a closer look into this.

One of the substances found in the Martian soil is perchlorate, which is consisting of chlorine and oxygen (ClO_4^-). On Earth, perchlorates' origin can be both naturally and industrially. Naturally, the production can happen in the atmosphere, where chlorine oxides react with ozone [Catling et al., 2010]. The highest naturally occurring consecration of perchlorates are found in the Chilean desert, Atacama. The concentration of perchlorates varies, some sources say between 0.03 to 0.1 % [Sellers et al., 2006], other sources states the concentration can be as high as 0.6 % [Catling et al., 2010]. Experiments made by the Phoenix rover showed a concentration of perchlorates at that test site to be between 0.4 and 0.6 % in regards to mass [Hecht et al., 2009].

The main concern when it comes to perchlorates is their toxicity and their impact on humans. Low level exposure of perchlorates can interfere with the uptake of iodide by the thyroid gland, high consecrations can instead affect the endocrine system. [Sellers et al., 2006]. Beside the risk of affecting the endocrine system, perchlorate is considered a "likely human carcinogen" by the U.S. Environmental Protection Agency (EPA) [Council et al., 2005]. In contrast to humans, there are organisms that can use perchlorate during respiration. These bacteria have been shown to use a wide range of organic but also inorganic compounds like ferrous iron [Bruce et al., 1999] to produce energy. Organisms that are able to use perchlorates can do this in the absence of oxygen, but when oxygen is present it will in turn represses the transcription of the perchlorate reducing gene [Susan et al., 2010].

Today, it is still unclear if there are any terrestrial microorganisms that could cope with these biocidal factors and if they could, would they then be able to replicate on Mars [Nicholson et al., 2013]. Searching for organisms in places on Earth where the environmental conditions are considered as extreme, is the best way to find candidate organisms that may survive martian conditions.

1.3.4 Life in extremes on Earth

There are some places on Earth considered as extreme environments. Places like these are the highest in the mountains, deep lakes, dry desert etc. But, there are some organisms, that not only survive but thrive there. One such place is the Atacama desert in Chile, this place is considered a desert due to the lack of rainfall through the year [Azua-Bustos et al., 2018]. Due to the absence of clouds and the high altitude the UV exposure is extremely high, with annual doses of UVA and UVB at 3.5 kWh/m^2, which is almost double of what is seen on other places on Earth [Cordero et al., 2018]. Another contribution to the aridity of this place, is the perchlorates as already mentioned above. In this, not a biology friendly environment, there are bacteria, archae and fungi that seem to have gained tolerance in this kind of environment [Azua-Bustos et al., 2018].

1.4 Terraforming and the ethical problems of space exploration

One idea for the future of Mars is terraforming the planet, this might sound as science fiction, but it might very well become a reality in the future. But with the possibility of terraforming Mars, ethical and moral questions arise. The following section takes a look at both terraforming of Mars and the ethics.

1.4.1 The idea of terraforming

The idea of terraforming the red planet is not new and has existed for some time. The idea was to make our neighbor planet, Mars, a sustainable second home for humans, and thereby making it possible to survive on the surface of the red planet without any kind of protection.

The habitability of the planet depends on its atmosphere. But how could one change an entire planetary atmosphere? This is not an easy task and it may lie somewhere between science fiction and a potential revolutionary epoch for humanity. In order to change the environmental parameters that has existed on a planet for a long time, the technological and engineering tools would have to evolve, and this may not necessarily be an easy task.

For all the reasons already discussed in previous sections, the Martian atmosphere is not hospitable for humans, as it is today. So the first step might be to change the atmosphere, and ideally making it as close to Earth's as possible. Since, as already mentioned, Mars once had a thicker atmosphere, some of this atmosphere might have been trapped into the ground [Jakosky and Edwards, 2018]. So, potentially releasing the carbon dioxide and the water vapour trapped in the sinks, a greenhouse effect might get created and this in turn could possibly lead to the creation of an atmosphere. But, since Mars has a weaker gravitational field than Earth, it is more likely to have lost the atmosphere into space, rather than absorbing the most of it. The idea of using the existed carbon dioxide sinks in order to terraform the atmosphere was recently studied by Bruce M.Jakosky and C. Edwards [Jakosky and Edwards, 2018]. With the most accessible carbon dioxide sink being the one at the polar caps, the scientists found that if explosives are used to release the available gases then it would lead to an increase in the atmospheric pressure from $\sim$6 mbars to $\sim$15 mbars [Jakosky and Edwards, 2017]. And this is not anywhere close to Earth's atmospheric pressure.

Another relatively recent suggestion for terraforming Mars is by the use of microbes [Friedmann and Ocampo-Friedmann, 1995]. The idea is based on the fact that Earth for long periods of time was dominated by bacteria (e.g cyanobacteria) that were able to produce enormous amounts of gases (e.g oxygen) [Kulasooriya, 2011]. This lead to a build up of gasses in its atmosphere and after thousands of years the atmosphere changed. One other possible way of achieving terraforming is by finding a way to produce greenhouse gasses that in turn might be able to change the atmosphere on the planet. But without a magnetic field, the atmosphere, will slowly be stripped away by solar winds. However one way of preventing this could be by the creation of a artificial magnetic field in a fixed point (lagrangian point), which will be able to protect the planet [Nasa, 2020b]. If this will be possible one day, it could potentially lead to the gradually thickening of the

atmosphere, this will cause the temperature to rise and in turn lead to the melting of the polar caps, releasing the trapped carbon dioxide [Nasa, 2020b].

If this idea one day could become a reality, then Mars will most likely be the first colonization of a terrestrial body beyond Earth, and that could lead to further terraforming and colonization in the galaxy.

1.4.2 Ethics and possible moral complications in space exploration

Humans are curious by nature and the exploration of space is rather unpreventable. But there are a number of possible complications as well as ethical questions that should be kept in mind when it comes to exploring the vast expanse. In 1967, the *United Nations Outer Space Treaty* came to be and with it, a policy in regards to microbes and space [Lopez et al., 2019]. Along with the treaty is also the Planetary Protection Guidelines (COSPAR) that works to prevent contamination. Essentially it means that one have to make sure that there does not exist any type of life before the first human contact [Lopez et al., 2019]. To try an ensure that no contamination happens, a key policy for the majority of exploration was implemented, it had to make sure that everything was sterilized [Lopez et al., 2019]. When using the word sterilize in a biological context this means that all types of living organisms will be eliminated completely. This in turn means that there will be limitations as to what can be sent to extraterrestrial locations [Lopez et al., 2019]. But why is there a fear of contaminating, sure one does not wish to conduct tests on the Martian surface getting a positive result only to realize that the organism detected was brought along from Earth. But the risk of disrupting emerging life should also be taken into account. When and if the first humans explores are send to Mars new microbial organisms will regardless of preparation be introduced to the Martian environment [Lopez et al., 2019]. And this raises the question of ethics and if there should be any limitations to future space exploration. Ethics plays an important role in everyday life - what is right and what is wrong. Some actions are pretty well defined, where other are not as black and white in terms of morality and the question “is it right or wrong?” will vary depending on the person asked. When it comes to ethics, space exploration and possibly colonization, a number of questions arise and the answers will most likely vary again depending on who is asked. An example of such a question emerge when diving in to the topic of exploration of Mars could be ”can we colonization?” But also the ethical question “should we colonize Mars and do we have the right to so?”. There are no simple answer to this type of question and many similar questions will arise when topics like these are discussed.

2 Aim

The main focus of this thesis is to take a look closer regarding the habitability of planet Mars. In the first section, using numerical calculations we wanted to approach the origin of Martian atmosphere and what could this indicate for the past history of the planet. Using two models based on the origin of the atmosphere, impact and outgassing scenarios, the time when the magnetic field lost can be calculated and based on recent studies from

Maven mission, the results can be compared and see which model gives a better estimate for that time. As an extension, the time in which Mars could have had conditions favorable for life before its atmosphere started to escape in space could indicate a past habitable history.
After a past history investigation, experiments based on today's Martian conditions will contribute and indicate if life can be present Mars. In the experimental section the primary goal was to see how microbial Earth based life extracted from Atacama desert was able to tolerate conditions that we face on planet Mars today. Starting from testing each individual parameter first, and by gradually exposing them in more than one extreme parameters we see how these affect their survival condition. Along with the experimental side of the thesis, a main purpose was to improve the Jens Martin Mars Chamber (JMMC) in order to simulate more precisely the various factors contributing to the Martian environment. This could help us to simulate conditions that we face on planet Mars and also improve the simulator for future experiments.
Additionally to the survivability experiments a step further is to see the residual gases coming from them while they exposed to the extreme conditions on Mars. The emission rate of residual gases coming from these organisms indicate how metabolic active they can be in such conditions compared to Earth's regular atmosphere. And the case of being active, it gives the "green light" for further investigation and detection of such compounds. Measuring what kind of gases microbial life leaves behind ,when facing the extremes, could guide us to search for these specific compounds not only on Mars, but also in the spectra of exoplanetary atmospheres. Also, searching and finding organisms that produce specific compounds that would help increase the greenhouse gases on planet Mars, could be used to change or contribute to the atmospheric cycle of the planet.

3 Investigation of the Martian atmosphere

In addition to the experiments that took place during this thesis, also a theoretical approach was attempted to investigate the atmospheric origin on Earth and Mars. Although some models and theories that are trying to predict the origin of the atmospheres, none of them has been proven. In the early history of terrestrial planets, their atmospheres experienced a series of events of mantle outgassing and erosions due to asteroid and cometary impacts [Schlichting and Mukhopadhyay, 2018]. The most prevalent theory is that the atmosphere either came from outgassing, from the planet itself, and/or was a result of an impact event due to a collision between a meteoroid and the planet [Drake, 2005]. In the early stages after the core formation, volatile bodies were beating Earth and they have been proposed as possible providers of volatiles that caused the formation of oceans, atmosphere as well as carrying essential organic elements necessary for life to emerge [Marty, 2012][Altwegg et al., 2016]. But, the origin of terrestrial volatiles remains unclear in planetary sciences.
In the following chapter an attempt of approaching the origin of the atmosphere on Earth and Mars was made.

3.1 Atmospheric mass ratios

In the following lines the ratio between the original atmospheric masses of Earth and Mars was found, based on the two assumptions for outgassing and collision scenarios. The mass of the atmosphere (Eq. 3.1) for the outgassing scenario is proportional to the radius of the planet to the power of three, since the volume of the planet is proportional to the radius to the power of three (Eq. 3.2). The mass is also proportional to the different mean densities of the planets (Earth and Mars) (Eq.3.1). For the collision scenario, the atmospheric mass is proportional to the effective area to the power of two based on Eq. 3.3.

$$M = \rho \cdot V \tag{3.1}$$

$$V = \frac{4}{3} \cdot \pi \cdot r^3 \tag{3.2}$$

$$S = 4 \cdot \pi \cdot l^2 \tag{3.3}$$

Following the above lines, the two proportional relations, for outgassing and collision scenarios ,respectively, were found (Eq. 3.4 , Eq. 3.5).

$$out \propto \rho \cdot r^3 \tag{3.4}$$

$$coll \propto l^2 \tag{3.5}$$

For the outgassing scenario, knowing the density and their radii parameters (table 1) of the two planets the desired ratio can be calculated (table 2).

Parameter	Earth	Mars	Units
Radius [R]	$6.371 \cdot 10^6$	$3.3895 \cdot 10^6$	$[m]$
Density $[\rho]$	$5.51 \cdot 10^6$	$3.93 \cdot 10^6$	$[g/m3]$

Table 1: Parameters for outgassing model

Parameter	Value
Mass $[M \propto \rho \cdot V]$	9.31
Volume $[V \propto R^3]$	6.64

Table 2: Atmospheric mass ratio assuming outgassing model

For the collision scenario the ratio of the atmosphere of the two planets demands the assessment of the effective area.

According to Eddington's accretion theory, the geometrical accreting area of the planetesimal can be described by Eq. 3.6, with r to be the radius of the planetesimal. Knowing that the dust particles are a bit outside of the geometrical area and they are gravitationally attracted to the planetesimal too, the effective area will be described by Eq. 3.8. The relative velocity of the particles which are moving in the accretion area is U_{rel}. The equation that shows the relation between the radius, r, the effective area, l, and the relative velocity of the body moving to the accretion area, U_{rel}, can be described in Eq. 3.7.

$$A_{geom} = \pi \cdot r^2 \tag{3.6}$$

$$A_{eff} = \pi \cdot l^2 \tag{3.7}$$

$$l^2 = r^2 \cdot (1 + \frac{2 \cdot G \cdot m}{r \cdot U_{rel}^2}) \tag{3.8}$$

If we apply this theory in our case, with the particles to be the collision body and the planetesimal to be planet Earth and Mars, then due to Eq.3.8, one can see the dependence of the effective area on the radius r, mass m, and the relative velocity of the colliding body, U_{rel}.

In order to find the relative velocity with which the meteorite hits the planets, then it was assumed free-fall motion. That means that the air resistance is negligible and also the object starts with zero velocity. The meteor will be affected by the gravitational force of the Sun, with mass M_S from the initial position until its final position, the planet. For the initial position of the incoming object, it was assumed to be the asteroid belt or the Kuiper belt.

The energy of the free falling object at its initial and final position, can be described by the sum of its kinetic and potential energy respectively (Eq.3.9, Eq.3.10, Eq.3.11, Eq.3.12). Then, the conservation of energy (Eq. 3.13) demands the energy in the initial position equals the energy in its final position (Eq.3.14). Where m_o is standing for the mass of the colliding object.

$$K.E_I = 0 \tag{3.9}$$

$$P.E_I = \frac{-G \cdot M_S \cdot m_O}{R_S E} \tag{3.10}$$

$$K.E_F = \frac{1}{2} \cdot M_S \cdot u_{ff}^2 \tag{3.11}$$

$$P.E_F = \frac{-G \cdot M_S \cdot m_O}{R_{SO}} \tag{3.12}$$

$$K.E_I + P.E_I = K.E_F + P.E_F \tag{3.13}$$

$$0 - \frac{-G \cdot M_S \cdot m_O}{R_F} = \frac{1}{2} \cdot M_S \cdot u_{ff}^2 + \frac{-G \cdot M_S \cdot m_O}{R_I} \tag{3.14}$$

By solving the last equation (Eq. 3.14), the free fall velocity for the infalling object was obtained (Eq. 3.15).

$$u_{ff} = \sqrt{2 \cdot G \cdot M_S \cdot (\frac{1}{R_F} - \frac{1}{R_I})} \tag{3.15}$$

Substituting the parameters from table 3 in equation 3.15, and using Python script, the velocities obtained for the impact scenario both for an object coming from the asteroid and Kuiper's belt (table 4).

Parameter	Symbol	Value	Units
Gravitational constant	G	$6.67408 \cdot 10^{-11)}$	$[m^3 kg^{-1} s^{-2}]$
Sun's mass	M_S	$1.989 \cdot 10^{30}$	$[kg]$
Final position, Earth	R_F	$150 \cdot 10^9$	$[m]$
Final position, Mars	R_F	$1.5 \cdot 150 \cdot 10^9$	$[m]$
Initial position AB	R_I	AB: $2.7 \cdot 149.597 \cdot 10^9$	$[km]$
Initial position KB	R_I	KB: $30 \cdot 149.597 \cdot 10^9$	$[km]$

Table 3: Parameters

Planet	Asteroid belt	Kuiper belt	Units
Earth	33382	41363	[m/s]
Mars	22900	33481	[m/s]

Table 4: Free fall velocities

The minimum required velocity an object has to has to penetrate a planet's atmosphere and be "trapped" by its gravitational field is the escape velocity. Adding the escape velocity of the planets to the free-fall velocities we end up with the final velocities (table 5). These velocities represent the maximum velocity an in-falling object has, coming either from asteroid or Kuiper's belt, landing on planet Earth and Mars.

Knowing the impact velocities, the radius effective area (Eq. 3.8) for the assuming impact scenario was calculated (Table 6).

Planet	Escape Velocity	Max. Velocity(AB)	max. velocity (KB)	Units
Earth	11186	44568	52549	$[m/s]$
Mars	5027	27927	38508	$[m/s]$

Table 5: Impact velocities

Planet	Effective area (AB)	Effective area (KB)	Units
Earth	6568602	6513744	$[m]$
Mars	3418589	3404831	$[m]$

Table 6: Radius Effective area

The proportion relationships for the effective areas between Earth and Mars for both scenarios were found (Table 7).

Scenario	Ratio
Object coming from asteroid belt	3.691
Object coming from Kuiper belt	3.659
Outgassing	9.310

Table 7: Ratios of effective areas for impact and outgassing model

Knowing the ratios between the two planet's original atmospheres based on the two models, the original Martian atmosphere was calculated (table 8). This was done by assuming that in the Martian atmosphere and also in Earth's atmosphere wasn't occurring any atmospheric escape. For the mass of Earth's atmosphere was used the value of $5.15 \cdot 10^{18}$ kg [Lide, 2004], and the fact that atmosphere is losing approximately $2 \cdot 10^{-6}$ kg/s [Saxena and Chandra,], which is extremely low compared to the one that happens on Mars, led us to consider it as stable .

Ratio	Martian atmospheric mass [kg]
3.691	$1.39 \cdot 10^{18}$
3.659	$1.40 \cdot 10^{18}$
9.310	$5.53 \cdot 10^{17}$

Table 8: Martian original atmosphere

After, knowing that the Martian atmosphere is about $2.5 \cdot 10^{16}$ kg [Nasa, 2020a], and its

atmospheric escape rate today is 2-3 kg/s [Jakosky et al., 2018], the demand time for this loss was found using Eq. 3.16 (table 10), based on the differences between the atmospheric mass today and the original that were found (table 9).

Dif1	$1.36 \cdot 10^{18}$ [kg]
Dif2	$1.38 \cdot 10^{18}$ [kg]
Dif3	$5.28 \cdot 10^{17}$ [kg]

Table 9: Mass differences

$$time = (dif/rate)/3.16 \cdot 10^{16} \tag{3.16}$$

Dif1	21.67 [Ga]
Dif2	21.86 [Ga]
Dif3	8.35 [Ga]

Table 10: Expected time

The results at the table 10 contribute to the fact that something must have accelerated the atmospheric loss of the Martian atmosphere during its history. Results from data coming from MAVEN and Mars express missions reveal the dependence of the martian atmospheric loss rate on solar variance [Jakosky et al., 2015b][Jakosky et al., 2015a].

In the next section (section 3.2), an approach was made on how the evolution and a more massive solar wind affected the atmospheric loss on Mars during its history.

3.2 Evolution of the Martian atmosphere

A former research based on atmospheric modeling measuring the mass-loss rates of solar-like stars has shown that the solar wind may have been one hundred times stronger when the Sun was young (section 1.2.3). The empirical relation that describes the mass loss evolution of cool main-sequence stars like Sun has been described in equation 1.1. A more massive young Sun profound a more massive solar wind, that may have had effects on planetary atmospheres [Wood et al., 2002].

Here, it has been assumed that the atmospheric loss of the Martian atmosphere compared to Sun's mass loss is scaling with a factor of R (eq.3.17).

$$\frac{dm}{dt} = R \cdot t^{-2} \tag{3.17}$$

Solving the equation with respect to scale factor R:

$$R = t^2 \cdot \frac{dm}{dt} \tag{3.18}$$

To find the factor R, a moment in time has been chosen in which we know the actual rate of its atmospheric loss. Today we know that Mars is losing its atmosphere at a rate of 2 kg/s. Substituting these values in equation 3.18, the scale factor R was calculated (eq. 3.19).

$$R = t_{now}^2 \cdot (\frac{dm}{dt})_{now} = 4.10 \cdot 10^{34}[kg \cdot s] \tag{3.19}$$

Finding the factor, R, was able to see how the mass loss of the martian atmosphere is scaling compared to Sun's mass loss, and then it was plausible to find the indefinite integral that describes the mass loss rate over time.

Beggining by expressing the mass of the atmosphere as the indefinite integral in terms of change in mass over time:

$$M_{marsatmo} = \int \frac{dm}{dt}\, dt \tag{3.20}$$

Substituting the definite limits in the integration between the now time, t_n, and the time, t_0, that broke the coherence of the original atmosphere of Mars that found in table 8 based on the models of collision and outgassing, was able to find the time where the atmosphere of Mars started losing mass in space (Eq. 3.23).

$$M_o = \int_{t_0}^{t_n} \frac{dm}{dt}\, dt \tag{3.21}$$

Substituting the mass loss rate from eq. 3.18

$$M_o = \int_{t_0}^{t_n} \frac{R}{t^2}\, dt \tag{3.22}$$

Solving with respect to time t_0:

$$M_o = R \cdot (\frac{1}{t_0} - \frac{1}{t_n}) \longrightarrow t_0 = \frac{R \cdot t_n}{M_o t_n + R} \tag{3.23}$$

Based on the last equation (eq.3.23) the time scales that they result due to the model of collision and outgassing appear at table 11.

These numbers represent the time when Mars lost its atmosphere based on these models if we assume that that had happened instantaneously.

Model	Atmospheric mass [kg]	t_0 [Ga]	t_{ago} [Ga]
Collision (AB)	$1.394 \cdot 10^{18}$	0.789	3.810
Collision (KB)	$1.407 \cdot 10^{18}$	0.783	3.816
Outgassing	$5.531 \cdot 10^{17}$	1.572	3.027

Table 11: Time atmosphere changed

3.3 Conclusion

An investigation about the origin of Earth's and Mars' atmosphere was made, including a few assumptions. Besides these assumptions the impact velocities were found to be in the range of the velocities values that were found by a previous study for comets orbiting around Mars [Ma et al., 2002]. Based on these two model assumptions the time where the Martian atmosphere started losing its atmosphere was estimated to be approximately 3.8 Ga ago for the collision model, and 3.02 Ga ago for the outgassing model. The more recent research studies give an estimation for the time that Mars lost its magnetic field at around 3.9 Ga to 4.1 Ga ago [Jakosky et al., 2015a]. Both of the two model assumptions give a good estimate compared to the given references, with the collision model to be exactly in the range. As a consequence, the conditions on Mars may have been favorable for life to emerge on the planet by the time the magnetic field disappeared. This period due to our models was estimated to be until the age of 0.78 Ga for collision and 1.57 Ga for the outgassing model. During this period the earliest known life form first appears on Earth [Betts et al., 2018][Dodd et al., 2017]. So, if Mars sustained its original atmosphere for that long, creating a form of atmosphere including greenhouse gases, protection from the UV radiation, and keeping its temperature high enough and approximately stable, then there must have been a possibility and a window of hope that the planet once had the opportunity to give rise to a form of life as we know it on Earth.

4 The Jens Martin Mars Chamber and experimental set up

In order to explore environments beyond Earth, like Mars, where the conditions differ from that we have on Earth we need simulators that could provide us with such environments. The space exploration of Mars during the last decades, using landers and orbiters, has provided us with numerous of data, making it now possible to experiment and simulate surface and atmospheric conditions in the laboratory.

There are a few of these simulators around the world and there is also one in the Mössbauer spectroscopy laboratory at Niels Bohr Institute (figure 3). The first version of the Jens Martin Mars chamber (JMMC) has been constructed by former students in 2011 under the supervision of Morten Bo Madsen. Later on, in 2014, it has been reconstructed to

improve and cover a more broad spectrum of testing parameters.

Figure 3: *The Jens Martin Mars Chamber*

The primary parts of the JMMC are:

- Glove box: A glove box has been set up surrounding the general structure of the chamber in order to achieve the minimum leaking. Also, experiments that demand only change in the atmospheric composition, as well as a modified relative humidity, can be executed in the glove box itself (numbered as 1 in figure 3).
- Main Chamber: The main chamber is a container inside of it Mars atmospheric analog experiments can be execute combined with a low atmospheric pressure (numbered as 2 in figure 3).
- Ante-chamber: An airlock cylinder is connected to the glove box in order to achieve an easy transfer of tools and samples in and out of the glove box, without affecting the modified environment. The airlock has been connected to one side to a smaller vacuum system and on the other side to the glove box. Vacuuming the airlock first and then letting air from the glove box through the cylinder, an easy transfer can be performed (numbered as 3 in figure 3).

A part of this thesis was also to modify and make the JMMC functional for various of our experiments. To test the isolated bacteria from extreme environments on Earth and examine if they are able to survive the extreme conditions of Mars, we had to make sure that the Mars chamber was working properly.
An ideally simulator chamber would be if all of the below conditions could co-exist at the same time. Due to the new corona restrictions, many of the tasks were delayed so we made sure to come up with alternatives and be as accurate as possible.

4.1 Pressure parameter

As already mention in 1.2.3 section, the atmospheric pressure on Mars ranges between 0.1 mbar to 7 mbar [Martínez et al., 2017], while the current atmospheric pressure on Earth has been measured to be approximately at 1 bar. This difference in the pressure could be

fixed by the already installed vacuum pump system which has been externally connected through the outlet backplate of the glove box, to the chamber (figure 4). The purpose of the vacuum pump is to reduce the atmospheric pressure inside the chamber while it is removing and vacuuming the system from the pre-existed gas molecules in the system. The lowest partial pressure the system can have using the vacuum pump when the outlet connection is sealed to avoid any leaking is at 0.8mbar. Also, it was possible to see how the pressure fluctuates using a pressure meter connected to the computer and through the OMEGA TRH software the real-time pressure was displayed on the screen.

Figure 4: *The vacuum pump*

4.2 Atmospheric composition regulation

When we talk about the atmosphere of the two planets, they are definitely different in terms of composition. With the Martian atmosphere being composed mainly from carbon dioxide (0.96%), argon (0.02%) and nitrogen (0.02%) [Franz et al., 2017] compared to Earth's that is mainly dominated by nitrogen (78%) , oxygen (21%), argon (0.93%) (and small amounts of other gases) [Tokunaga and Cox, 2000].
This alteration in the atmospheric composition can be simulated in the laboratory in the chamber. This can be done by removing through the vacuum system the primarily mostly dominated atmosphere by nitrogen and oxygen, while at the same time there is a purging gas inlet system that is connected to gas cylinders and is able to insert other preferable gases in the system (figure 5). For our experiments, gas cylinders of carbon dioxide and nitrogen were used. The goal was to decrease the amount of oxygen in the box at the minimum while purging it with the desired gas.

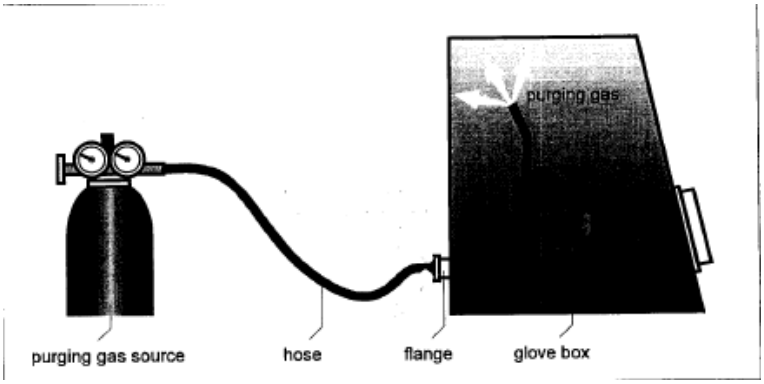

Figure 5: *Purging system*

In order to regulate the flow of the gas volume that was needed to purge the glove box a ping pong ball flow meter (figure 6) there was connected to the gas cylinder showing the volume per time ratio of the gas. Keeping the flow at approximately 5 m^3/h and at the same time vacuuming the system, the purging procedure was completed in under 30 minutes.

Also, for safety reasons and in order to be sure that none of these gases weren't leaking through the laboratory while purging, we activated an oxygen sensor that is able to measure the oxygen levels in the room and ring an alarm while the amount of oxygen decreases.

Figure 6: *Ping pong flow meter*

4.3 Temperature

As the temperature on the surface of Mars fluctuates depending on the location and seasonality, an average of the temperature has been found at $\sim$ 210K [Martínez et al., 2017]. Along with the fact that JMMC hasn't the ability to measure the exact temperature in the chamber yet and with the temperature having such a big range, the experiments started from a room temperature and expanded to lower temperatures using incubators that exist in the Microbiology department.

4.4 UV radiation

As already mentioned at section 1.3.3, Mars due to its thin atmosphere is not able to block the harmful part of the UV radiation that reaches the surface. In contrast to Earth's surface that due to the ozone layer both with Raleigh scattering that is able to block and scatter most of the UVB and UVC radiation, with only the most of the UV

radiation that reaches the surface to be the UVA. Due to this fact, we are aware of the extreme UVB and UVC exposure that an organism has when laying on the surface of Mars.

In order to include this parameter to our experiments UV lamps (Appendix A.1) were placed to a moving box covered with tinfoil (figure 7). Using these lamps we achieve to expose the isolate cultures in UV radiation using both lamps at the same time covering the wavelengths of 254nm and 366nm which are in the spectrum of UVC and UVA respectively.

Figure 7: *UV installation*

4.5 Soil analogues

The fact that no soil samples from exploration missions on Mars have been returned to Earth, yet, the necessity for creating simulated soil was arose. Even though, the sample return synergy mission between ESA and NASA has been scheduled for the end of 2030 [ESA, 2020], the amount of the sample material won't be enough (and probably too expensive) for bulk laboratory studies. This demand has brought scientists to create analogs for different areas of Mars based on data provided from previous orbiters and landers. In the following experiments, Mars soil simulants for Gale and Jezero crater delivered by Exolith lab were used as testing media for the different kinds of bacteria. The exact mineralogy and bulk chemistry for each of these analogs can be seen in the appendix section (Appendix A.2).

5 Survival experiments

At this chapter we examine isolated bacteria from environments here on Earth that the environmental conditions are characterized as extreme. The environments that were chosen they used as a Martian analog environment. The idea was based on the assumption and expectation that the forms of life that we found on these environments on Earth will have a better chance to survive the extreme conditions that we face on planet Mars. Firstly, the isolated bacteria will be subjected to some of these parameters, individually, and secondly to all of the parameters that can co-exist at the same time in the Martian chamber. The purpose of the first phase was to understand how the isolates cooperate with these parameters and then how all of them affect their growth.

5.1 Isolation of bacteria

The soil samples that were extracted from Atacama desert were available at the microbiology lab to work with. In the first place and in order for the bacteria from these soils to be examined, they needed to be isolated. This was done by taking 2 gr of soil and then mix them with 5ml of PBS media (Appendix A.3), vortexed them in a falcon tube, and then rest the mixture for approximately one hour. After this time, 200 μL of the mixture was inoculated on agar plates of regular TSB. (Appendix A.3) and placed at room temperature. After three to four weeks, bacteria cultures were visible. To distinguish variety of bacteria that were overlaying, each of these cultures was re-streak to a new plate to get the isolated cultures - the isolates. After growing the isolates on plates, they were restricted to liquid TSB media and incubated at room temperature while standing on a shake table.
The same procedure was followed for soil samples extracted from the Yungay area in Chile, at 2 meters below the surface. The respective ten isolates named after 201 to 208, and while they were re-streaked from culture 202 and 206 derived two kinds of isolates, 202-1 202-2 and 206-1 206-2 named respectively.

5.2 Perchlorate tolerance

The derived isolates were placed in TSB liquid media containing 0.6% and 1% of perchlorate. This experiment was able to make us conclude which of the isolates are able to survive in an environment enriched with perchlorates.
The isolates that survived the condition were 16 out of 20. In this way, we limited our next experiments including only the isolates that were able to survive in the presence of perchlorate.

5.3 Relative humidity and anaerobic experiment

At this experiment, we wanted to see how the isolates cooperate in an anaerobic environment with a lower relative humidity approach that on Mars, while their media was enriched with 1% perchlorate. To examine only these two parameters, the atmospheric pressure was kept at 1 bar and the temperature at 23.5 ° (room temperature).
The isolates were prepared overnight in fresh TSB liquid media. The next day each of the isolates' density measured using as a spectrophotometer. Putting a sample of each isolate culture in the spectrophotometer, the optical density was measured for each of them at a wavelength of 600nm (OD_{600}). The optical density of the samples was necessary to estimate the concentration of bacteria in the liquid media. Here a 0.4 of optical density was attempted for all of the isolates exactly before plating.
The testing samples were splitted into two groups, one with starved isolates and one with media. To prepare the starved cells, 1ml of liquid media was vortexed at 8000 rpm for approximately three minutes and the previously TSB media was replaced by PBS. As the analog soil wasn't available by the time we started this experiment, the soil was substituted by sand from Amager strand (Copenhagen region). The testing samples were made

by adding 100 µL of liquid media on top of 3 gr of autoclaved sand.

This experiment was executed in the JMMC while exactly the same groups were placed also outside of the chamber as control samples, in room temperature with 43% of relative humidity. To achieve a lower humidity with an anoxic environment, the glove box was purged with N_2 (while the CO_2 wasn't available yet) from the gas cylinder. The humidity was measuring daily and after four days had dropped to 17%. To measure the relative humidity in the glove box, a newly purchased humidity sensor was installed (figure 8).

Figure 8: *Humidity sensor*

While in the JMMC had been established the desired conditions (anaerobically and 17% humidity) then the samples were ready to be placed in the glovebox. The samples stayed in the glove box (and out for the control samples) for about two days. After two days, the samples were added to falcon tubes with 10ml of PBS, stayed in place for 30 minutes. Then, the samples were vortexed and stayed for another 30 minutes and in the end were vortexed again and centrifuged at 8000 prm for 30 seconds. Finally, to see which and how many of them survived the anoxic and lower humidity environment, 1ml from each sample was placed to an Eppendorf tube while diluted for times (dilution from 10^{-1} to 10^{-4}) and then placed on TSB agar plates.
The observed results after a couple of days was that the colonies on the agar plates were too many to count. Besides that we didn't have exact results about how many of them survived in numbers, we did know that all of the samples could tolerate the conditions of an environment without oxygen and lower humidity in a room temperature at 1 bar atmospheric pressure. The procedure was very helpful to improve our methods for the next experiments.

Due to time demand factors, we decided to reduce the number of isolates from 16 isolates to 6 of them. These 6 primary isolates (5, 6, 9, 10, 201, and 202-1) were chosen based on criteria that indicate better survival options. Two of the primary isolates (201 and 202-1) showed that they are potential candidates for perchlorate reduction (research that was made by a former master student) [Madsen, 2020].

5.4 UV experiment

In this experiment, we built our own UV box as explained in the section (4.4). The UV

light lamp was considered as an isotropic UV point source that emits in empty space. The radiation emitted from a point source decreases with the distance from the source (r) as the light spreads over the area ($4\pi r^2$). The irradiance described as the ration between the power emitted from the lamp (in watts) divided by the surface area (in m^2) (Eq. 5.1).

$$Irradiance = \frac{Power}{4\pi r^2} \tag{5.1}$$

The structure of the UV experiments started from a 20cm distance from the source, with that driven the irradiance at 15.9 W/m^2. The cultures were diluted to 0.4, making dilution from 10^{-1} to 10^{-4} while placed in petri dishes. Then, the petri dishes were placed in the UV box with their lid open. In this first set of the UV testing, time was started from 5 seconds and then was increased until 20 seconds, with a step of 5 seconds.
The second set of the UV experiments, included exactly the same procedure as the first with an addition of the perchlorate parameter. In the samples was added 1% of perchlorates in 100 µm of demineralized water which was autoclaved before.
In the third set, the exposure time increased to 30 seconds, 1 minute, 1.5 minute and 2 minutes.
Since it wasn't observed any effect on the cultures' growth with in the following days, between the exposed and the control samples a fourth and final experiment took place. The samples were exposed to UV radiation corresponding to a 20.7 W/m^2 irradiance, since the distance from the source decreased to 17.5 cm. The exposure time also increased to 1.5, 2.5, 3.5 and 4.5 minutes respectively. The dilutions that exposed for each of the isolates was from 10^{-1} to 10^{-9} and the same for the controls. Following the exposure to UV radiation all the colonies were checked daily and then they were count using the click counter. The colony forming unit (CFU) for each of the isolates appear in the next figures (figure 9 - 14).

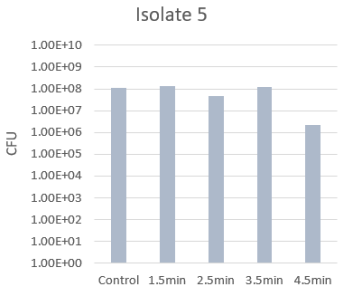

Figure 9: CFU isolate 5

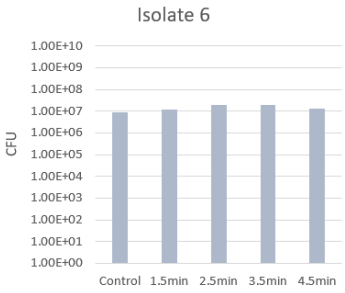

Figure 10: CFU isolate 6

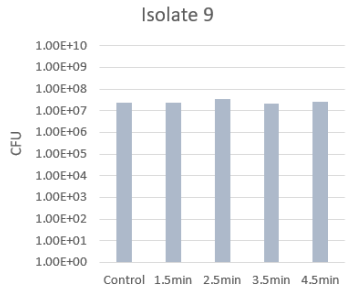

Figure 11: CFU isolate 9

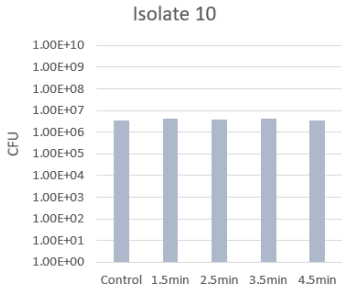

Figure 12: CFU isolate 10

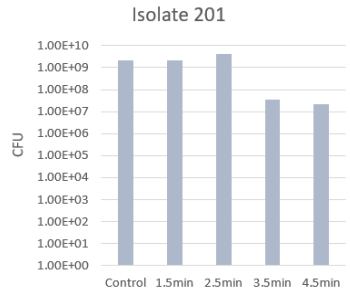

Figure 13: CFU isolate 201

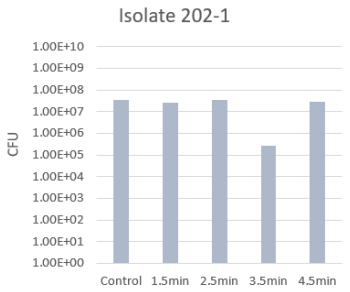

Figure 14: CFU isolate 202-1

As seen in the graphs represented the colonies as function of time compared to the control samples, there is no clear effect on the isolates due to the received UV radiation. In more details, isolates 6,9,10 and 202-1 showed almost non decrease due to radiation, while isolates 5 and 201 showed a decrease in the growth of their colonies by a factor of 10 units by exposing them at 3.5 minutes and 4.5 minutes.

In general, cells develop DNA repair mechanisms to overcome the damage coming from UV exposure and from environmental changes that are not favorable for their growth [Madigan et al., 2015]. This is a mechanism that life needs to overcome harsh environments and the extreme exposure to ultraviolet radiation without any DNA alteration. Another mechanism that certain bacteria develop is the formation of a structure called endospores. This mechanism help the cell to overcome and be protected not only from radiation but also from heat and harsh chemicals [Madigan et al., 2015]. This could be a possible explanation due to the resistance that we observe compare to the growing colonies.

5.5 The Martian chamber experiment

The purpose for this experiment is to expose the isolates to as many Martian conditions at the same time as it was possible. At the first part is described the experiment that was started a couple of days before the COVID-19 lockdown restrictions were applied and thus the results and the procedure was affected by them. At the second part, and while

we had permission to work limited time at the laboratories a more organised experiment took place while having numerical results to discuss.

5.5.1 Atmospheric composition of N_2 17% RH, 1% atmospheric pressure and Martian analog soil

To prepare the 6 isolates for exposure, we made sure that first were diluted to an OD corresponding to 0.3. Samples that were made were splitted into two groups. Half of them were starved and half of them (placed separately) with food. Three different soil analogues were used in this experiment, each of them simulate soils from two different crater regions on Mars, Gale crater (G) and Jezero crater (J), and an additional one represented soil from Gale crater enriched with sulfur (GS). The Mars Global Stimulants had to be mixed with perchlorate to receive the desired perchlorate percentage. To achieve that, 1% wt of perchlorate was added to the soils. Then a tiny amount of soil (that wasn't weighed) was added to a microtiter plate (figure 15), half of them with perchlorate (GP,GSP,JP) and the other half without a perchlorate addition (G,GS,J). To plate the isolates in the microtiter, 100 µm of the fed isolates (F) where placed in autoclaved perchlorate soils (GP,GSP,JP) and 100 µm of the starved isolates (NF) placed in the soils without perchlorate (G,GS,J).

Figure 15: Microtiter plates with Martian analog soils

The Mars chamber was prepared one day before the samples were inserted to it. This started by purging the chamber with N_2 and by placing silica gel (figure 16) on petri dishes inside the glove box. Using silica gels it was a faster way to reduce the relative humidity inside the chamber. The silica gel can absorb moisture starting from a blue color which means it is dry, and while absorbing moisture turns into a pink color which means is saturated. While the relative humidity dropped at 17% and the glove box was filled with N_2, the microtiter plates were inserted in the chamber. After the placement, the chamber was sealed of and the pressure was reduced gradually. Starting from a pressure of 1 bar, the final desired pressure came a couple of hours later. The reason for the slowly decrease of the pressure, and not a steep one from 1 bar to 0.01 bar, was necessary in order to avoid the sharp exposure of the cultures into a different environment causing a potential death of them. The same amount of samples (duplicates) were also placed outside of the chamber to act as control samples.

Figure 16: *Silica gel*

The initial goal for this experiment was to leave the isolates in the chamber for about 14 days and then observe how they behaved in the conditions of 1% atmospheric bar, anaerobic environment flushed with N_2 at 17% of relative humidity, in the presence of analog soils with or without perchlorate percentage. The day after the experiment was set to run, the restrictions due to COVID-19 were applied also to the University and the laboratories. The exposed isolates stayed in the JMMC for more than a month without any supervision. In one of us was given special permission to visit the laboratory after a month, but without extra bulk of agar plates the result were not possible to be consistence. At the same time with the JMMC experiment were also running other various experiments testing the UV radiation on the samples, UV with the presence of perchlorates, perchlorate tolerance and anerobic enviroment. The amount of the different kind of experiments (approximately 1500 agar plates were prepared) waiting for measuring the final growth in the isolated culture was heavy duty to be done under the lockdown circumstances. Thus, the results from this experiment proven to be inconclusive. Besides the fact that we had no results, the experiment proved to be a vary good guide for us in order to get better and improve our methods for the upcoming experiments.

5.5.2 Atmospheric composition of CO_2 17% RH, 1% atmospheric pressure and Martian analog soil

During the following experiment the six primary isolates were exposed to 1% atmospheric pressure mainly composed of CO_2 with in a 17% relative humidity placed in analog soil with and without perchlorate, with and without food. Based on the previous experiment experience, this time the soils were weighted and the purchased CO_2 gas cylinder made our experiments more efficient and consistent to be analysed and the environmental conditions to be closer to the Martian atmosphere, respectively. Also, distributing the number of the isolates equally into three microtiters rather than one, proved to be more convenient for the samples to be analysed later.

After the long period away from the lab, the original isolates were kept in the incubator at -8 Celsius degrees in agar plates. To restore the cultures, a small amount of them where transferred from the incubator to falcon tubes with 50 mL of TSB media. The

restored isolates were kept in the room temperature for a few days to gain enough food for growing and be able to used afterwards. After the isolates have grew enough, the OD was adjusted at 0.4 for a total media and isolate solution of 5 mL, and then serial dilutions were made from 10^{-2} to 10^{-7} for each of the isolates.
The analog soils were weighted at 17gr for each alcove and placed in to the microtiters. Afterwards, a 160 µL of each isolate was inoculated in the microtiters.

To prepare the Martian chamber for the cultures exposure, the below steps were followed. Firstly, the new gas cylinder of CO_2 was connected to the glove box as the new purging system in the place of the previous N_2 gas cylinder. The constant flow of the CO_2 in the glove box and at the same time the operation of the vacuum pump that is connected to the airlock chamber made possible the decrease of the relative humidity from 50% to 17% in less than 30 minutes. Also, being aware that the grease used to seal the perimeter of the chamber could potentially increase the relative humidity with time, silica gel in petri dishes used to low the humidity even more reaching the value of 12% RH. At the same time the glove box was flushed with CO_2 so the total percentage of the atmospheric air in the glove box was almost pure CO_2. The microtiters were inserted in the glove box throught the airlock and were placed in the chamber (figure 17) while the lid was sealed.

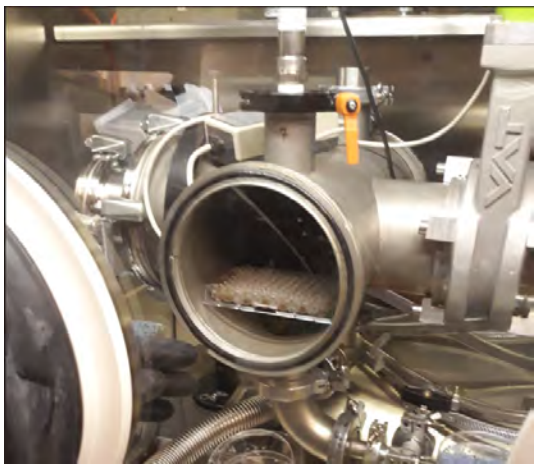

Figure 17: Microtiters filled with soil and isolates in the chamber

After the placement, the main vacuum started pumping air from the chamber reducing the internal pressure from the original 1 bar. Since the isolates need time to acclimatize to the new lower pressure, the decrease was made in steps. Starting from 1 bar atmospheric pressure and letting air out from the chamber through the vacuum, the pressure dropped to the final 0.08 bars. Due to leaks occurring in the system and in order the pressure to maintained at 0.01 bars throughout the experiment, an every day adjustment was needed. At the 8th day of the experiment the pressure gauge crashed and the reason was unknown. The software was attempt to be installed in a different computer but it didn't work. With the pressure gauge wasn't working properly, after the 8th day the adjustment in the pressure to be mainted at the desired value wasn't possible.
By the end of day 12 of exposure the isolates were suspended and mixed in PBS in the microtiter plates. Then, they stayed for 30 minutes at room temperature and then serial

dilution (from no dilution to 10^{-9}) occured following inoculation on agar plates. The plates stayed at room temperature and observed daily.

Results

After leaving the isolates to grow a couple of days the results obtained from colonies counting are displayed in figures 18 - 20. The graphic figures represent the colonies forming unit per analog soil (GS, G and J) both for the control samples outside of the chamber in regular conditions and for the experimental ones inside of the chamber.

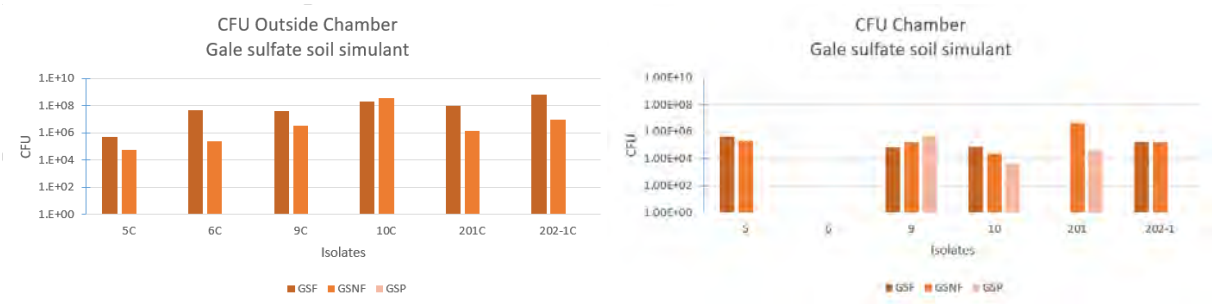

Figure 18: CFU for Gale sulfate soil

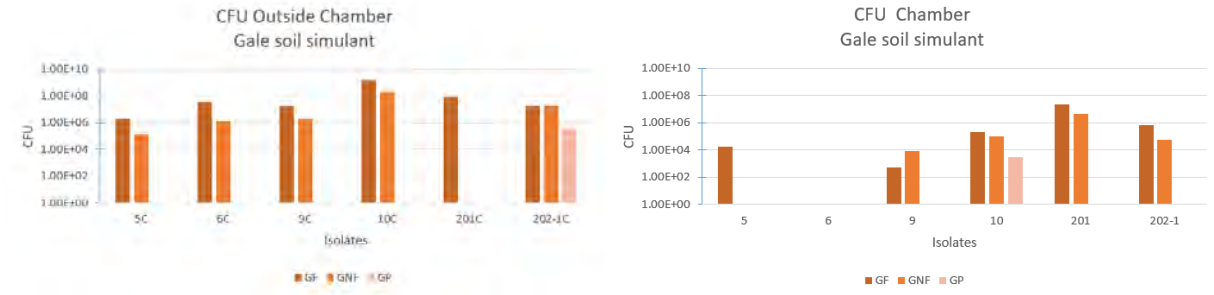

Figure 19: CFU for Gale crater soil

Figure 20: CFU for Jezero soil

Observing the colonies counting in the three different soil simulants we can see how the

different isolates behave under the Mars simulated conditions compared to the conditions found regularly on Earth's environment. The isolate 5 showed growth in all three analog soils with food and without food in regular environment while in the chamber showed growth with and without food for the GS and J soil, and only with food surviving in the G stimulant. The isolates 9, 10 and 202-1 out of the chamber showed a higher CFU bar compared to them inside, while a similar pattern observed also for isolate 201. The isolate 6 showed growth only in the control samples but no growth at the samples under Martian conditions. With the presence of perchlorate in the soils the only isolates that showed any growth were 9, 10 and 201 in the Gale sulfate analog, the isolate 10 in the Gale analog and for the Jezero soil the isolates 10, 201 and 202-1.

In general, in the absence of nutrients the isolates decrease their activity resulted a lower number of final colonies. The cells low their activity due to the lack of nutrients as they struggle to find available sources to sustain their energy. This can be clearly seen as in the most of the isolates the growth without food is lower than the one with food. n addition to, subjecting the cells in dry environments will lead the bacteria to be unable to reach the nutrients resulting their death due to starvation [Dechesne et al., 2010].

Also, regarding the isolates' response to the perchlorate in the soil, the data showed that in sixteen out of the eighteen exposed cases outside of the chamber contributed as an non growth factor. In contrast to the cases inside the chamber that showed eleven out of eighteen cases non growing after the exposure. In the cases that seem to overcome the presence of the perchlorates in terms of growing, appeared overall to have less forming colonies compared to the non-perchlorate soil. The perchlorate in the soil, appears to inhibit the growing ability in these cases. The interesting fact in the case of the perchlorate is that the isolates survived better in the presence of it exposed to Martian conditions compared to the ones exposed in regular conditions. In some cases, the presence of perchlorate could contribute to the raise of the humidity in the soil resulting a moist environment favorable for the organism [Gough et al., 2012]. This could explain the fact that isolate 9 showed a appear to grow better in the presence of perchlorate in one the soils (18, right panel).

Regarding the lower pressure in the chamber combined with the carbon dioxide atmosphere in a anaerobic environment, the isolates appear to tolerate them. Carbon dioxide has been proposed to inhibit microbial grow causing alteration in the cell [Debs-Louka et al., 1999] something that could explain, among other factors, the decrease of the isolates' grow in the chamber. Another study focused on Martian conditions in terms of low pressure combined with carbon dioxide has showed that microbial organisms were not be able to recover from previous exposure [Schwendner and Schuerger, 2018].

An interesting result is that although the isolate 201 presented as a possible contamination, based on a former analysis which showed a potential common skin bacterium, had a better performance overall compared to the other isolates.

6 Residual gases experimental set up and metabolic activity experiments

In one of the introductory chapters (1.3) mentioned that organisms use energy sources and carbon based material to function. The process of how organisms use the available sources from the surrounding environments is not a simple pathway and it can lead to many different reactions depending on the sources that they can utilize and metabolite. The product elements that arrive from these kind of metabolic networks (intermediate or final) are called metabolites. From an astrophysical point of view, these metabolites consider as bio-markers meaning the identification or the absence of them in the spectra of a planetary atmosphere could identify the presence of life on a planet or specify particular kinds of living organisms [Des Marais et al., 2002]. On Earth, life as we know it, is carbon based and by that carbon based chemistry provides methods to identify life that exists or life that has already extincted. The majority of biosignatures related studies concentrate on simple molecules like oxygen or methane, but Earth's biosphere produce a more broader range spectrum of gaseous compounds into the atmosphere. A part of this spectrum is also the volatile organic compounds (VOCs) which are more complex molecules also related to biotic processes that could be useful as complementary biosignatures. The same identification method of life could provide answer not only for planet Earth but also for Mars and for other exoplanets around other host stars. As far as we know, there are no confirmed identified bio-signatures on Mars like the one we have on Earth. The environment on Mars and the atmospheric composition, as well the energy sources differ from that on Earth, so if a potential microbial life on Mars uses different energy source, will have also a different atmospheric print and impact on the spectra. But in order to understand how microbial life behaves and metabolizes the available energy sources from the surrounding environment and what potential gases releases through this process, the study of residual gases coming from microbial life in extreme environments emerged.

In the previous chapter (5) bacteria isolated from the extreme environment of Atacama desert on Earth were considered as potential microbial life may able to survive the extreme conditions on planet Mars as we see it today. Some of the isolates showed growth in all or some of the testing parameters. By testing the bacteria cultures in these conditions, we are not be able to know if they are active while they exposed to them or they just went under a dormant metabolically inactive state. In this part of the thesis the goal was to observe the residual gases coming from the isolates while they were being exposed to Martian simulated conditions and from that to see how their metabolic rates changed compared to regular conditions.

6.1 Methods for measuring the residual gases

Indentifying the smallest amount of gases coming from a small sample of bacteria demands instruments sensitive enough and capable of measuring and identifying the chemical spectrum of these compounds.

6.1.1 The Quadrupole Mass Spectrometer

The Martian simulator chamber (JMMC) is equipped with a quadropole mass spectrometer (QMS), the PrismaPlus QMS, that is connected directly to the analyzer chamber and from that to the main chamber itself. The QMS is an open ion source that is subjected to the same partial pressure with the rest of the system while it is connected to it. The maximum operating pressure of the system is at 10^{-5} mbar (10^{-4} torr) [Pfeiffer, 2020].

The principle in mass spectrometry is that uses an analytical method to determine the mass to charge ration in a mixture of ion gases with the measurements presented in a spectrum of intensity as a function of them. The mass spectrometry method is used to determine the chemical composition from simple molecules to more complex chemical compounds. This method is applied in a big variety of different fields, in research and industry, that wants to perform isotopic analysis of simple or complex mixture of gases. The smallest of them that they attached to high vacuum system are also used to detect leaks and contamination in the systems, called residual gas analyzers (RGAs). A mass spectrometer like those, is the one that is attached to the JMMC.

The gas container (analyzer chamber) has to reach a maximum of an 10^{-5} mbar atmospheric pressure in order for the QMS to operate safely. Once the pressure drops under the minimum pressure then the desired atmospheric analog or the desired amount of residual gases can enter through an inlet system to the gas container. Through the gas container the gases go through the ion source in which they become ionized by a constant bombardment of electron flux (numbered as 1 in figure 21). Then a source heater ,attached to ion source, heats up a cathode filament forcing the electrons to be accelerated. In this way gas molecules that enter the ion source get ionized by the collisions between them and the electrons [Pfeiffer, 2020].

Figure 21: *Quadrupole Mass Spectrometer*

The accelerated ions after the ion source they follow the Quadrupole mass filter (QMF) (numbered as 2 in figure 21). The role of the mass filter is to collect positive and negative ions with a specific mass to charge ratio. The mass filter is made up of four parallel squared placed quadrupole rods, two of them having positive voltage and the other two

negative voltage, that create an electric field. The ions with specific mass to charge ration while entering the QMF will be able to reach the connected detector and obtain a mass spectra.

The QMS owns two kind of detectors depending on the type of measurement someone wants to obtain. The first detector, Faraday cup, is able to identify ion currents with specific intensities converting the ion current to voltage (by an amplifier) and sending the signal to the analysis system. This detector is suggested for long period measurements at high temperatures but is unable to detect fast measurements and lower quantities of ions. The second detector, Secondary Electron Multiplier (SEM), is suggested to detect small amounts of ions. The difference between this detector and the first, is that the SEM causes a secondary electron emission by hitting the ions onto metal sheets. The degradation of the metal sheets can effect the capacity of the detector to operate.
After the detection process the ion current is sent to the system where one can obtain the mass spectrum visualized by a software installed in the computer, the Quadera software. This mass spectrum is the spectrum obtained from the separation of ions according to their mass to charge ratio. The spectra peaks can easily correspond to compounds with the help of the spectra library that is part of the Quadera software. Using the QMS and the computer software it is easy to detect in real time the residual gases or the atmospheric analog composition.

Experimental set up using the QMS

Having an instrument like the QMS available in the JMMC the next task was to set a proper connection sequence in order to serve our purpose. The below steps were followed to achieve the desired experimental set able to measure residual gases coming from microbial life.

- Since the QMS has been already used in the past but not recently, the system run a test trial to figure out what was missing or what was out of date and it was in need of optimization. One of the first things that was noticed, was that the computer was old enough that wasn't able to support a newer version of software environment. This scenario confirmed by technicians so the need of a new computer was prioritised in the list of improvements.

- The next step followed, we wanted to make sure that the vacuum pump system connected to the chamber was able to reduce the pressure to the minimum required in order for the QMS to operate safely. Running the vacuum pump while was connected to the chamber and using the pressure transducer the installed Omega TRH Central software was able to measure absolute or negative pressures. Several trials for reducing the pressure to the wanted range (10^{-5} mbar) were executed. The pressure couldn't drop below the value of 3 mbar. Then, we suspected that there must be some kind of leak between the connection in the system. So, firstly all of the o-rings bands and flanges connecting the various parts of the system were replaced by new ones. When the O-rings are over-used or after some time they get altered/deterioration, so they could let some air in and prevent the system from

reaching a higher vacuum state. Replacing and cleaning all of the intermediate parts didn't solve the problem.

- The next thought was to look at the vacuum pump itself (Appendix A.4) and make sure that it is in a operated condition. The oil necessary for the pump to work was checked and replaced with a new one. The pressure after these alterations dropped to 0.8 mbar.

- Following the above improvement the pressure still wasn't fixed at the demand range. The next was to measure the pressure connecting the pressure transducer in every possible connection. After a lot of modifications and adjustments there wasn't any noticed leak between them. Asking for a further advice from Claus Birger Sørensen, who was the person that the laboratory borrowed the vacuum pump from, we managed to borrow a new more precise pressure meter to test the vacuum. The pressure value measured to be at $3 \cdot 10^{-4}$ mbar. Finding out that the problem was issued to the pressure transducer, a new one that would be able to measure lower range values was ordered and installed (A.6.

- After fixing the problem with the pressure measuring, and with the arrival of the new computer, with the valuable help of Jannis Nikolas Bouchikas, computer specialist of the department, we managed to install a newer version of the Quadera software.

- Along with the previous steps, also a dosing valve was order (A.5). The purpose of the valve was to be connected to a special designed needle (build in colloboration with the mechanical workshop) that would be able to insert the rubber lid placed on the flasks containing the isolates with soil. By opening the valve the desired amount of air would be transferred to the gas container and get analyze by the QMS.

The design and improvement of the experimental setup using the QMS and the JMMC for residual gases measurements took longer than expected, while the new pressure transducer arrived during the last weeks before the completion of the project.
For the above reasons the attention focused to find other possible methods to analyze the samples.

6.1.2 Gas chromatograph

The Microbiology department owns a Gas chromatograph (7890A GC system, Agilent Technologies 22) that was kindly offered to operate the analysis of the samples in the requested time.
Gas chromatography (GC) is an analytical method that is used to separate and determine the presence of the various chemical compounds of a mixture. This method is widely used for quality control in the industrial field and also in the research field from product analysis to meteorites analysis.
The desired gas mixture is injected in the GC from the autosampler or the inlet system (autosampler) using a carrier unreactive gas, that is usually nitrogen or helium. The GC separates the various volatile compounds into individual components in the oven and from

there the detection of them occurs in the detector part. When one of each compound is inserted into the detector is subjected to an electrical signal. This signal then is forward to a data analysis system and it is translated as a peak on a chromatogram. This peak indicates the presence of a chemical compound.

The available detected gases using the GC in the lab is CO_2, CH_4 and N_2O.

Figure 22: *Gas chromatograph* [Technologies, 2021]

- Detection of CH_4: The production of methane -methanogenesis- is the metabolic result coming from the microbial metabolic pathway in anaerobic conditions, in a process called fermentation. The CH_4 product is resulting mostly from respiring CO_2 or from breaking acetate, CH_3COOH to CH_4 and CO_2. The organisms that manage to perform methanogenesis belong to the domain Archaea -methanogens [Schwieterman et al., 2018]. Besides anthropogenic methane production, such as fossil fuel and biomass burning, and agriculture, methanogenesis as a product of microbial metabolism contributes to Earth's atmosphere. Methane has been proposed as a potential biosignature on Mars also [Krasnopolsky et al., 2004].

- Detection of N_2O: The main production of N_2O detected on Earth's biosphere occurs as an intermediate gaseous product from denitrification of nitrate NO_3 to N_2 gas [Schwieterman et al., 2018]. Bacteria causing the denitrification reaction are mainly heterotrophic bacteria living in soils and sediments. These bacteria in the absence of oxygen utilize NO_3, which origin is commonly from fertilizers and explosives [Laue et al., 2000], to N_2 in environments. On Earth's atmosphere the contribution of N_2O coming from abiotic reactions are limited [Oyarzun and Oyarzun, 2007], and for this reason has been proposed as a strong biosignature.

  Regarding to Mars, nitrogen first detected on the surface of Mars using the SAM instrument equipped on the Curiosity rover. The examined samples resulted from drilling the John Klein and Cumberland region as well as sand and dust from Rocknest region all of them siting within the Gale crater. The detected nitrogen was in the form of nitric oxide indicating the presence of nitrates in sedimentary rocks with an approximate estimation of 1,100 ppm of NO_3 [Stern et al., 2015]. It is

important to be noted that the abundance of nitrates that found on Mars consist of remnants of previous formation of nitrates probably from thermal shock due to volcanic activity or due to impact events [Stern et al., 2015]. The importance of this result lying on the fact that nitrogen has to be bound to other organic or inorganic elements in order to be utilized and participates in metabolic pathways from living organisms.

- Detection of CO_2: Carbon dioxide is part of Earth's atmosphere as well on the Martian atmosphere from planet formation due to outgassing. For this reason is not considered as a biosignature gas [Seager et al., 2012]. The volatile compounds resulting from life on Earth are not limited in the biosignature gas list. Although carbon dioxide is not constituted as biosignature gas, it could be generated not only as a result from abiotic sources but also as a result from metabolic processes by living organisms [Seager et al., 2012]. Carbon dioxide production from bacteria occur as a result from the process called cellular respiration. During the process of cellular respiration in the cell of organisms, energy from nutrients and especially oxygen released to form adenosine triphosphate (ATP) by breaking down larger to smaller molecules, a process called catabolism. This process is essential for providing the cell with energy needed for cellular activity. In the absence of oxygen the process followed is called fermentation. During this process carbon dioxide and ethanol can be released by breaking down glucose.

The testing isolated bacteria from the Atacama desert are not restricted to the Archaea domain, so we do not expect to see any methane production as well the testing soil didn't contain any form of nitrates so the detection focused on the carbon dioxide emission.

6.2 Metabolic activity experiment

The configuration for measuring the residual gases coming from the bacteria exposed to Martian conditions is divided in four main procedures. Firstly, the preparation of isolated bacteria in the microbiology laboratory, then the preparation of the Martian chamber to regulate the conditions in which the bacteria will be exposed to and and the actual exposure time, the extraction of residual gases, lastly the analysis from the laboratory owning the GC and following the data analysis resulting the GC analysis.

Bacteria selection

The criteria of choosing the proper isolates for this experiments was based on the results from the previous chapter. The survival conditions results contributed to which of these bacteria will be more possible to cooperate in the extreme analog conditions in the simulator chamber. Due to lack of time this part of the research project focused on two of the five isolates that seemed to survive better in some or all of the exposed conditions.

Due to figures 18,19 and 20 the growth of the primary isolates' colonies in each condition (different, soil, food, no food and perchlorate) can be seen and compared. Isolate 5

rejected because showed no growth in the presence of perchlorate in none of the analog soils, isolate 6 showed no growth at all, isolate 9 showed growth in one of the three analog soils, isolate 10 showed growth in all of the conditions only in the Gale crater analog soils while isolate 201 and 202-1 showed growth in all circumstances only in the Jezero crater analog soil. Bearing in mind the above comparison, the isolates that were selected to be exposed again in extreme conditions with the purpose of detecting their residual gases were isolate 201 and isolate 202-1.
An extra contribution to the specific selection was the analog soil that was chosen to work with. Firstly, the growth results based on the type of analog soil that was used, wasn't seemed to play a tremendously role on the isolates' growth and secondly the fact that the chosen isolates (201,202-1) appear to grow in all conditions in one of the three analog soils, led us to decide to work with the Jezero simulant soil. Also, a third reason for choosing the specific soil was arise from the selected landing location of the Perseverance mission on Mars [Perseverance, 2020]. The Jezero cater is located in the region of Isidis Planitia. There, a crater has been formed as an impact of a meteorite collision with the planet and later a similar event created a smaller crater within the region, the Jezero crater with a diameter of 45 km. For this region there have been studies showing that river flow was present in the ancient Jezero region, that they were able to form delta. For this reason it is believed that the water could have drifted any form of microbial life from the surrounding area and thus accumulate them in the crater. So the choice of operating the experiments in such analog soil, makes the results of great interest.

Bacteria preparation

Based on the master thesis work of Poul Kari Madsen, the two primary isolates' DNA was sequenced and the two isolates showed identification results for the genus and the species they belong to. Isolate 201 identified to be in the genus of Staphylococcus and species of capitis and the 202-1 as Bacillus mojavensis [Madsen, 2020].

These two strains were incubated in Erlenmeyer flasks with 150 mL of fresh autoclaved TSB media, for 24 hours in the 37 Celsius degrees incubator and then for another 24 hours in the 24 celcius degrees incubator. After two days and while the strains had been multiplied enough, they placed in Eppendorf tubes, they got centrifuged to separate the media from the cells and then were inoculated in 15mL falcon tubes with fresh TSB media while the optical density adapted to 0.6. At the same day, the number of growing colonies measured for both isolates right before they were ready to be exposed. That was possible by making serial dilution from 10^{-2} to 10^{-7}, plating them in regular agar plates in the room temperature incubator and observe on a daily basis. Following the dilution process, the Jezero simulant soil was mixed with perchlorate in order to obtain a 0.6% wt of perchlorate in the final soil. The soil was autoclaved and 6gr of it were placed in each of the flasks that were also autoclaved beforehand. Finally, 150 μL of each isolate mixture were inoculated in the flasks.

The experiments were divided in three main categories based on the exposure conditions. In each group we had 5 flasks for each isolate, containing the Jezero analog soil enriched with perchlorates plus the amount of liquid cultures. Besides the flasks containing the

isolates also control samples containing only the analog soil were placed in each condition. Also, to avoid experimental bias or some random error for every flask we made triplicates. In total for each condition we had 45 flasks.

In the first group, the bottles were placed in the chamber containing only N_2 as an atmosphere, while the percentage of relative humidity was at 15%. After the exposure the isolates were incubate in room temperature. In the second group, the temperature parameter introduced. The isolates were exposed also in N_2 with 15% of relative humidity, but after the exposure time they were placed in the 4°C incubator. Finally, the third group was exposed to regular atmospheric conditions while incubation occurred in room temperature.

Preparation of the Martian chamber

Since the available detection for residual gases using the GC are CO_2, CH_4 and N_2O, the configuration of the experiment was based on the idea that in order to detect the smallest changes in the atmosphere, the atmospheric analog should be governed by other gas than CO_2. For this reason the gas that was selected to work with as an analog atmosphere was N_2.
In order to create the desired atmospheric analog, the N_2 gas cylinder was connected. While the glove box was sealed, a constant flow of N_2 was purging the system. At the same time, to make the purging procedure faster but also to help the humidity in the glove box to decrease, while purging the system the small vacuum pump that is connected to the airlock was opened. Also, silica gels were placed in the glove box to contribute maintain the humidity at a low percentage.
After 20 minutes and while the humidity has steady at 15% the 30 flasks were inserted to the glove box using the airlock (figure 23). The lids on the flasks were opened to let the nitrogen-ed air flow in them. After half an hour an extra purging run was held to make sure that most of the regular atmosphere that was dominated before in the flasks was replaced by nitrogen gas. The flasks stayed opened for an extra half an hour. After that time all the lids were placed again on the flasks and gradually all of them inserted out of the system. Also to make sure that the lids were sealed enough and wasn't happening any leak to the bottles, every lid was secured using a bottle sealer. Half of the samples were placed in the room incubator while the other half in the 4° C incubator.

Figure 23: *Samples inserting the GB through the airlock*

Residual gases extraction

After the exposure of the samples in a dominated nitrogen 15% RH environment the samples stayed in the incubators and the gases were extracted once a day for five days in a row. While the same procedure was occured for the samples that exposed only to regular atmosphere. Every day one triplicate of each group (24° C, 4° C and 24° in JMMC), was taken in the laboratory for the gas extraction.
The gas extraction procedure was following the same steps in every round. Firstly, the flask was unsealed by removing the metallic cap while keeping the rubber lids on them. Then with a regular syringe inserting the lid ,carefully, the air in the bottle was blend for about 5 to 10 seconds. After achieving an equally distributed air, and using the syringe, 3ml of the gas in the bottle were extracted. This air sample was inoculated in small vacuumed flasks specially designed for inserting the Gas Chromatograph to get analysed (figure 24).

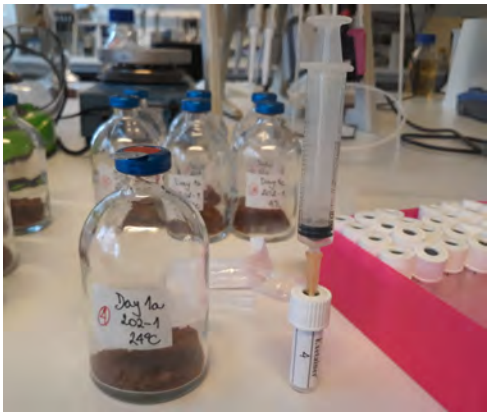

Figure 24: *Residual gas extraction*

This procedure took place everyday for five days to see if any changes in the residual gas composition was changed. In addition to the residual gas extraction, afterwards all of the testing samples were placed in agar plates to see their survival condition by measuring their CFU.
The flasks were completely opened under a Laminar flow cabinet, in sterile conditions. From each sample 2gr of soil were taken and placed in falcon tubes. Additionally, 5mL of autoclaved PBS was added to the soil. Then, the samples were being vortex for about 20 seconds. The samples were left in room temperature for an hour. For measuring the CFU, the samples had had to be diluted. This was done by inoculating 100muL of the PBS, that was mixed previously with the soil, with 900muL of steriled water and then plating 100muL of them on agar plates while evenly distributed using glass beads. This was dilution 10^{-1}. Serial dilutions up to 10^{-7} were carried out for each of the flasks, all triplicates for five days of measurements.

Data analysis

The first results from the dilution process for the liquid cultures that were made before starting the exposure experiments were available a couple of days after. From each isolate it was chosen the number of dilution that was appeared to have 30 to 300 colonies, as they observed and counted with the click counter.

For isolate 201, the number of colonies counted from dilution 10^{-2} was 223 CFU per 1 μL so the liquid media that were inoculated in the flask to be exposed were $223 \cdot 150 = 3.345$ CFU at $150\mu L$, for isolate 201. Respectively, for isolate 202-1 the number of bacteria colonies calculating from dilution 10^{-4} were $184 \cdot 10^2$ CFU per 1 μL, so the total were $276 \cdot 10^4$ CFU.

The results from the gas measurements were available from the laboratory three days after. The results from each sample can be seen in Appendix (A.7).

From each measurement it was subtracted the average value that was issued from the control triplicates for each day. Then from each group of triplicates the average was found with the corresponding standard deviation using Excel Spreadsheet Software.

The dependence of the CO_2 in μg that produced by the isolates as function of time was found. The CO_2 represented in units of ppm (moles of CO_2 per million parts of air) was converted to units of atmosphere (atm) using equation (6.1). Then with the use of the equation of state for the our ideal gas (equation 6.2) the number of moles were found for the production of CO_2 for each isolate in every expose condition. And afterwards the moles of CO_2 converted to μg using equation 6.5.

$$1atm = 10^6 ppm \tag{6.1}$$

$$P \cdot V = n \cdot R \cdot T \tag{6.2}$$

Where, P is the pressure of the gas due to CO_2 emission , V the volume of the gas, n the number of moles contained in the V , T the temperature of the gas and the R is the ideal gas constant. The parameters used are summarized in table 12.

The volume of the gas was calculated subtracting the volume of the Jezero simulant soil from the total volume of the flask, $V_{flask} = 0.118L$. The bulk density of the soil is $1.54gr/cm^3$ (31), meaning that the volume of the 6gr of soils occupy 0.00389L. So the volume of the gas will be $V_{gas} = V_{flask} - V_{soil} \rightarrow V_{gas} = 0.1141L$.

Applying the equation state for our gas, the resulting moles, n, appear in table ref as well their corresponding number of μg, $n_{\mu g}$, using equation 6.5 .

$$n_{\mu g} = n \cdot M_{CO_2} \cdot 10^6 \tag{6.3}$$

Parameter	Value	Units
P	changes in every measurement	atm
V	0.10876	$Liters$
$T_{24^\circ C}$	297.15	K
$T_{4^\circ C}$	277.15	K
R	0.0821	$atm \cdot L/mol \cdot K$

Table 12: Parameters used in equation of state

$$M_{CO_2} = (12.01 + 2 \cdot 16.00)\frac{gr}{mol} = 44.01\frac{gr}{mol} \tag{6.4}$$

$$n_{\mu g} = 44.01 \cdot 10^6 \cdot n \tag{6.5}$$

Where M_{CO_2} represents the molar mass of CO_2.

The dependence of the amount of μg of the produced CO_2 from bacteria ,201 and 202-1, as a function of time appears in figures 25 26 and 27.

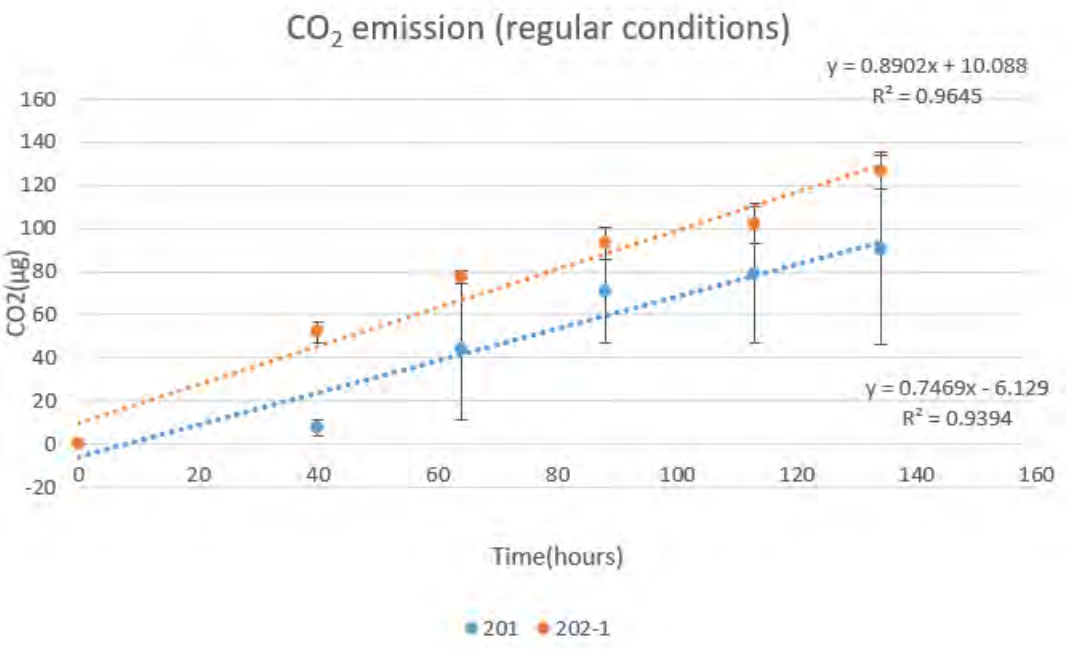

Figure 25: CO_2 released from bacteria in regular atmospheric conditions

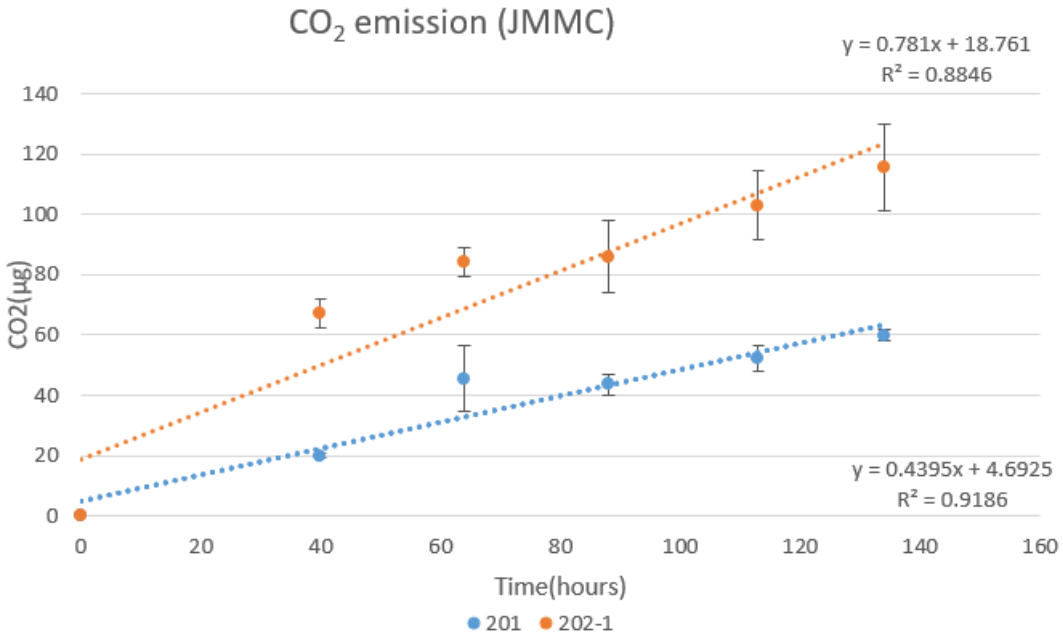

Figure 26: CO_2 released from bacteria in 15% RH and N_2 atmosphere

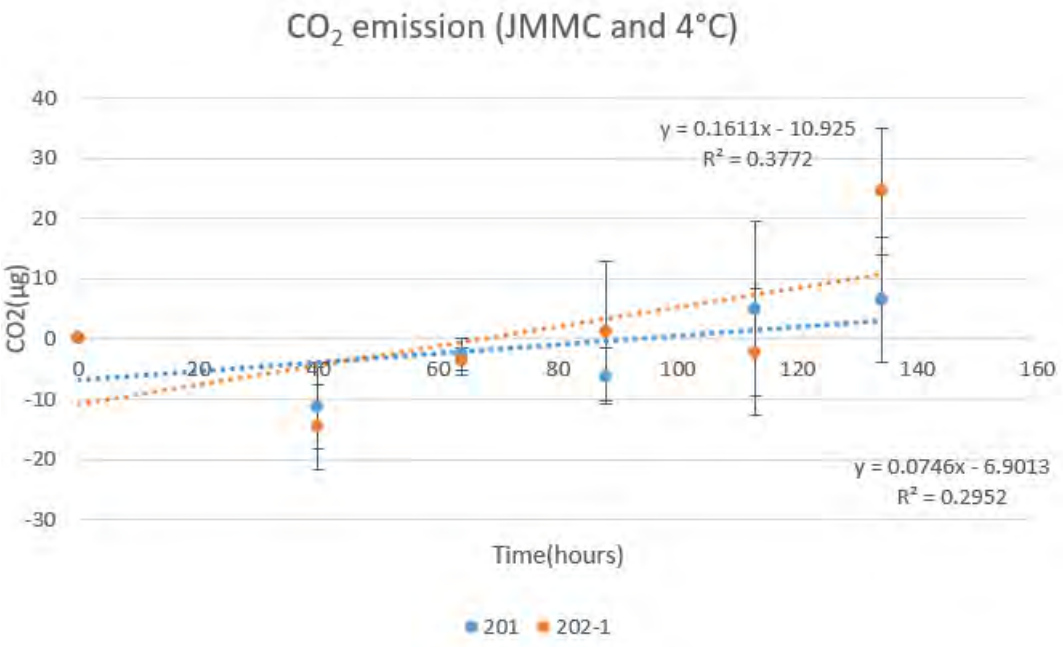

Figure 27: CO_2 released from bacteria in 15% RH and N_2 atmosphere at 4o C

In the figures 25, 26 and 27 is presented the amount of CO_2 in μg, that was produced by the isolates 201 and 202-1 in three different exposure conditions in the duration of five days.

In the first figure, the CO_2 emission follows an almost clear linear dependence with time for both strains. And the emission rates found by the slope are 0.89μg/hour for isolate 201 and 0.74 μg/hour for isolate 202-1.

The isolates with the absence of oxygen in a N_2 dominated atmosphere while the relative humidity was 15%, also follow a linear dependence with time regarding their production in CO_2, as seen in figure 26. Isolate 201 with a slightly dropped rate in CO_2, at 0.78 μg/hour and isolate 202-1 with a more steep drop at 0.43 μg/hour, compared to regular conditions.

In the third exposure condition, the addition of a lower temperature seemed to affect the CO_2 production. As it is shown in figure 27 bacteria struggled to produce CO_2, but after the third day it appears to have a small increase in the production. The metabolic rate of CO_2 is small as 0.16 μg/hour for isolate 201 and 0.07 μg/hour for isolate 202-1. The abrupt change in the temperature probably shocked the cells while further measurements could had helped to see if they actually cooperate with the low temperature environment. The negative values represented in the graph, means that the control sample which its measurement subtracted from the isolate sample have a greater value.

In addition, we have to consider that some of the CO_2 could have been dissolved in the liquid media in all of our testing groups, but the solubility of it could not justify all of the observed production. At a temperature of 25° in an atmosphere of 1 atm the carbon dioxide solubility calculated at 1.45 gr/L [Dodds et al., 1956]. This value corresponds to $217 \cdot 10^{-3}$ ppm. With a lower temperature in the third set, the solubility of the CO_2 in the liquid increased [ToolBox, 2008], so more CO_2 could be dissolved and thus not able to detect in the form of gas.

In addition to the gas analysis,the survival analysis after the exposure didn't show any results. The exact protocol was observed and it is still unknown what went wrong with the procedure. So, we don't know how many of the isolates were able to survive this time.

Overall, we can clearly see that produced CO_2 was detected in all three conditions. The production was gradually increased over time for exposure to low humidity and N_2 environment while in the same conditions with lower temperature there is a trend for gradually increase without a confirmed linear dependence over the time.
The detection of the carbon dioxide indicates the ability of the isolates to undergo of the process of fermentation. Fermentation is a case of anaerobic catabolism, in which the energy is resulted from electron exchange only from the organic compounds and without the presence of external electron acceptor.
The importance of the result lies in the fact that the isolates were able to overcome any of the limited factors during their exposure and generate their metabolic activity producing residual gases.

7 Conclusions and Prespectives

Life as a concept in the Universe is not well-defended yet. However, it is this force that keep us searching for the meaning of it in many terms. The limits of life are depended on the conditions of the environment and since there is no evidence for an existing form of life different from what we know on Earth, searching for environments similar to ours or with a common climate history stands for the reason.

One could not be able to approach the starting point of the cycle of life without bearing in mind how the the elements and the particles from the interstellar medium ended up on being planets and atmospheres of the planets. The way in which the atmospheres of the planets formed in our solar system, beginning from the collapse of the nebula gas forming the protoplanetary disc is still under research and none of them has given a precise answer to this complex question. Besides the study of the origin of the planetary atmospheres in the phase of planetary formation, an additional proposed mechanism for delivering essential compounds on the planets is the impact scenario due to asteroids and/or comets [Robert, 2001][Drake, 2005]. The fact that Mars is believed that once had a more denser atmosphere, an evidence coming from weather erosion observed in the highland craters [Catling, 2014] along with the fact that water was abundant in the Noachian period to form valley systems [Ehlmann et al., 2016, Solomon et al., 2005] points towards to question if life had the opportunity and the time to arise on the red planet during this period.
During this thesis, an investigation regarding the origin of the atmosphere both on Earth and Mars was attempted. During this approach we based our study in two models, the outgassing model and the collision model. Based on these models, the outgassing effect for Mars appears to be almost ten times smaller compared to that that could have happened on Earth. While for the collision model the proportionality between the two planets revealed a value close to four. The former could be an suggestion that if the atmosphere came from collision from an object carrying relative same compounds for both Earth and Mars, the created atmosphere would be the same for both planets. The Martian atmosphere today resulting from these two models can not be justified only from today's escape ratio (2-3 kg/s [Jakosky et al., 2018]). This lead us to calculate in what time the atmosphere of Mars interrupted based on the two models knowing that solar activity in terms of solar wind was stronger than it is today causing Mars losing its atmosphere due to magnetic field loss [Wood et al., 2002] [Jakosky et al., 2018]. That time found to be at 3 Ga ago for the outgassing scenario and approximately at 3.8 for the collision model. The difference compared to what it has been already found, 3.9 Ga to 4.1 Ga ago [Jakosky et al., 2015a], is not big enough to come to a conclusion for the atmospheric origin. It is however noticeable that simple calculations based on free fall motions and proportional relationships driven us to valuable results. By that, we found that the Martian atmosphere might had favorable conditions for life to arise for approximately 0.8 Ga to 1.5 Ga until the planet lost its dense atmosphere. These numbers are in agreement with previous studies focusing on the first arise on Earth [Betts et al., 2018] [Dodd et al., 2017] and a possible arise of life on Mars [McKay et al., 1992].
Future studies could be focused on the composition of the asteroids and comets and also to the more complex equations regarding the motions of these objects around the solar

system. This along with further research of how planetary atmospheres were created into the protoplanetary discs could help us to understand further the origin of the atmospheres following the origin of the life itself.

Today's Martian atmosphere differs from the one that we talked about in the former part. The impact erosion events on its surface along with the loss of its magnetic field have lead the atmosphere to seems hostile for life to emerged. The dry environment, the extreme UV and cosmic radiation, along with the thin atmosphere and the presence of perchlorates are some of the 14 inhibitory factors are now present on its surface [Schuerger et al., 2013]. This has established an environment difficult to habitat from humans. The search for microbial life that could be able to survive the harsh Martian conditions has been proposed during the last years with a direct emerge of finding places on Earth which mimic the Martian conditions.
In this thesis, we exposed bacteria coming from the Atacama desert, which among others considered to be a very good analog of the Martian environment. Starting from testing the perchlorate and UV tolerance we expanded our parameters into one experiment including 17% of RH, anaerobic conditions, a mostly dominated carbon dioxide atmosphere, pressure that was reached the 8mbar using also analog soils from two different region on Mars, Gale and Jezero crater, using the Jen Martin Martian chamber for a duration of 12 days. The results showed that in terms of UV radiation 3 out 6 isolates showed no decrease in growth while the rest of them appeared to grow slower after 3.5 to 4.5 minutes of exposure. In terms of testing multiple parameters at the same time showed that some the isolates tolerate one or more parameters found on Mars, proving that the Martian environment could be tolerated from microbial life found on Earth. For a more depth analysis is suggested to test each parameter separately and also the including triplicates for each measurement to minimize any random error.

Testing the survivability in the previous part was an guidance to conclude which of the isolates tolerate better the Martian conditions. Barring in mind those results, two of the isolates that seemed to have had a better response on them where chosen in order to measure their probable emission in carbon dioxide for a period of five days. The exposure of the isolates showed the production and detection of carbon dioxide using the GC. The carbon dioxide emission from the exposed isolates to anaerobic conditions in a nitrogen environment with 15% of RH using as analog soil the Jezero crater simulant, showed a decrease compared to regular conditions. A more steep fall in the emission rate was seen when the temperature factor was inserted as an additional parameter, indicates that the exposure to lower temperatures affects the metabolic activity of the isolates. The fact that the isolates managed to produce enough carbon dioxide to be detectable indicates that cells weren't under a dormancy state while they were able to metabolize the nutrients from the environment. Suggestion for future experiments could to be the detection of the residual gases for a extended period of time to see how the isolates respond to the condition for longer periods. Also, the use of the QMS in the Martian chamber could be used to detect a more broaden range of gases, such as alcoholic compounds (resulted from fermentation). In this process the Jens Martin Martian Chamber was upgraded to support future gas analysis experiments. Regarding this, it is suggested to test the system once the water supply is available for supporting the turbo vacuum pump which

is necessary for the QMS to start operating. Once the QMS is ready the changes in the atmospheric analog could be observed through the monitor real time, improving our perspective regarding of how effective and how fast the analog fulfilled our purpose. Also, the new UV lamp has been recently arrived so future UV experiments will be more accurate since its spectrum expanded to parts of the spectra that is considered even dangerous for the cell and hasn't been examined in our experiments.
The ability of microbial life to produce volatile compounds in environments beyond Earth could be useful in terms of terraforming the red planet. It has been already suggested that microbes could be a solution for increasing the atmospheric gases on Mars [Friedmann and Ocampo-Friedmann, 1995] and in the far future this could establish a more hospitable place for life. Also, the detection of these volatile compounds produced by microbial life is known to influence the atmosphere [Leff and Fierer, 2008] [Peñuelas et al., 2014]. So, the detection of them in simulated conditions in the laboratory, could serve as guide for detection of exo-life in planetary systems nearby stars using their spectra.

From the Viking mission to the latest Perseverance mission on Mars, human kind will never stop searching for past or future possible habitable planets. From landers to satellite missions and all the data that could be provided to us are always a step closer to the truth, opening our perspectives for future exploration and understanding our position in the place that we call cosmos.

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Abbreviations and notations

JMMC = Jens Martin Mars Chamber
GB = Glove Box
RH – Relative Humidity
QMF = Quadrupole Mass Filter
QMS = Quadrupole Mass Spectrometer
SEM = Secondary Electron Multiplier (when mentioned under the Quadrupole Mass Spectrometry chapter)
TMP = Turbomolecular Pump
Wt% - weight percentage
EMH – Enrichment Media Halophile
TSB – Tryptic Soy Broth
G – Gale crater soil simulant
GS - Gale crater soil simulant with added sulfur
J – Jezero crater soil simulant
ppm = parts per million
F – Food
NF – No food
MGS – Mars Global Simulant
MGSF – Mars Global Simulant Food
MGSNF – Mars Global Simulant No Foo
MSC – Mars Soil Control (Isolates placed outside the Mars chamber)
MSM – Mars Soil Mars (Isolates placed inside the Mars chamber)

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A Appendices

A.1 Appendix - UV lamp

Scientific Education | Technical Training and Education | Trade | LEYBOLD | LEYBOLD DIDACTIC GmbH

7/98-V5-

Instruction sheet 665 635

UV Analysis Lamp (665 635)

The analysis lamp for short-wave and long-wave ultraviolet radiation is used to investigate fluorescence in numerous organic and inorganic materials.

Ultraviolet radiation cannot be seen by the naked eye; only a blue-violet shimmer can be seen through the filter of a UV lamp. This is the proportion of "visible light" which the burner generates and which is absorbed by a filter with the exception of this weak residue. This lamp lets you make use of two groups of ultraviolet radiation:

Short-wave range: 254 nm
farthest from the visible spectrum. The short-wave energy is preferred for chemical analysis applications and cold sterilization.

Long-wave range: 366.0 nm
closest to visible light (also called black light). The long-wave radiation excites fluores-cence in numerous natural substances and materials made from them. It is relatively harmless to the skin and eyes.

Function

The two UV burners are switched on separately using two push-button switches on the side of the device. Do not switch off the lamp during brief interruptions, but leave it burning. Note that the maximum radiation intensity is only reached after approx. five minutes have elapsed. To ensure maximum service life, always allow a cool-down time of at least five minutes after switching off the device before switching it on again.

Technical data

Wavelength:	254 nm and 366 nm
Burner power:	2 burners, 4 W each
Dimensions:	205 x 70 x 55 mm
Weight:	1.0 kg
Power:	220 V/50 Hz

Figure 28: *UV lamp*

A.2 Appendix - Soils

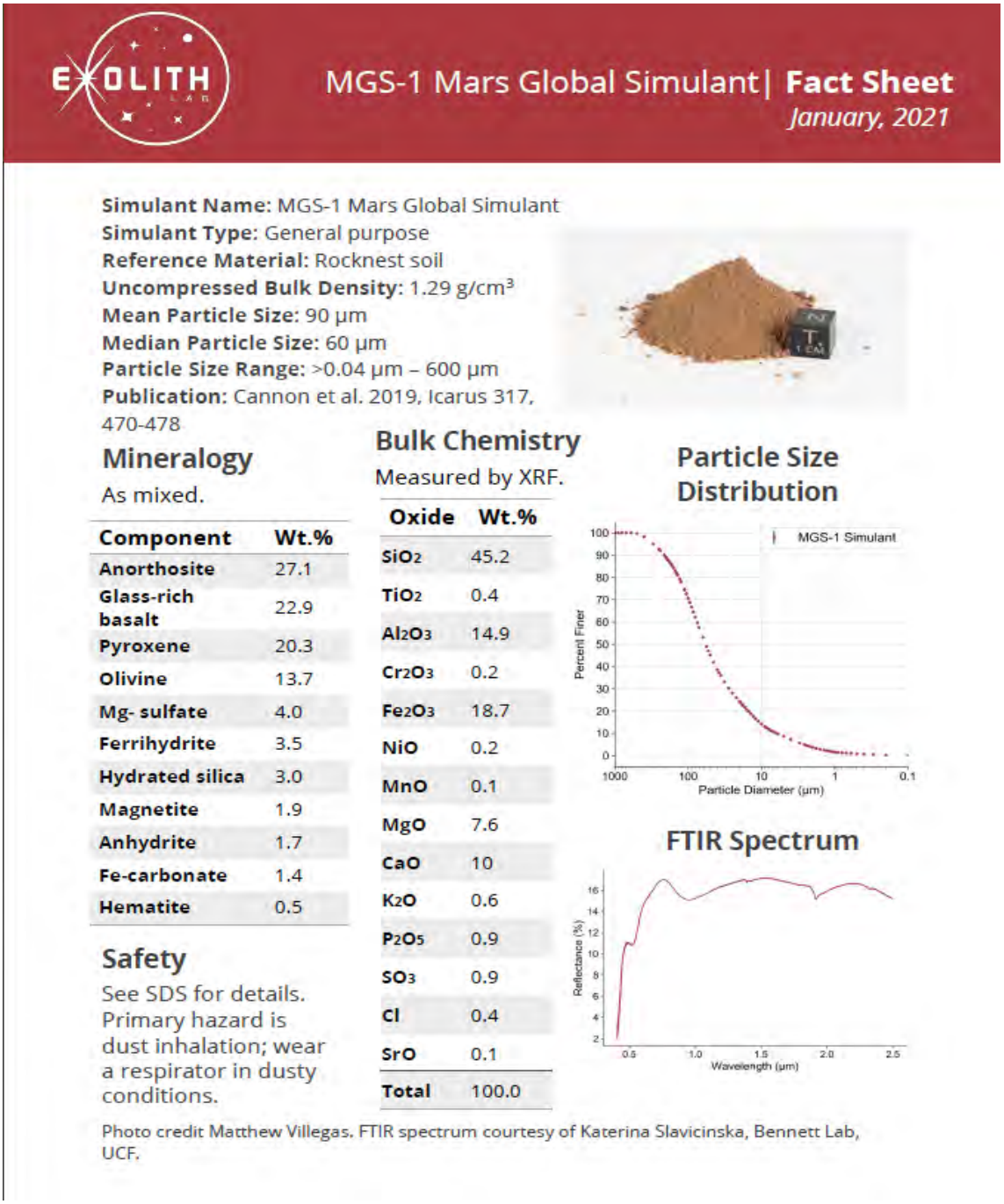

Figure 29: *Gale simulant*

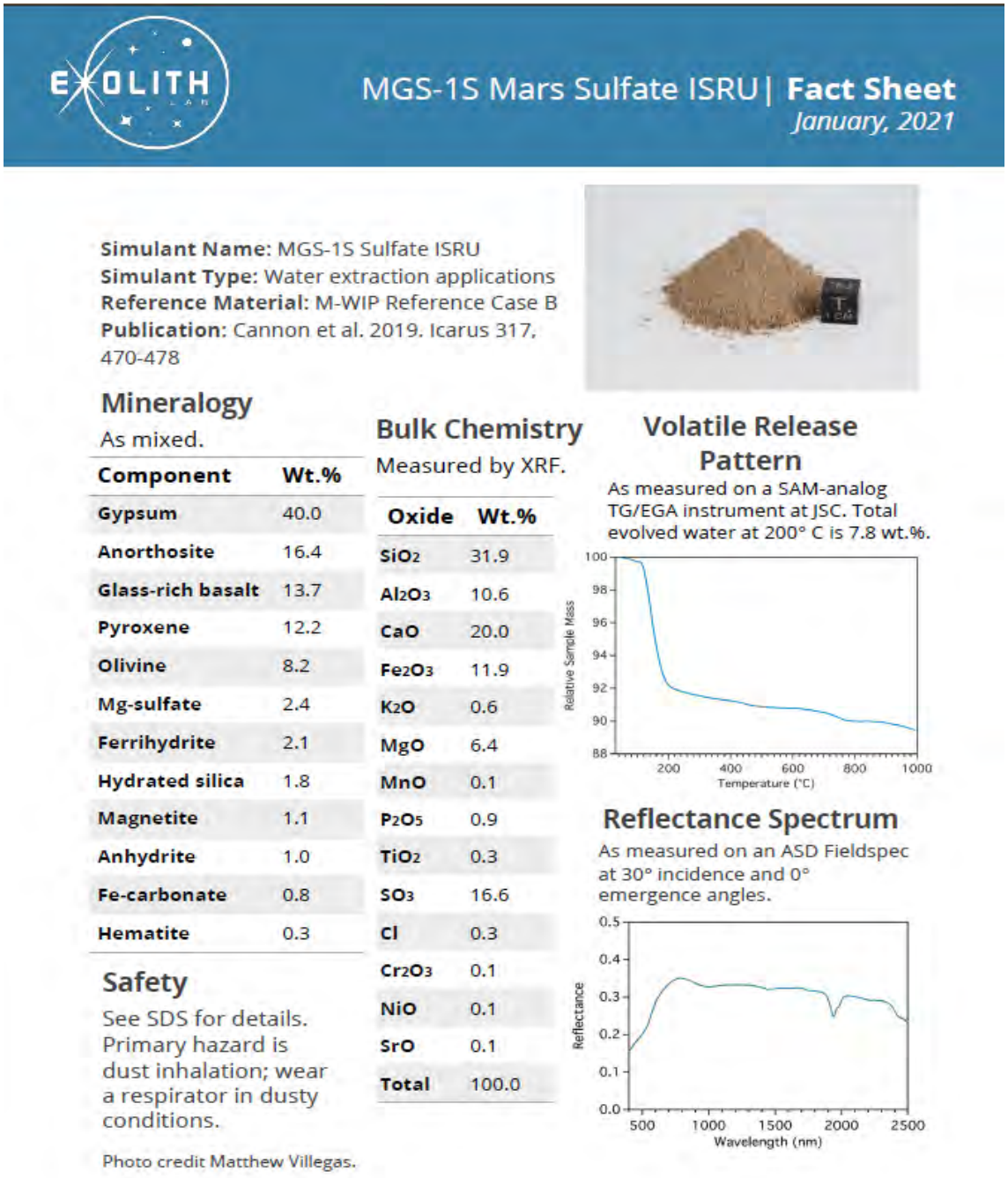

Figure 30: *Gale simulant enriched with sulfate*

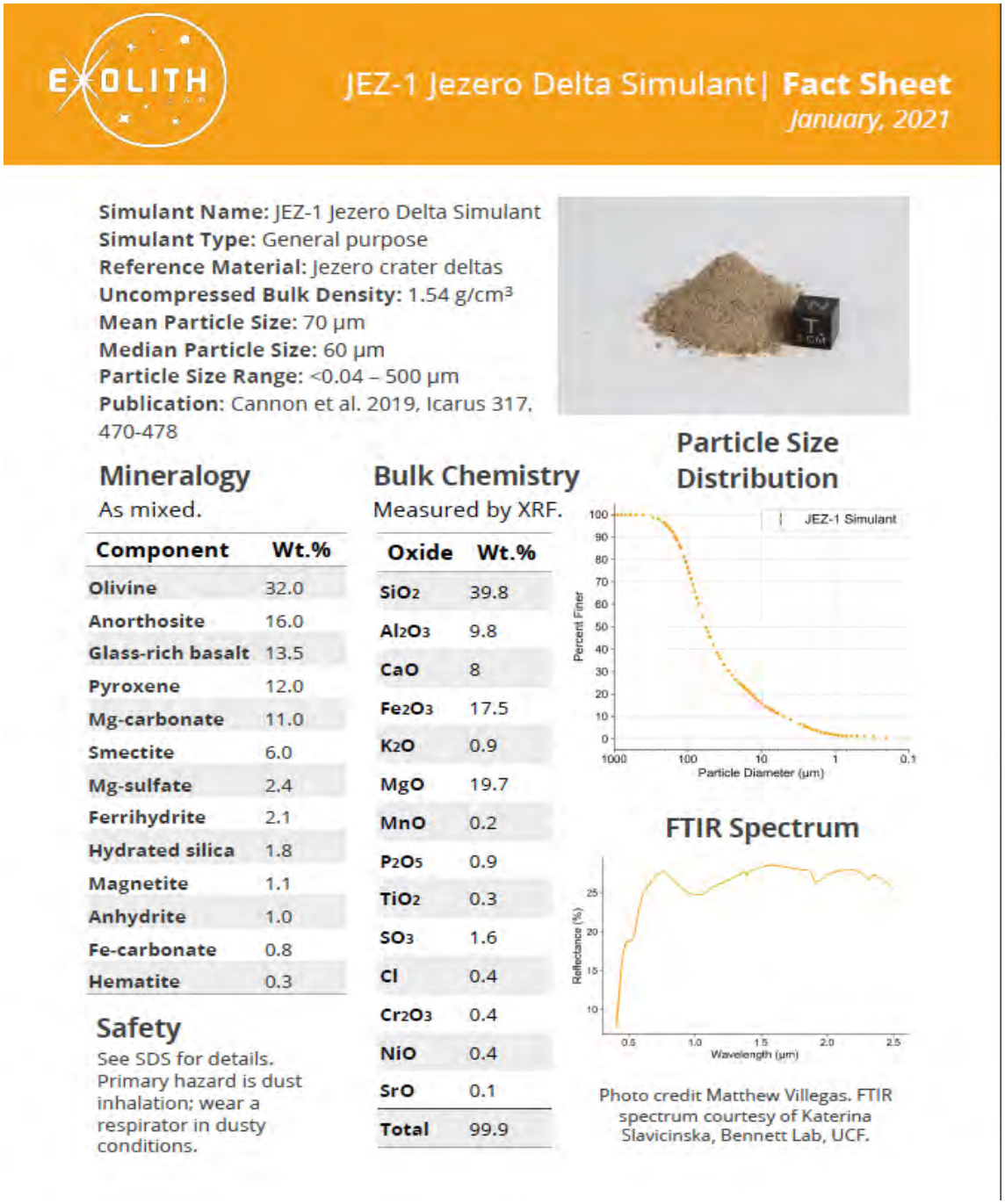

Figure 31: *Jezero simulant*

A.3 Appendix - Media recipe

- "Phosphate buffered saline (PBS) [1L]" :1.75g Cl^-, 38.4g SO_4, 3.1g NO_3, 14.8g ClO_4, 960 mg Mg, 2.4g Ca, 8.8g Na

- "Tryptic Soy Broth (TSB) [1L]": 30g Tryptic Soy Broth,3g Yeast extracts (for solid TSB plates, 15gr of agar added)

A.4 Appendix - The Vacuum pump

https://www.manualslib.com/manual/1507581/Leybold-Trivac-B-Series.html?page=9#manual

A.5 Appendix - Dosing Valve

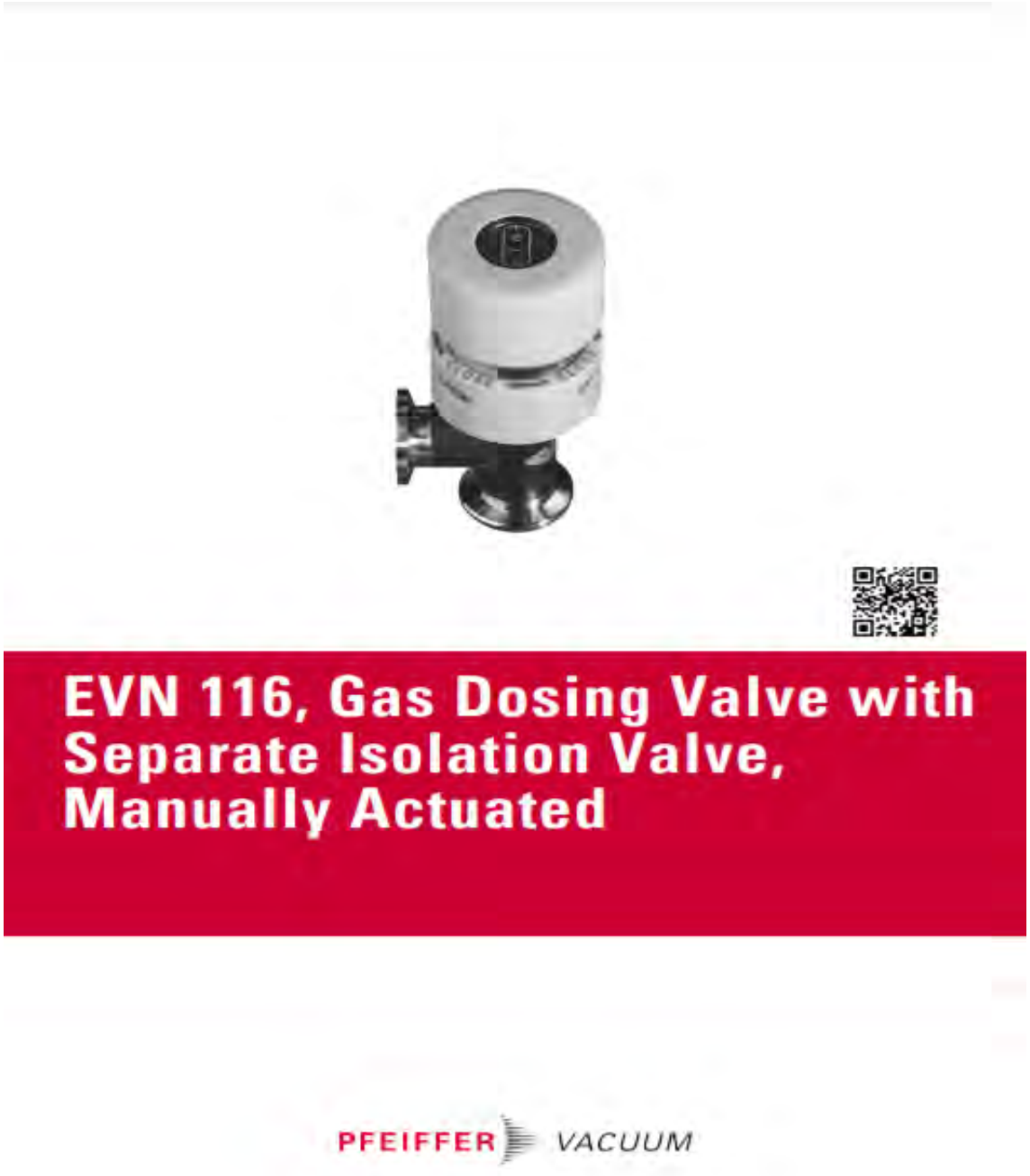

Figure 32: Dosing valve 1/2

Technical Data	EVN 116, Gas Dosing Valve with Separate Isolation Valve, Manually Actuated
Actuator	Manual
Bakeout temperature: flanges	150 °C | 302 °F | 423 K
Dead volume	0.032 cm^3
Differential pressure	2500 hPa
Dosing sleeve	Fluorplastomer
Housing	Stainless steel
Housing/needle/filter	Stainless steel
Max. controllable gas flow	$1 \cdot 10^{3}$ hPa·l/s
Min. controllable gas flow	$5 \cdot 10^{-6}$ hPa·l/s
Nominal diameter	DN 16 ISO-KF
Scope of delivery	With separate shut-off valve
Seal	FKM
Tightness	$1 \cdot 10^{-10}$ Pa m^3/s | $7.5 \cdot 10^{-10}$ Torr l/s | $1 \cdot 10^{-9}$ mbar l/s
Type	Gas dosing valve
Weight	0.4 kg | 0.88 lb

Order number	
EVN 116	PF I32 031

Figure 33: Dosing valve 2/2

A.6 Appendix - Pressure transducer

Figure 34: Pressure transducer 1/3

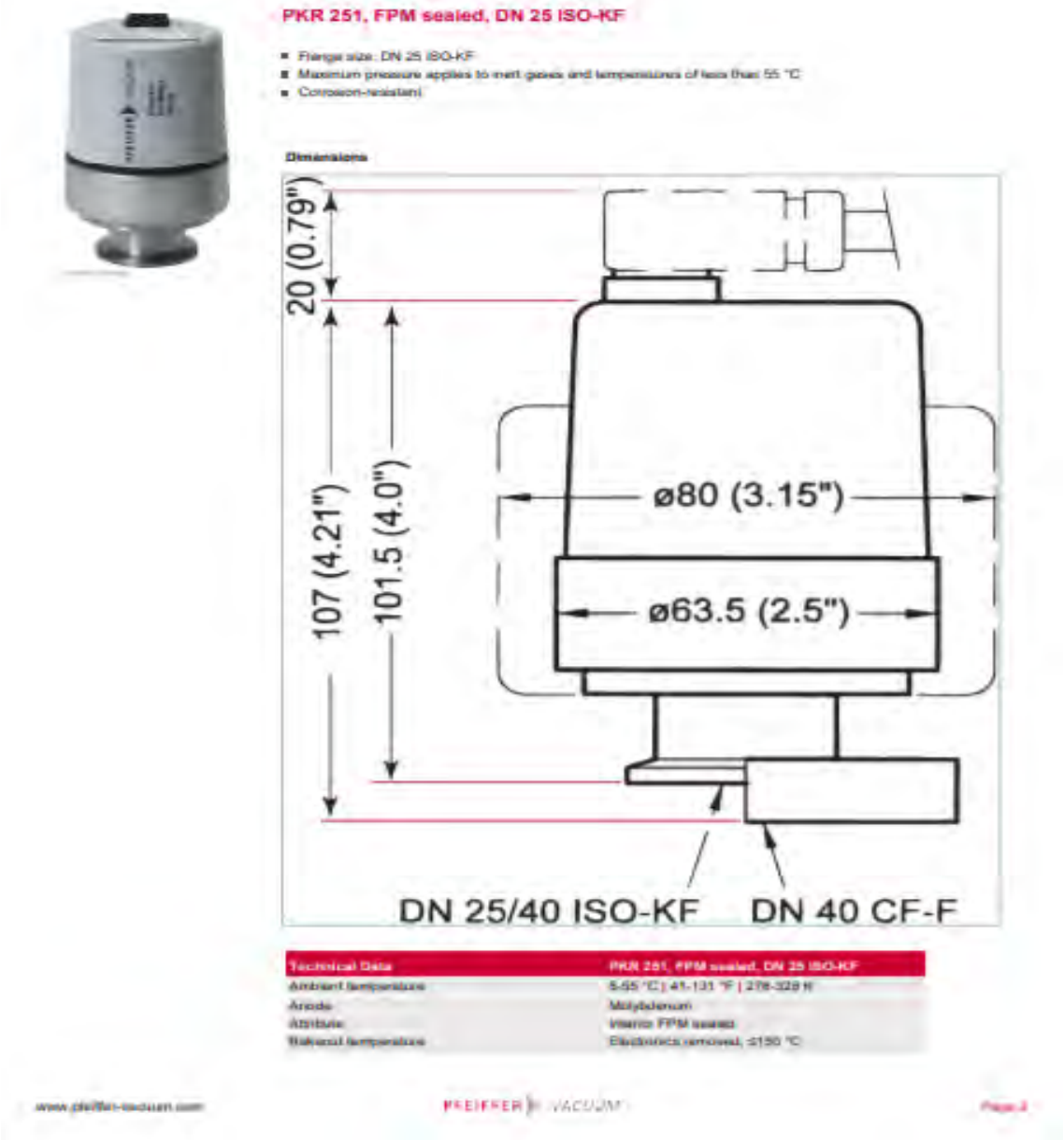

Figure 35: Pressure transducer 2/3

Technical Data	PKR 251, FPM sealed, DN 25 ISO-KF
Feedthrough	Al_2O_3, Glass
Filament	Tungsten
Flange, material	Stainless steel
Measurement cable length	300 m
Measuring range	$5 \cdot 10^{-9}$ – $1 \cdot 10^{3}$ hPa
Nominal diameter	DN 25 ISO-KF
Output signal: Measuring range	1.8 – 8.6 V
Output signal: Minimum load	10 kΩ
Precision: $1 \cdot 10^{-4}$ – $1 \cdot 100$ hPa	± 30 %
Pressure max.	10,000 hPa | 7,500 Torr | 10,000 mbar
Repeatability: $1 \cdot 10^{-4}$ – 100 hPa	± 5 %
Seal	FKM
Supply: consumption max.	2 W
Supply: Voltage V DC	15 – 30 V DC
Temperature: Storage	-40-65 °C | -40-149 °F | 233-338 K
Volume	20 cm³
Weight	700 g

Order number	
PKR 251	**PT R26 000**

Accessories	
Adapters (electrical)|Adapter Measurement	
Mating connector, 6 pole	**B4107283MA**
Cables|Measurement cables	
Measurement cable, 3 m	**PT 448 250 -T**
Protection|Magnetic shield	
Magnetic shield for IKR and PKR 2xx	**PT 443 155 -X**

Figure 36: Pressure transducer 3/3

A.7 Appendix - CO_2mission from isolated bacteria

Sample	Bacteria	Day	Replicate	conditions	Exposure time (hours)	CO2 (ppm)
1	201	1	a	24°C,regular atmosphere	40	372,24
2	201	1	b	24°C,regular atmosphere	40	398,69
3	201	1	c	24°C,regular atmosphere	40	407,05
4	202	1	a	24°C,regular atmosphere	40	633,28
5	202	1	b	24°C,regular atmosphere	40	587,03
6	202	1	c	24°C,regular atmosphere	40	601,38
7	201	1	a	N2, 15% RH	40	293,51
8	201	1	b	N2, 15% RH	40	292,57
9	201	1	c	N2, 15% RH	40	285,95
10	202	1	a	N2, 15% RH	40	515,94
11	202	1	b	N2, 15% RH	40	544,18
12	202	1	c	N2, 15% RH	40	497,89
13	201	1	a	4°C,N2, 15% RH	40	162,16
14	201	1	b	4°C,N2, 15% RH	40	116,87
15	201	1	c	4°C,N2, 15% RH	40	97,95
16	202	1	a	4°C,N2, 15% RH	40	138,42
17	202	1	b	4°C,N2, 15% RH	40	71,61
18	202	1	c	4°C,N2, 15% RH	40	119,10
19	control	1	a	24°C,regular atmosphere	40	404,55
20	control	1	b	24°C,regular atmosphere	40	324,33
21	control	1	c	24°C,regular atmosphere	40	334,65
22	control	1	a	N2, 15% RH	40	191,39
23	control	1	b	N2, 15% RH	40	184,64
24	control	1	c	N2, 15% RH	40	206,35
25	control	1	a	4°C,N2, 15% RH	40	190,45
26	control	1	b	4°C,N2, 15% RH	40	166,33
27	control	1	c	4°C,N2, 15% RH	40	185,82
28	201	2	a	24°C,regular atmosphere	64	401,72
29	201	2	b	24°C,regular atmosphere	64	646,78
30	201	2	c	24°C,regular atmosphere	64	700,78
31	202	2	a	24°C,regular atmosphere	64	735,33
32	202	2	b	24°C,regular atmosphere	64	756,81
33	202	2	c	24°C,regular atmosphere	64	362,52
34	201	2	a	N2, 15% RH	64	411,86
35	201	2	b	N2, 15% RH	64	495,49
36	201	2	c	N2, 15% RH	64	395,55

Table 12 continued from previous page

37	202	2	a	N2, 15% RH	64	642,53
38	202	2	b	N2, 15% RH	64	595,87
39	202	2	c	N2, 15% RH	64	627,92
40	201	2	a	4°C,N2, 15% RH	64	94,85
41	201	2	b	4°C,N2, 15% RH	64	120,36
42	201	2	c	4°C,N2, 15% RH	64	110,16
43	202	2	a	4°C,N2, 15% RH	64	107,24
44	202	2	b	4°C,N2, 15% RH	64	110,96
45	202	2	c	4°C,N2, 15% RH	64	89,73
46	control	2	a	24°C,regular atmosphere	64	417,56
47	control	2	b	24°C,regular atmosphere	64	347,19
48	control	2	c	24°C,regular atmosphere	64	344,90
49	control	2	a	N2, 15% RH	64	213,07
50	control	2	b	N2, 15% RH	64	215,39
51	control	2	c	N2, 15% RH	64	211,82
52	control	2	a	4°C,N2, 15% RH	64	118,57
53	control	2	b	4°C,N2, 15% RH	64	113,24
54	control	2	c	4°C,N2, 15% RH	64	130,76
55	201	3	a	24°C,regular atmosphere	88	590,05
56	201	3	b	24°C,regular atmosphere	88	776,56
57	201	3	c	24°C,regular atmosphere	88	797,08
58	202	3	a	24°C,regular atmosphere	88	861,75
59	202	3	b	24°C,regular atmosphere	88	793,09
60	202	3	c	24°C,regular atmosphere	88	834,69
61	201	3	a	N2, 15% RH	88	6,42
62	201	3	b	N2, 15% RH	88	436,03
63	201	3	c	N2, 15% RH	88	459,39
64	202	3	a	N2, 15% RH	88	587,30
65	202	3	b	N2, 15% RH	88	692,65
66	202	3	c	N2, 15% RH	88	680,07
67	201	3	a	4°C,N2, 15% RH	88	87,67
68	201	3	b	4°C,N2, 15% RH	88	124,10
69	201	3	c	4°C,N2, 15% RH	88	128,99
70	202	3	a	4°C,N2, 15% RH	88	84,25
71	202	3	b	4°C,N2, 15% RH	88	185,50
72	202	3	c	4°C,N2, 15% RH	88	179,54
73	control	3	a	24°C,regular atmosphere	88	431,18
74	control	3	b	24°C,regular atmosphere	88	362,27
75	control	3	c	24°C,regular atmosphere	88	340,29
76	control	3	a	N2, 15% RH	88	228,43
77	control	3	b	N2, 15% RH	88	231,55
78	control	3	c	N2, 15% RH	88	246,87
79	control	3	a	4°C,N2, 15% RH	88	122,23
80	control	3	b	4°C,N2, 15% RH	88	159,18
81	control	3	c	4°C,N2, 15% RH	88	148,92

Table 12 continued from previous page

82	201	4	a	24°C,regular atmosphere	113	863,98
83	201	4	b	24°C,regular atmosphere	113	579,00
84	201	4	c	24°C,regular atmosphere	113	818,49
85	202	4	a	24°C,regular atmosphere	113	889,04
86	202	4	b	24°C,regular atmosphere	113	815,79
87	202	4	c	24°C,regular atmosphere	113	897,57
88	201	4	a	N2, 15% RH	113	479,61
89	201	4	b	N2, 15% RH	113	519,02
90	201	4	c	N2, 15% RH	113	507,67
91	202	4	a	N2, 15% RH	113	757,42
92	202	4	b	N2, 15% RH	113	688,35
93	202	4	c	N2, 15% RH	113	799,28
94	201	4	a	4°C,N2, 15% RH	113	132,20
95	201	4	b	4°C,N2, 15% RH	113	234,79
96	201	4	c	4°C,N2, 15% RH	113	100,30
97	202	4	a	4°C,N2, 15% RH	113	177,63
98	202	4	b	4°C,N2, 15% RH	113	176,39
99	202	4	c	4°C,N2, 15% RH	113	151,35
100	control	4	a	24°C,regular atmosphere	113	406,95
101	control	4	b	24°C,regular atmosphere	113	357,08
102	control	4	c	24°C,regular atmosphere	113	350,59
103	control	4	a	N2, 15% RH	113	259,88
104	control	4	b	N2, 15% RH	113	240,66
105	control	4	c	N2, 15% RH	113	244,92
106	control	4	a	4°C,N2, 15% RH	113	128,94
107	control	4	b	4°C,N2, 15% RH	113	137,78
108	control	4	c	4°C,N2, 15% RH	113	128,33
109	201	5	a	24°C,regular atmosphere	134	992,99
110	201	5	b	24°C,regular atmosphere	134	902,79
111	201	5	c	24°C,regular atmosphere	134	584,66
112	202	5	a	24°C,regular atmosphere	134	1.037,41
113	202	5	b	24°C,regular atmosphere	134	1.021,32
114	202	5	c	24°C,regular atmosphere	134	957,11
115	201	5	a	N2, 15% RH	134	535,98
116	201	5	b	N2, 15% RH	134	552,99
117	201	5	c	N2, 15% RH	134	550,99
118	202	5	a	N2, 15% RH	134	859,02
119	202	5	b	N2, 15% RH	134	855,12
120	202	5	c	N2, 15% RH	134	736,13
121	201	5	a	4°C,N2, 15% RH	134	191,58
122	201	5	b	4°C,N2, 15% RH	134	102,57
123	201	5	c	4°C,N2, 15% RH	134	188,01
124	202	5	a	4°C,N2, 15% RH	134	87,82
125	202	5	b	4°C,N2, 15% RH	134	91,27
126	202	5	c	4°C,N2, 15% RH	134	177,42

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127	control	5	a	24°C,regular atmosphere	134	410,09
128	control	5	b	24°C,regular atmosphere	134	369,34
129	control	5	c	24°C,regular atmosphere	134	388,96
130	control	5	a	N2, 15% RH	134	251,46
131	control	5	b	N2, 15% RH	134	270,60
132	control	5	c	N2, 15% RH	134	245,41
133	control	5	a	4°C,N2, 15% RH	134	129,64
134	control	5	b	4°C,N2, 15% RH	134	129,24
135	control	5	c	4°C,N2, 15% RH	134	129,34