The Effects of Photosynthesis on Martian Atmospheric Composition in a Closed System

Abstract

Martian habitability is a problem for NASA and other space agencies that will need to be solved before we can send people to Mars. This project consists of attempting to transform current Martian atmospheric composition into a composition in which humans could potentially inhabit within an enclosed environment. This idea has the potential to solve the problem of the inhospitable Martian atmosphere. Two airtight boxes were constructed using plexiglass and a strong adhesive, and Lepidium sativum (Garden Cress) was planted in regular potting soil in one box and planted in Martian soil simulant in the other. The container was flushed with 100% carbon dioxide and sealed, and the plants were allowed to grow for approximately 20 days. The resulting atmospheric composition was monitored over all of these days using carbon dioxide and oxygen gas sensors and recorded to a LabQuest device. The results of the experiment show that after 20 days, the oxygen and carbon dioxide levels leveled off to very near that of Earth. The experiment may have been affected, however, by the fact that the chambers could have been leaking, and other life might have been present in each box. Because of this, it cannot be concretely determined that the results were directly the result of the plant growth. Despite this, the experiment did yield the change in the gas concentrations predicted over the 20 days. From these results of this experimentation, it can be determined that if this is done on a larger scale, the same effect could be achieved on Mars. Further work of this project includes recreating the experiment with more control over the variables, which includes autoclaving the martian and

earth soils so that the growth of the plants can be shown as the determining factor in gas composition change, as well as the use of other plants and more accurate gas sensors.

Introduction

As humanity readies itself for manned missions to Mars, the creation of livable spaces on the red planet and research into how to do it the best is and will continue to be extremely important in the coming years. Therefore, it is necessary to research how this could potentially be done in as many ways as possible. An idea for one way this could work is to somehow put some sort of glass dome on the surface and simply plant crops and see if they could produce an environment with a an amount of carbon dioxide and oxygen that could sustain human life. To my knowledge, this way of a creation of breathable air on Mars has not been researched thus far, so therefore it is necessary to see how viable such a plan could be.

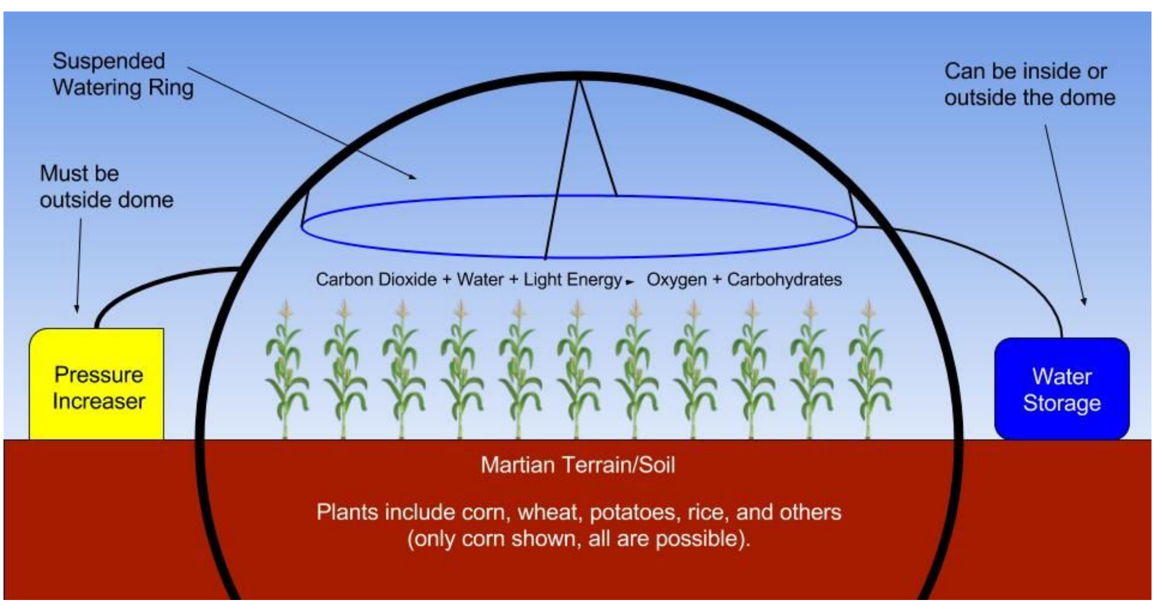

Figure 1

Figure 1 is a mock-up of how the afore mentioned Martian habitat could look. This system would work by first setting up the dome. The creation of this dome and the process for setting it up would be determined by further research and adaptation of current technologies. The yellow box in the picture represents a machine that would increase the pressure inside the dome, allowing for the plants to grow. The blue box represents a chamber that would store water. This could be located inside or outside the chamber, but for practical purposes, it would more likely be located outside so it could be refilled by some sort of Martian vehicle. The plants would either be watered by the "ring" system as shown in the diagram, in which the water would be pumped above and "rained" down upon the plants. Alternatively, the plants could be watered by a more common (on Earth) irrigation system on the ground.

This idea could be very cost effective if put into effect on Mars, as man-produced oxygen would not be needed after a certain period of time. This project researches a much smaller version of this. There has been enough research done around this idea to make it valid, and not just some sort of sci-fi dream. Much research has been done on the composition of Martian soil. This is shown in an article by M.H. Hecht and several others, as well as another by B. Sutter and more, which gave the composition of the soil from the tests done by the Phoenix Lander on Mars. The soil seemed to have more perchlorate ions in it than originally thought, and it was of concern to scientists as to if the soil could support healthy plants and produce edible food (M.H. Hecht, S.P. Kounaves, et. al., 2009; B. Sutter, W. Boynton, et. al., 2012). However, this problem was found to be easily fixed, as the perchlorates can be simply washed out of the regolith with water due to water polar properties. In fact, the perchlorate could even be heated enough to release oxygen. There has been extensive research done as to how well plants could grow in

Martian soil (in part because of this) shown by a peer reviewed research report. This article researched how well different plants could grow in Martian soil replicant, and showed that all plants they experimented with could be grown in the soil (G.W. Wamelink, Joap Y. Frissel, et.al., 2014). The Martian soil simulant that was used for this experiment was developed by NASA/JPL scientists, and its characteristics are shown in a article also showing how to even further improve the similarity of the soil towards real Martian regolith (G. Peters, W. Abbey, et. al., 2008).

Another concern for planting on Mars is the possible effects that the lower gravity might have. An article by J. Kiss of the University of Mississippi shows how the gravity of different planets can also affect plants, and it concludes that lower gravity would have a minimal effect on plant growth (J. Kiss, 2014). This helps to eliminate one variable in how well plants could grow on Mars, and thankfully it benefits this idea and removes another variable from my experiment.

An interesting question in this research is what plants are the best to use in this experiment. An article gives insight into what plants will grow the best on mars now by showing what grows around the NASA mars research area Utah, helping me to choose it for what plant to use in my experiment (P. Sokoloff, 2016). These were all good; however, they were all too big for the scale of which I intend to do the experiment, though they could potentially be used on the actual dome habitat on Mars described earlier. The earlier mentioned article determining if plants could grow in martian soil (the simulant at least) used quite a few different plants, and from that I will be using garden cress, a fast growing edible herb (G.W. Wamelink, Joap Y. Frissel, et.al., 2016).

With many variables removed and specifics of the experiment found, we now turn to the justification for this project's hypothesis. The composition of the martian atmosphere, according to the Mars Curiosity Rover, is about 96 percent carbon dioxide (P.R. Mahaffy, C.R. Webster, et.al., 2014). A study found that with the global increase of carbon dioxide, the rate of photosynthesis of corn plants in the U.S. corn belt grew (A. D. B. Leakey, C. J. Bernacchi, et. al., 2004). This indicates that the high levels of carbon dioxide like the one present on Mars could actually lead to a high rate of oxygen production, therefore making this idea even more valid. Another study found that increased amounts of carbon dioxide in an enclosed space led to increased strawberry quality and yield at low temperature, while high temperatures led to decreased quality and yield (P. Sun, N. Mantri, et. al., 2012). This works to our benefit on Mars, as temperatures are generally low. This condition cannot be used in this experiment, however, as it is sharing a growth chamber with plants of a different project needing a higher temperature than what would be ideal for this experiment. One would assume, however, that the actual "dome" sent to Mars would have some sort of heat controlling system.

One limitation of this project is the fact that tools were not available to show how one might recreate the growth of plants in the pressure of the Martian atmosphere. A research study showed that reducing the amount of pressure on a plant in growth inhibits its ability to grow and makes the plant become stiff and rigid (Daunicht, H., & Brinkjans, H., 1992). This actually helps my project along with the heat control assumption in that I can assume that there is some sort of way to make the martian atmospheric pressure equal to that of earth's mechanically in the "dome" to keep the plants from dying. Also, it is not advisable for humans to breath just oxygen or just carbon dioxide, so more research into how much nitrogen would need to be added to the atmosphere in this bubble to make the air safe for humans would need to be conducted, as well as a possible Martian source for nitrogen. One possible solution would be if the oxygen could be removed from the bubble and then put into a pressure about a third of earth's, which is breathable as shown on the Apollo missions (E. Michel, J. Waligora, D. Horrigan, & W. Shumate, 1970). This would mean that the Mars explorers could no longer grow plants in the "dome" however, which not preferable.

Analyzation of the martian soil after plants have grown in it could be useful in determining later the long term effects of the use of this type of system on Mars. Methods on how this could be done are described in a scientific article by Foing and Kidd. (G.B. Foing, R. Kidd, et. al., 2011). It could also be done using high performance liquid chromatography. This would be important to the incorporation of this system on Mars. Overall, using all of the outside information found above, this project aimed to determine if a system such as this could be effective in its purpose on Mars through its replication on a smaller scale on Earth.

Research Question

Are *Lepidium sativum* able to photosynthesize and produce oxygen in a system containing a simulated Martian atmosphere and soil simulant?

Purpose

The purpose of the project is to analyze how much an enclosed artificial Martian atmosphere changes over 20 days with the introduction of *Lepidium sativum* to calculate how long an environment such as this on actual Mars would take to support life, or if it even could. This could potentially lead to its implication on a larger scale on Mars in the future.

Hypothesis

The planting of *Lepidium sativum* (commonly known as garden cress) in an environment with martian soil simulant and atmospheric composition will lead to, through the photosynthesis of the plants, a decrease in the amount of carbon dioxide and an increase in the amount of oxygen in the atmosphere that, after long enough, may be breathable by humans.

Null Hypothesis

The planting of Lepidium sativum in an environment with martian soil simulant and atmospheric composition will not lead to a decrease in the amount of carbon dioxide and an increase in the amount of oxygen in the atmosphere, yielding an atmosphere that will not be breathable by humans.

Materials

- Small plexiglass sheets cut and drilled from a single larger sheet (3 ft x 4 ft)
  - 2 - 8 x 10 inches
  - 2 - 10 x 12 inches
  - 2 - 8 x 12 inches
- Acrylic Glue (IPS 3 quart Weld-On 3 quart)

- Hypo 25 Applicator for Weld-On Hypo 25 industrial solvent adhesive (a.k.a. IPS 3 quart Weld-On 3 quart size)
- Large Funnel for Water
- LabQuest Original - Vernier
- Carbon Dioxide Sensor Probe - Vernier
- Oxygen Sensor Probe - Vernier
- Martian Soil Simulant - “Mojave Mars Simulant” from The Martian Garden
- Seeds of *Lepidium sativum* - 40 used
- Small Sprinkler Tubing (Black)
- PVC Valves - 6
- Clear ⅝ inch Tubing - 2
- ⅜ inch attachment to Valve for gas line - 1
- Carbon Dioxide Canisters (12 gram) - 16 used
- Square Pot Carrying Trays - 15 slots, 20.25x12.25x2 inches (cut down to fit in chambers)
- Square Injection Molded Pots - 4”
- Laptop Computer
- Logger Lite Software
- Teflon Sealant Tape
- Liquid Sealant - General Electric
- Electrical Tape - Black
- Soapy Water in Hypo - 65 Applicator
- Paper Towels

- Scissors
- Ryobi Drill
- Drill Bits - ⅞ inch and 1 ⅛ inch
- Growth Chamber - Thermo Scientific Precision

Methods

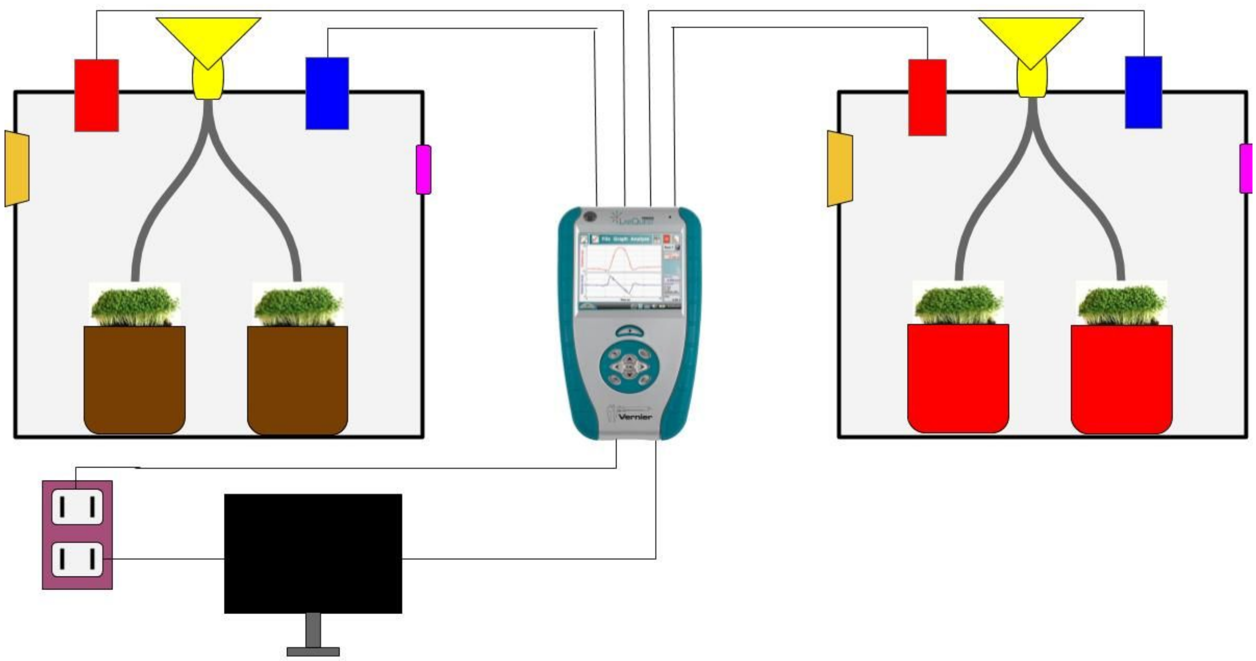

Figure 2

Diagram of the setup for the experiment - The yellow box and triangle on top of the box represent the valve and funnel set up for watering these plants, and the grey lines extending out of them towards the plants represent the tubing that will guide the water to each plant. Though it does not look like it, each plant in each box actually represents two (as one would be behind the one in the front), and the same goes therefore for the grey water-carrying tubing. The brown pots *contain regular potting soil, while the red pots contain the martian soil simulant. On the side of the boxes there are small purple rectangles which represent a valve which will let out the air while the carbon dioxide is being flushed into the chamber from another valve, represented by the yellow trapezoid. The large purple square that looks like a power outlet is just that, and everything connected to it with a line needs power. The computer screen represents a computer which will record the data of the carbon dioxide and oxygen concentrations. The red and blue boxes are the oxygen and carbon dioxide probes, respectively. The monitor screen represents the laptop that took the data from the LabQuest. The picture of the LabQuest represents itself.*

Part 1 - Construction of Two Airtight Growth Chambers

The two chambers were constructed by cutting the plexiglass to the dimensions needed (shown in materials). The following illustrates the construction of a single chamber, but two were made the exact same way. A hole in each side piece (4 8x10) was drilled and 3 holes were drilled in each lid piece (2 10x12). This was done by using drill bits of the sizes necessary to be able to insert the carbon dioxide and oxygen sensors into the lid. These were both ⅞ inch, so two holes of the three on the lid were drilled that size. The holes on the side and the remaining on on the lid were sized to allow the PVC valves to be inserted.These were each ⅞ inch. The sides of the chambers were then glued together in the necessary pattern to form the “shoebox” shape needed. The lid was not glued, as it needed to be removed, so it was later sealed with electrical tape. All seams in the chamber were then sealed with sealant. The oxygen and carbon dioxide probes were secured in the chamber by wrapping them with Teflon tape, and then placing them in the holes on the lid. These were then also sealed with the clear sealant. The watering system was installed by inserting the small PVC pipe into the hole and screwing on the sprinkler hoses on one side and the and a short, clear length of ⅝ inch tubing on the other side. By keeping the large tubing always filled partially with water, the gas concentration inside the chambers was maintained. The lid was then secured and the chambers were then tested for leaks.

Part 2 - Testing for Leaks

Leaks in both of the boxes were checked by slightly pressurizing the chamber using a bicycle pump attached to the nozzle attached to one of the side valves. Soapy water was put on all of the seams of the chamber to see if there were any sort of leak. There were leaks on the seals of the gas monitors on both boxes. Because of this, both chambers were further sealed using the clear sealant, and tested again. The second time, there were no leaks, so the experimentation was started.

Part 3 - Plant Growth and Atmospheric Change

After the chambers had been shown to be airtight, approximately 400 ml of martian or potting soil simulant was put into each plastic pot (400g and 150g respectively due to density difference), totaling 4 pots of each soil type. In each pot, 25 ml of water was then poured. Previously, clear plastic wrap had been taped to the bottom of each pot to make sure the soil did not escape. Then, 9 seeds of garden cress were planted in a square pattern into each pot. This number was chosen because it allowed a generally large number of seeds to be planted in each chamber while still being small enough to chart the growth of each seeds and differentiate the sprouts. The pots were then placed into a tray designed for those specific pots purchased from the same distributor. A tray of only one type of soil was then placed in each chamber, Martian and potting. The lid was sealed on and the carbon dioxide was flushed into the container. This process used 16 small canisters in total. After this, the carbon dioxide gas was not used again. These plants were each watered with 40 ml of water per chamber 3 times per week using the PVC watering system. The growth period for the plants was determined by previous research to be about 18 days to harvest, so experimentation was carried out for 20 days. During these 20 days. with the probes took constant measurements, recording a data point every 4 hours. After the experiment, the materials were disposed of in the proper waste containers.

Part 4 - Data Collection and Analysis

The data was then obtained from the LabQuest device. This was done through connecting the LabQuest device to the laptop using a mini USB cable. The data was able to be analyzed using the LabQuest online software, Logger Lite 1.9.1. This was downloaded from the Vernier website. The software created a data table of all the data points, as well as a graph showing the results of the experimentation. These results are shown in the Results/Data section of the report.

Results/Data

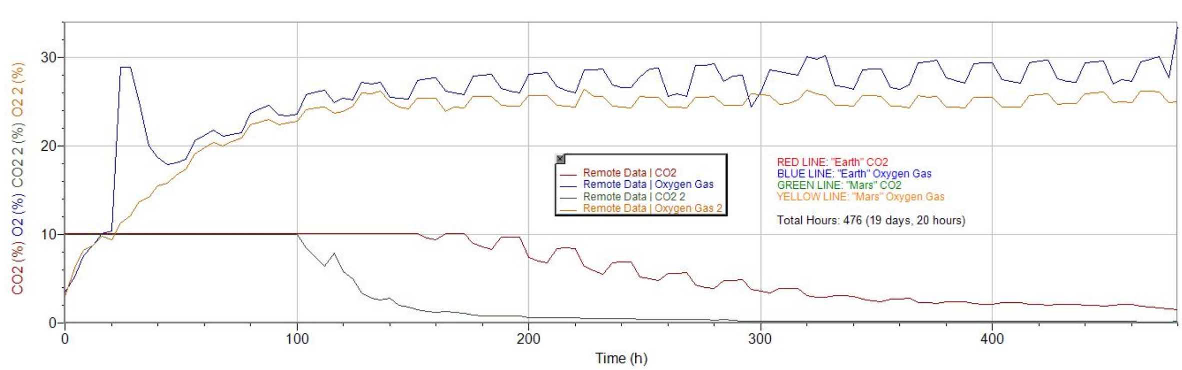

Figure 3

In figure 1, the gas concentrations are shown over the time of experimentation. Overall, the gas concentrations of carbon dioxide fell and the concentrations of oxygen gas rose due to photosynthesis. The starting point of both carbon dioxide levels are shown as 10% for both the Mars and Earth chambers, due to the fact that the carbon dioxide sensor probes are not able to detect over 10%. Small fluctuations occurring every 24 hours, approximately. are due to the day night cycle present in the growth chamber used in experimentation. During the "day," photosynthesis led to a small, approximately 3% increase in oxygen levels in the "earth" chamber, while the "mars" chamber had an approximately 2% increase during the "day." As for the carbon dioxide levels, the "earth" chamber did have matching fluctuations from all data after levels had decreased below 10%. This effect did to occur in the "mars" chamber's readings of carbon dioxide, though it is not as evident as the others. As also seen in figure 3, from 20 to 45 hours, the "Earth" oxygen gas was much higher than it should have been. The conclusion can be made then that something went wrong with the oxygen probe, as the probe seemingly corrected itself after a certain amount of time and continued matching the general trend of the oxygen gas. The same oxygen graph rises sharply near the end of the graph (final 6 hours). This abnormal occurrence is due to the chamber being breached before experimentation was finished. The breach, however, did not have any affect on the overall conclusion of the experiment.

Along with all of this data from the oxygen and carbon dioxide probes, a chart of which seeds of the 36 in each chamber sprouted. At the halfway point of the experiment, 10 seeds had sprouted in the "Earth" chamber, and 23 seeds had sprouted in the "Mars" chamber, leading to a quicker decline in the amount of carbon dioxide in the "Mars" chamber that in that of the "Earth" chamber. By the end of the experiment, only the same 10 had sprouted in the “Earth” chamber, while 25 had sprouted in the “Mars” chamber. The effects of this difference in sprouting between the two chambers can be seen in the chart with the difference in the time it took for the carbon dioxide gas concentration to decrease. These results are overall what was expected to be found from this experiment as far as that gas, but it would have been better for the experiment if more garden cress had sprouted in the “Earth” chamber.

Discussion

The graph shows the oxygen levels rose significantly and the carbon dioxide levels declined significantly in each chamber. As shown, the carbon dioxide levels are 10% at maximum. This is due to the probes only being able to measure up to 10%. This is due to the need to stay within a budget, as the probes for both carbon dioxide and oxygen were available for free. A probe able to go to 100%, however, would have been better for consistency, however. This is important to research on the topic in the future. The oxygen levels in each chamber generally followed the same path, both starting at approximately 4% and ending at 30% for “Earth” and 25% for “Mars”. This exact data is included in the data log book, but as it is 121 data points, it has not been included in this paper. The carbon dioxide levels were much different from each other, in contrast with the two oxygen levels. The “Mars” level went down very quickly, while the “Earth” level took much longer, though there is an explanation for that: many more garden cress seeds sprouted in the “Mars” chamber. This is due to a small amount of mold that grew, preventing growth of the garden cress in the “Earth” as well as producing some carbon dioxide. This likely contributed to the longer time for the carbon dioxide level to decline in the

"Earth" chamber. As can also be seen in figure 3, the oxygen levels immediately increased once the chamber was sealed. This data is suspicious, as the first sprouts did not appear until the fifth day. Further research into the ability of *Lepidium sativum*'s ability to photosynthesize before sprouting should be researched further before an official conclusion can be made specifically on the plant's effect on the gas concentrations. It is more likely that the chambers started to leak than some sort of photosynthesis before the plant actually sprouted. This is countered by the fact that the chambers were well sealed, however. Repetitions of this experiment will contribute to a better understanding of the outcomes of this experimentation, as well as more accurate sensors with a wider detection range. Despite all of this speculation of what could have happened inside both of these chambers, they were airtight, and the desired effect on the gas concentrations was achieved. This ultimately leads to the conclusion that this system works.

Conclusion

Ultimately, though there were some flaws in the experiment, the overall goal of transitioning the synthetic Martian atmosphere from largely carbon dioxide to oxygen was achieved. This shows that this method of changing Martian atmosphere into something humans could breath with plants could be a viable alternative to other, more costly methods of oxygen production on Mars. The technology could also be used in conjunction with these other methods of atmospheric conversion to create the best system of living on Mars.

Further Work

Further work for this project includes doing mainly the same experiment in order to confirm the conclusion of the experiment. Multiple tests of this would likely yield the most convincing results as to the viability of this system for actual use on Mars. Also, further research into Lepidium Sativum are also important in order to see if a change in carbon dioxide and oxygen levels is actually possible before the seeds of the plant sprout, or if it was in fact a leak or other organism creating the change. Variations on the project are also important in order to prepare for every circumstance that could possibly be on Mars. The necessary further research to make the system better tested would include multiple variations on the original research. One necessary variation would include the use of a carbon dioxide sensor able to sense the full range of concentration, as it would allow for more accurate readings. Also, the use of simple dirt instead of potting soil may be able to provide a better control for the experiment, as it would help to eliminate the possibility of mold or other microorganism contamination. Analyzation of Martian soil after plants have grown in it could also show how the plant growth affects Mars, which could be good or bad for potential martian habitat. This data would be important to the longevity of this system. Potentially, the soil would need to be replenished in nutrients, so further research into the long term effects of the system would overall be very beneficial to the goal of the system. Also, a bigger version of this experiment with crops, such as corn, rice, or wheat, would be beneficial to to actual use of the system on Mars. This would have to occur before the use of this design could be used on Mars for safety issues. Other necessary research for this to be a viable system is needing to design or adapt a system to increase the pressure within the dome, designing the exact watering system, as well as designing the dome itself.

Acknowledgements

- Dr. Nikki Malhotra, Thousand Oaks High School
- Mr. Jeffrey Lewis, Thousand Oaks High School
- Dr. Kate Ashton, Amgen

References

Daunicht, H., & Brinkjans, H. (1992). Gas exchange and growth of plants under reduced air pressure. Advances in Space Research, 12(5), 107-114. doi:10.1016/0273-1177(92)90016-Q

Foing, B. , Kidd, R. , Orzechowska, G. , Stoker, C. , Ehrenfreund, P. , et al. (2011). Analysis of mars analogue soil samples using solid-phase microextraction, organic solvent extraction and gas chromatography/mass spectrometry. *International Journal of Astrobiology*, *10*(3), .

Hecht, M. , Hecht, . , Kounaves, S. , Quinn, R. , West, S. , et al. (2009). Detection of perchlorate and the soluble chemistry of martian soil at the phoenix lander site. *Science*,*325*(5936), 64.

Kiss, J. , Kiss, J. , & Legue, V. (2014). Plant biology in reduced gravity on the moon and mars. *Plant Biology*, *16*(s1), 12.

Leakey, A. D. B., Bernacchi, C. J., Dohleman, F. G., Ort, D. R. and Long, S. P. (2004), Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO2] rich atmospheres? An analysis of diurnal courses of CO2 uptake under free-air concentration enrichment (FACE). *Global Change Biology*, 10: 951–962. doi:10.1111/j.1529-8817.2003.00767.x

Mahaffy, P. R., Webster, C. R., Atreya, S. K., Franz, H., Wong, M., Conrad, P. G., ... & Owen, T. (2013). Abundance and isotopic composition of gases in the martian atmosphere from the Curiosity rover. *Science*, *341*(6143), 263-266.

Michel, E., Waligora, J., Horrigan, D., & Shumate, W. (1970). *SP-368 Biomedical Results of Apollo - Chapter 5 - Environmental Factors*

Peters, G. H., Abbey, W., Bearman, G. H., Mungas, G. S., Smith, J. A., Anderson, R. C., . . . Beegle, L. W. (2008). Mojave Mars simulant—Characterization of a new geologic Mars analog. Icarus, 197(2), 470-479. doi:10.1016/j.icarus.2008.05.004

Sokoloff, P. , Freebury, C. , Hamilton, P. , & Saarela, J. (2016). The "martian" flora: New collections of vascular plants, lichens, fungi, algae, and cyanobacteria from the mars desert research station, utah. *Biodiversity Data Journal*, .

Sun P, Mantri N, Lou H, Hu Y, Sun D, Zhu Y, et al. (2012) Effects of Elevated CO2 and Temperature on Yield and Fruit Quality of Strawberry (Fragaria × ananassa Duch.) at Two Levels of Nitrogen Application. PLoS ONE 7(7): e41000. doi:10.1371/journal.pone.0041000

Sutter, B. , Boynton, W. , Ming, D. , Niles, P. , Morris, R. , et al. (2012). The detection of carbonate in the martian soil at the phoenix landing site: A laboratory investigation and comparison with the thermal and evolved gas analyzer (tega) data. *Icarus*, *218*(1), 290-296.

Wamelink, G. , Frissel, J. , Krijnen, W. , Verwoert, M. , & Goedhart, P. (2014). Can plants grow on mars and the moon: A growth experiment on mars and moon soil simulants. *PLoS One*, *9*(8), urn:issn:1932-urn:issn:6203.