

The Biosphere Observation eXperiment II (BOX II)

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ABSTRACT

This purpose of this project is to improve on an existing design for the closed ecological system in which plant life and possible insect life may be self-sustained. The main goals of this project are to demonstrate both an increase in oxygen level in the biosphere and a reduction in the carbon dioxide level. The biosphere is self-sustaining with one uniform environment. This paper discusses the construction of a table-top experimental designⁱ, the changes made to the original design and measuring devices used to quantify gas levels and other environmental variables, such as relative humidity, temperature and light intensity. The system is completely enclosed from its beginning to its conclusion. The main instruments contained in the sealed unit are oxygen and carbon dioxide sensors along with hobo data loggers. Oxygen is created using a mixture of two plant types: Fern (*Bostoniensis* and *Pteris Cretica*) and Croton (*codium*). These plants are grown in sterilized potting soil. This paper reports on our initial findings, the changes implemented and the underlying scientific principles of creating a biosphere. The BOX II is an attempt to improve the understanding of enclosed systems and to create a system that produces more consistent results over a longer term. By making slight changes to the design, any noticeable variations in results will be more meaningful.

I. INTRODUCTION

As the nearest habitable planet, Mars has held our attention for a long time. From a series of uncrewed exploration missions using rovers and satellites, we know that the surface of Mars has an average temperature of -60 C and an atmosphere composed of 95% CO_2 . This will not sustain human life as it is; therefore methods must be developed to allow humans to live for extended periods of time on the surface of Mars.

One such method is the biosphere, a self-contained, self-sustaining environment. There have been many versions of biospheres created, from the simple to the complex. Some of the more complex ones even involve humans living inside the biosphere for several years. This paper chronicles one attempt to create a table top biosphere using no life forms and only plants, and the results and conclusions that were reached. The previous paper contains an extensive list of references on aspects and history of biospheres. The reader should refer to the paper in reference (i) for a list of publications.

II. BACKGROUND

A. Previous Experiment

In an experiment conducted by Konrad et al.ⁱ in the previous year, a setup was devised that used a 20 gallon aquarium and various other materials. The original BOX used a single type of plant, the Arabidopsis Thaliana, which is a proven high oxygen producer and has a very fast life cycle. They started with seedlings of this type of plant, and recorded oxygen levels, relative humidity, temperature (internal and external), and light intensity. They faced various difficulties and eventually the plants died.ⁱ After the failure it was observed that mold began to grow along the glass wherever the dead plants touched and moisture gathered. Also the soil itself began to grow mold.

It was decided to duplicate this experiment, but with minor changes in an attempt to determine where the breakdown in the system might have occurred. To this end, it was decided that while the Arabidopsis Thaliana was a high oxygen producer, it was not tolerant of the high humidity that quickly built up inside the biosphere. Also, while the oxygen level was of interest to show if the plants were increasing the amount of oxygen inside the sealed system, there was a need to determine if the levels of carbon dioxide were also dropping.

B. Equipment

In addition to the previous equipment used for the BOX, a carbon dioxide sensorⁱⁱ was added. The experiment had an oxygen sensor, an internal HOBO that collected temperature, light intensity, and relative humidity data from inside the BOX II and an external HOBO that recorded the data from the oxygen sensor, carbon dioxide sensor, and the external temperature. To provide an optimal growing environment, a 'grow' light was used on a 12 hour on/off timer. A grow light is a full spectrum light bulb that most closely resembles the spectrum of the sun and is believed to provide the necessary energy to plants for their life cycle.

There was no attempt made to control the temperature inside the BOX II, but it was in an internal room with no direct or indirect sunlight able to reach the plants. A significant difference was recorded in the light and dark cycle temperatures that seem to be consistent throughout the experiment, except for a 2-week period when there was no light supplied to the BOX II. Relative humidity also followed a cycle with the light and dark, and the build up of humidity during the light cycle could be observed visually.

Due to the fact that the BOX II was a rigid container, an inflatable lung was attached to help regulate the internal pressure and prevent any leaks from occurring. The BOX II was constructed from a "20 gallon" aquarium, which measured 11.75 inches wide, 23.5 inches long, and 15.75 inches high and in fact held 18.75 gallons. It was constructed of glass, with a non-permeable aquarium sealant used to join the planes of glass. A Plexiglas top was constructed to allow for the wiring to join the measuring devices and power supplies. When the BOX II was closed, an aquarium sealant was used to seal the top, as well as the channel for the wiring. A

wooden brace was constructed to further hold down the ends of the top and prevent warping due to the heat from the light.

All measuring devices were capable of connecting to a computer for data downloading. The HOBO was intended to store up to nine (9) days worth of data at a time before a download was needed. The oxygen sensor was also capable of being connected directly to the computer and real time data could be seen.

III. EXPERIMENTAL DESIGN

The design of this experiment was largely based upon the previous work done on the first BOX. Much of the materials were reused to defray setup cost and time. In the previous experiment, the small size of the biosphere was decided upon due to several factors. They were as follows:

1. Smaller sized biospheres decrease the possibly gas and atmosphere exchange that would compromise the integrity of the experiment,
2. Fewer internal monitoring devices would be necessary to fully sample the environment and provide an accurate representation of the gas levels,
3. A larger biosphere leads to a more complex system, which would require more resources to monitor and introduce other variables into the system.

It was determined that the system would need an air exchange system, in this case a computer fan was used to circulate the air, and a gas bag, or lung, (Qubit systems, G122) made of heat sealed, gas impermeable, nylon polyethylene laminate with a fully inflated volume of 30 liters provided for gas expansion inside the biosphere. Initially, the lung was emptied of the majority of its air. There was a minimal amount left in the lung and after the biosphere was sealed, it was squeezed several times to mix in the extra carbon dioxide into the air inside the system.

The data was collected using a HOBO data logger, which “is a four-channel data logger that provides temperature, relative humidity, relative indoor light level measurement and accepts one external input. ...[it] offers 12 bit resolution, high accuracy, 64k memory and direct USB connectivity.”ⁱⁱⁱ Two such devices were used, one inside the biosphere and the other outside the biosphere. The internal HOBO was battery powered and the battery was changed before the system was sealed. The external HOBO used a 12-volt power supply. The HOBOs were used to collect data from the various sensors, but there was an issue with them that will be discussed later in the paper.

Light for the system came from a 125-Watt fluorescent grow light. Grow lights provide the full spectrum that plants need to efficiently complete the photosynthesis process and promote overall plant growth. The light fixture measured 19 inches long by 13 inches wide and was placed two inches above the top of the biosphere on a wooden brace to allow for some of the heat of the lamp to dissipate. The previous experiment saw the Plexiglas top warping from the heat of the light. To counteract this, the BOX II included a wood and wire brace that effectively tightened down the two ends and prevented any warping or breaking of the seal. The top was made of acrylic (Plexiglas) that was about 0.5 inches thick. The lid was chosen in this material because it is easier to work with and several holes were drilled in it to accommodate the wiring as well as

the attachment of the lung and the syringe that injected the carbon dioxide. The wiring was routed through a cable gasket that was filled with the aquarium sealant when the wires were in their final position.

IV. FLORA, FAUNA, AND INTERNAL SETUP

A. Flora

In the original experiment, *Arabidopsis Thaliana* was chosen for its many positive characteristics, but the plant eventually failed possibly due to humidity intolerance. In an attempt to prevent this, a more primitive and highly humidity tolerant plant was chosen, a fern. Also, it was decided that by including more than one variety of plants, there was a higher probability of at least one plant surviving. To this end, a total of three different plants were chosen. Two were from the fern family, *Bostoniensis* and *Pteris Cretica*, and the third was *Croton Codium*, a leafy plant that has a high humidity tolerance. To begin the experiment, the plants purchased were chosen for their larger size and healthy appearance. It was decided that larger plants would have more surface area to facilitate the photosynthesis process and would possibly show any benefits in less time than a growing plant would.

Even though the plants were not placed immediately into the BOX II after purchase, they still retained their healthy appearance and did not appear to have any insect life on them or possible other contaminants. Figure 1 shows the plants.



Figure 1: Plants Used in the Experiment

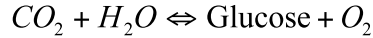
B. Fauna

No insect or animal life was intentionally introduced to the biosphere. Any pollination of the plants would have to be accomplished by the internal air movement provided by a small fan. Since ferns reproduce by spores, this was not seen as a hindrance. Any insect life included was incidental, and nothing moving was observed inside. The dirt used for the plants was Miracle Gro ® potting soil that was heated at 400 F for approximately 6 hours. It was hoped that this irradiation would kill any bacteria living in the soil as well as any other unwanted guests. There were possibly bacteria around the roots of the plants when they were planted, but the amount of dirt transferred with the roots was kept to a minimal. The water used in the BOX II was

unfiltered tap water, which contains a high level of chlorine and has been treated to remove the majority of harmful organisms so that it is fit for human consumption.

C. Internal Setup

Since the goal of this experiment was to create oxygen and lower the carbon dioxide levels, there needed to be an optimal environment for photosynthesis. Also, there needed to be an accurate way of measuring the various elements that would affect photosynthesis. The essence of the formula for photosynthesis is as follows:



The air inside the BOX II was 'normal' air with a mixture of gases. Extra carbon dioxide was introduced into the system after it was sealed to raise the levels and provide possibly more materials for photosynthesis. The humidity level in the BOX II was negligible when it was sealed, due to the low relative humidity of the surrounding environment, but after the water in the closed system was allowed to evaporate, humidity levels increased to the point of creating condensation on the glass surfaces. The internal layout of the BOX II is shown in Figure 2 below.

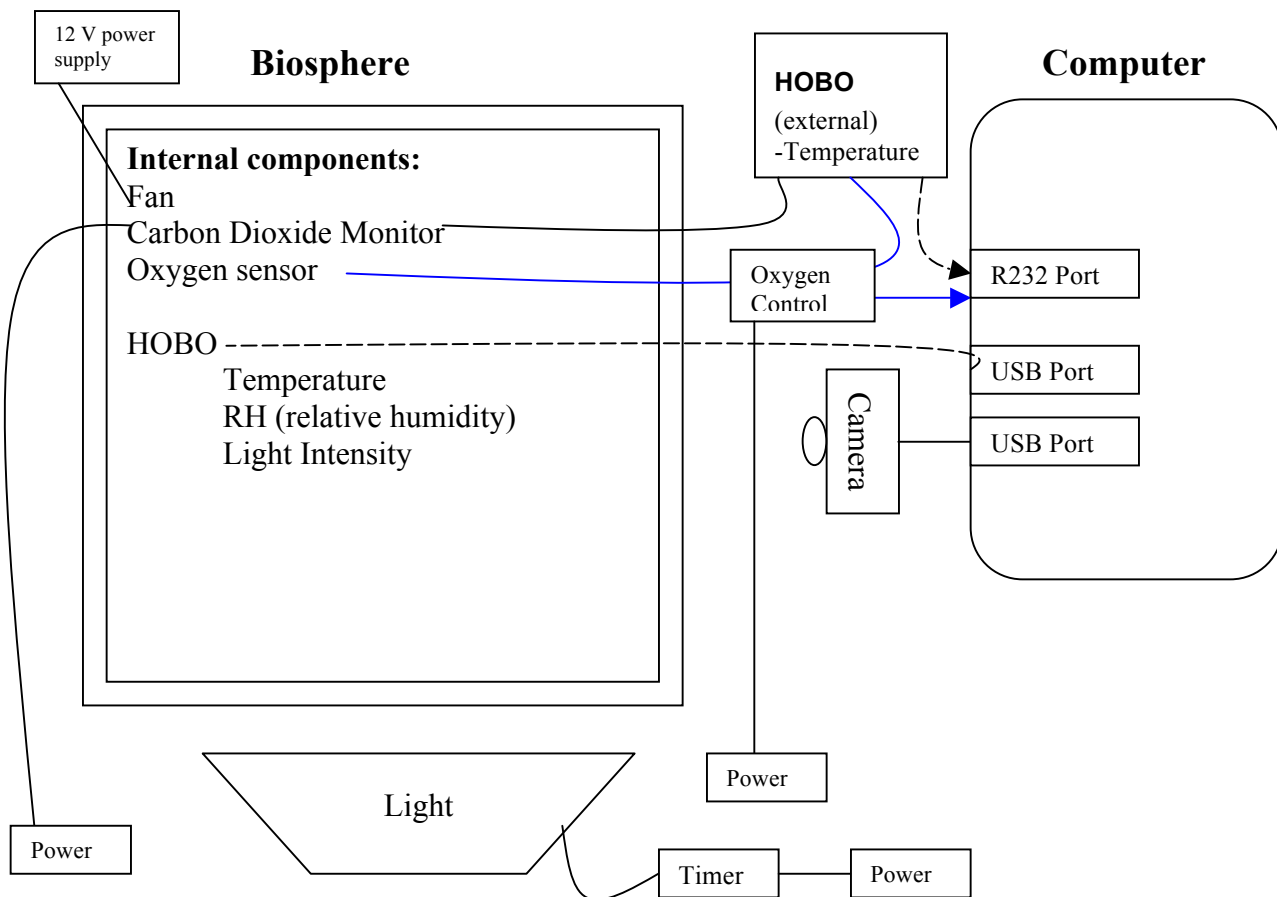


Figure 2. Schematic of the BOX II

V. Final closure procedure and data collection

A. Closure procedure

When all the pieces of the BOX II were finally gathered, it was time to seal the system. Before beginning, the aquarium was thoroughly cleaned from the previous experiment and allowed to dry completely for several days. A partition was put in place to separate the dirt side from the water side. Twenty-five pounds of river rocks were washed and placed on one side of the partition to hold it in place and provide an area for the water to gather and evaporate. The irradiated potting soil was placed on the other side of the barrier and slightly sloped to allow for any water runoff. Finally the plants were placed in the dirt. Two of the Croton Codium plants were placed at opposite corners from each other, and one each of the two ferns were placed in the other corners. After the plants were in, a large rock with the internal HOB0 attached to it was put on the side with the rocks. The carbon dioxide sensor was also placed on the rock, with the readout screen facing the end of the tank. After this, the lid was temporarily placed on top to make sure that all the wires reached where they should and there was no binding of anything. The carbon dioxide sensor, oxygen sensor, and HOB0 were tested to make sure that valid data was being collected and was able to be retrieved. Also, the internal fan was tested and was in working order.

After the testing, 200 mL of water was added to the tank and the lid was placed back on but not sealed. The BOX II was allowed to run for a week in this semi-closed status to ensure that all data devices were working correctly. After this time, approximately 40 more mL of water was added and the BOX II was sealed using an aquarium sealant. After the edge was sealed, the wires were pulled through the plug to the correct length, and sealant was poured around the wires to fill the gap in the rubber gasket. After securing the top with the wood and wire brace, carbon dioxide was added to the system using a syringe and a two-way valve. Since carbon dioxide is heavier than air, the gaseous carbon dioxide was simply placed into an upside down syringe and then attached to the valve and injected in. 150 mL of carbon dioxide was injected into the system, and it was immediately recorded on the sensor. To insure a more uniform mix of air, the inflatable lung was compressed several times. There was no noticeable amount of air that went into the lung when the carbon dioxide was injected, but it was assumed there would be a small amount in there.

B. Observational Results

Initially the BOX II appeared to be operating correctly. However, from the initial setup there were difficulties with the carbon dioxide sensor. The HOB0 recorded output voltage from the carbon dioxide sensor, but it records a maximum voltage of 2.5 Volts. The carbon dioxide sensor converted the part per million (ppm) of carbon dioxide directly to voltage. Unfortunately, when we injected in the carbon dioxide, our ppm went to 4848 and by day three was at 5345 ppm. The values began to drop eventually, but they were still quite high. This high voltage interfered with the HOB0 and all data from when the carbon dioxide sensor was connected to it was corrupted. This high level of carbon dioxide was unforeseen and it was not immediately recognized that the HOB0 could not handle it. After this problem was noted, the carbon dioxide sensor was disconnected from the HOB0 and visual readings were recorded from the screen on a

bi-weekly basis. Also, at some point in the experiment the HOB0 switched from showing ppm and went to percentages. Since it was inside the sealed biosphere, there was no way to adjust the sensor or reset it. Percentage of Carbon Dioxide inside the Biosphere II is shown in Figure 3.

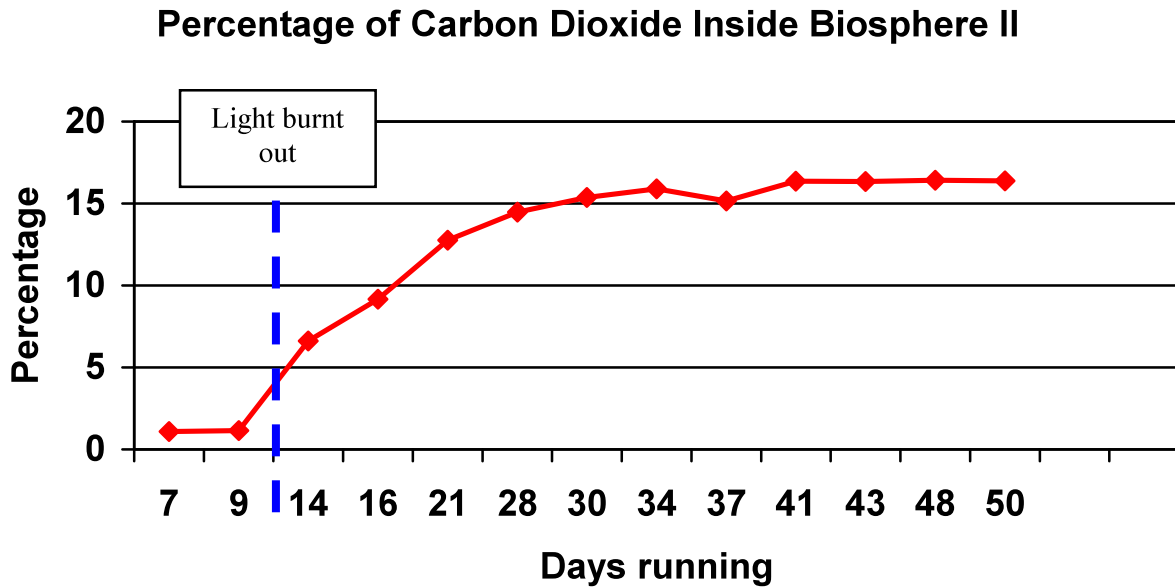


Figure 3. Percentage of Carbon Dioxide inside the Biosphere II

Oxygen levels appeared to be slightly decreasing during the beginning of the experiment, with slight variations in the levels during the day and night cycles until the light burned out (Figures 4 and 5). This variation could have been due to the air more completely mixing, or the levels of oxygen actually decreasing. Unfortunately not enough data was collected to prove or disprove either theory. Temperature followed a definite pattern and the swings in temperature directly corresponded to the light and dark phases of the experiment (Figure 6). Relative humidity inside the biosphere also followed this pattern, as shown in Figure 7. Figure 8 shows the intensity of the light throughout the experiment. It is immediately obvious where the light burned out and the length of time where no light was seen by the plants. In the first weeks of the experiment the plants appeared to be thriving and new growth and plants were visually observed.

During the experiment, there was a one-week period when the system was unable to be monitored due to schedule constraints and it was at that inopportune time that the light bulb in the grow light burned out. A new bulb was ordered, but it was almost two full weeks before the system again was running with light. Unfortunately during that time, the plants began to die and the carbon dioxide levels began to drastically increase. The percentage of carbon dioxide went from under 5% to over 10% in just one week. After this time, the system was unable to recover and all the plants eventually died. During this decline, the carbon dioxide levels were shown to continue increasing, but they appeared to level out around 17% concentration. Also at this time the oxygen sensor began reading decreased oxygen and finally showed a value of 0.0% oxygen in the system. It is undetermined at this time if the sensor was in fact reading no oxygen or if it had failed again. Before starting the experiment the oxygen sensor used in the previous

experiment was found to be 'bad' and it was replaced. The oxygen sensor operates at very high temperatures and it was believed to have burned out from being left on for over a year.

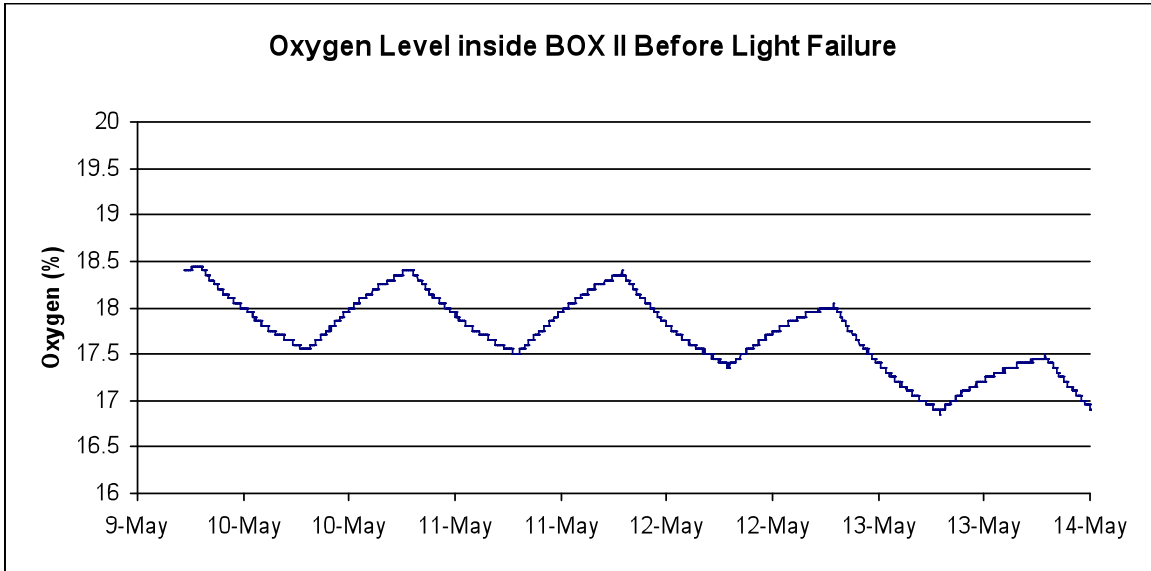


Figure 4: Oxygen Levels for Beginning of Experiment.

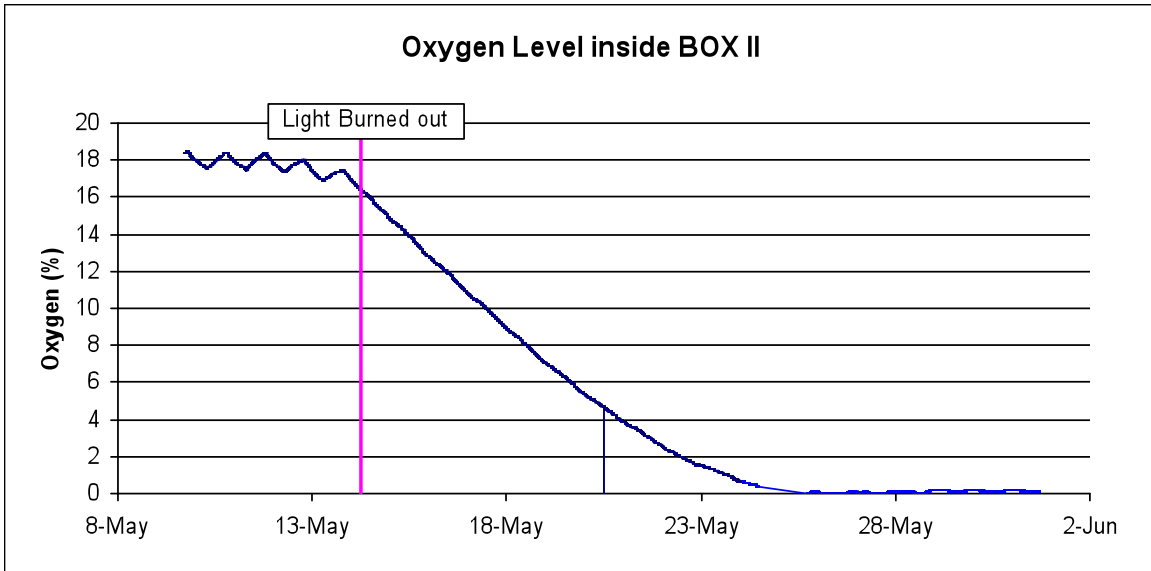


Figure 5: Oxygen Levels Throughout The Experiment

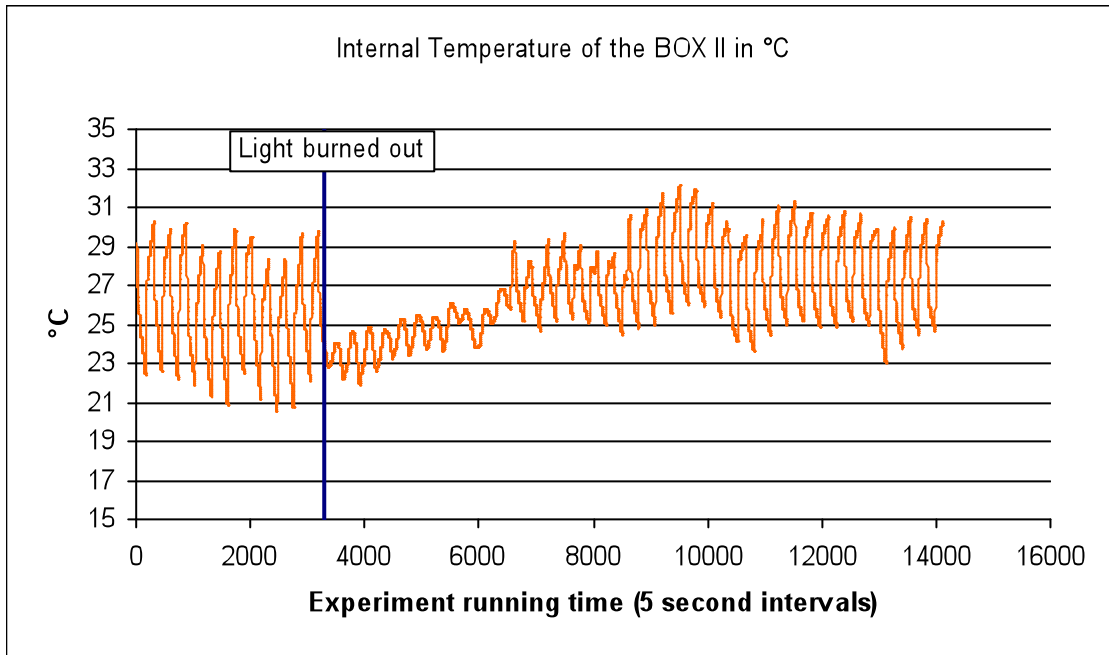


Figure 6: Internal Temperature of the BOX II

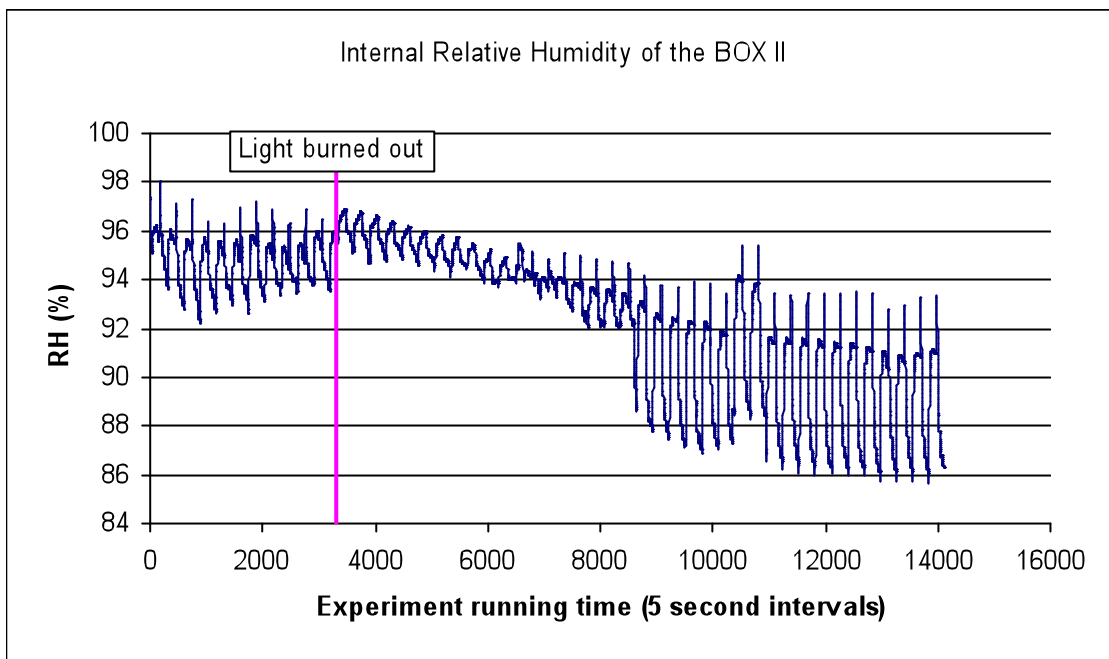


Figure 7: Internal Relative Humidity of the BOX II

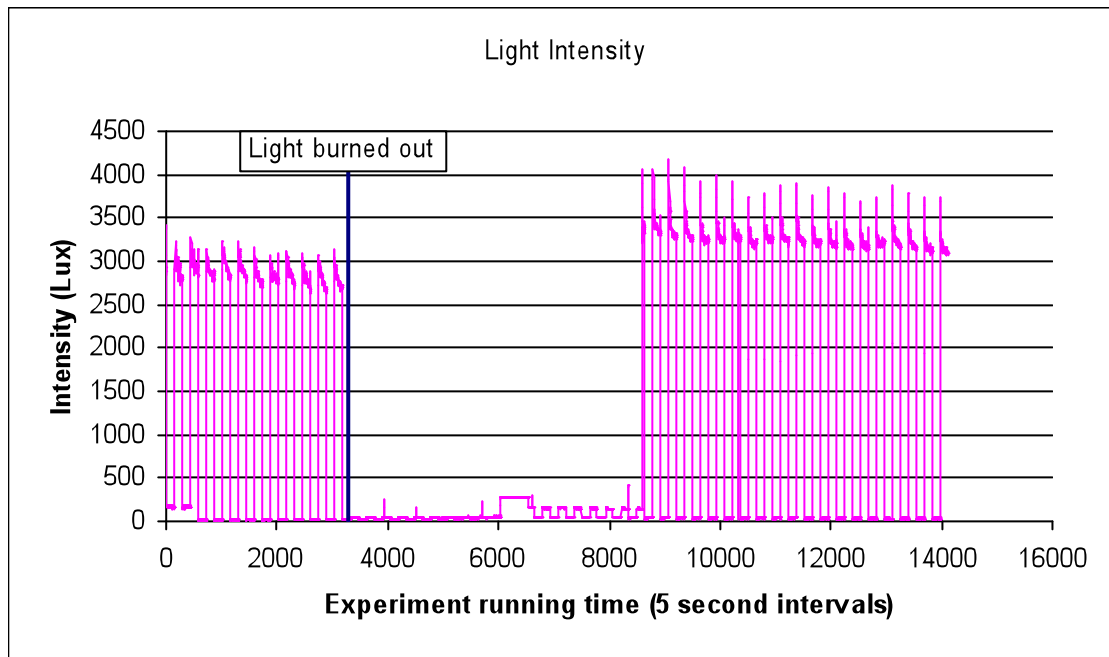


Figure 8: Light Intensity of the BOX II

VI. CONCLUSION

This experiment was in many ways a success, even though it eventually failed. It showed how slight changes, such as removing the light source can have irrevocable consequences. Once the light was off, the plants were unable to complete the photosynthesis process and even when the light was returned, it was not soon enough and the plants were unable to recover. One factor in this failure might be that during the time the light was off, the humidity levels decreased drastically, which led to less water in the air and the plants began to dry out. After the light was replaced the humidity never reached the same levels and the plants were already much too dry to recover.

In conducting this experiment, it was observed that what might appear to be a minor obstacle can lead to the downfall of the system. There is an opportunity to further develop this experiment and expand on it. A larger biosphere could be used to include more climate and plant types, and possible life forms could be included as well. Further monitoring equipment could be used to more accurately observe and record the gases inside the biosphere and provide more data on an enclosed system. A biosphere must be hardy and adaptable to survive, and this experiment has hopefully lead one step closer to the dreams of mankind to one day live on the surface of Mars.

ⁱ Konrad, T., Sauer, W., and Sarper, H., "The Biosphere Observational eXperiment (BOX)"

ⁱⁱ Onsetcomp.com, "Carbon Dioxide (CO₂) Data Logger Guide," 2006, Onsetcomp.com, 30 October 2006
http://www.onsetcomp.com/Products/Product_Pages/HOBO_H08/external_sensors.html#co2sensor

ⁱⁱⁱ The Data Logger Store.com, "HOBO U12 Temp/RH/Light/External Data Logger," 2005, MicroDAQ.com, 6 February 2005
<http://www.microdaq.com/occ/u12/u12-012.php>

APPENDIX

This section contains four pictures showing various phases of the experiment.



