The Effects of Atmospheric Conditions on the Output of CO$_2$ in _Saccharomyces Cerevisiae_ During Ascent and Descent to and from the Stratosphere

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Abstract

The purpose of this experiment is to send yeast, _Saccharomyces Cerevisiae_, to the stratosphere and measure the CO$_2$ productions of a control that stays on the ground and a variable sample that ascends to and from the stratosphere. _S. Cerevisae_ is placed inside a payload that is attached to a balloon satellite that ascends to and from the stratosphere. A hypothesis that _S. Cerevisae_ will produce a lower CO$_2$ level compared to a control _S. Cerevisae_ that was left on the ground was formulated. Post launch measurements of the CO$_2$ productions are performed at ground-level. This measurement showed an 86.4% increase of CO$_2$ productions compared to the control _S. Cerevisae_ on the ground. Measurements of the CO$_2$ productions are done with a solution of 10% glucose and a solution of 10% sucrose prepared with 0.5g of _S. Cerevisae_ yeast, one prepared for the control and one for the variable sample that traveled to the stratosphere. The prepared solutions are placed into an incubator for increments of 15 minutes until 45 minutes elapses. The conclusion that the increase of CO$_2$ levels related to change of atmospheric pressure and temperature during the ascent to and from the stratosphere is drawn. This assumption could be attributed to the transposable elements, or “jumping genes,” found in _S. Cerevisae_, which might result in genetic modification and a higher output of CO$_2$ because of the stressful environment. Further experiments would need to be done to test this conclusion with a more restricted medium for experimentation.

Keywords: transposable elements – jumping genes – glucose – sucrose – payload - stratosphere

Introduction

_Saccharomyces Cerevisiae_, which is commonly known as Brewer’s yeast or Baker’s yeast, is a unicellular organism that is considered one of the most important fungi in the world because of its applications in baking and wine/beer productions. _S. Cerevisiae_ is found in the wild growing on grape skins and a variety of other types of fruits. This species of yeast can reproduce asexually and sexually, which is a rarity amongst the phylum Ascomycota. The name _Saccharomyces Cerevisiae_, when translated, literally means “sugar fungus.” This species acquired its name because glucose is the main source of food for ATP production. _S. Cerevisiae_ is able to breakdown glucose through two different methods: aerobic respiration and anaerobic fermentation (alcohol fermentation), which allows this species to live in a large variety of places, such as environments lacking adequate oxygen for survival. When no oxygen is available _S. Cerevisiae_ goes through alcohol fermentation, which produces two ATP with two byproducts: CO$_2$ and ethanol [10].

Huge environmental stressors when ascending and descending to and from the stratosphere are the lack of oxygen, high levels of pressure, extreme temperature changes and bombardment from different
forms of radiation, such as gamma rays, ultraviolet radiation, and x-rays produced from the sun. A majority of these forms of radiation are absorbed through the ozone, which 90% of which is located within the stratosphere approximately 16 to 48.3 kilometers above the surface of the earth. Each form of radiation has detrimental effects on biological life [2]. Ultraviolet Radiation is the most prominent in this layer of the Earth’s atmosphere, which causes biological damage to the DNA within organisms and alters the shape of DNA molecule’s causing the distortion of proteins and the death of cells [6].

In an age of space exploration, a plethora of tests have been conducted on biological systems to further understand the way species survive and adapt to harsh environments that are not found on Earth, which is a large basis for the experiment on the productions of CO$_2$ in *Saccharomyces Cerevisiae* in the harsh conditions of the stratosphere. NASA and other space industries have tested *S. Cerevisiae* under the conditions of low earth orbit to understand their response to low levels of gravity and how life responds to different physical stimuli and phenomenon, which are done for many different reasons, such as acumen involving cancer research as well as understanding different factors that might affect crews who are sent to space [9].

The main purpose of this experiment was to observe how atmospheric conditions to and from the stratosphere effect the CO$_2$ productions in *Saccharomyces Cerevisiae*. It was predicted that the higher levels of atmospheric conditions, such as atmospheric pressure, the lack of oxygen, extreme temperature changes, and the different forms of radiation on *S. Cerevisiae* would cause the species to produce lower productions of CO$_2$ compared to the control sample left on the ground. This experiment was implemented by attaching a payload to a balloon satellite and sending a variable sample of *S. Cerevisiae* to the stratosphere, which was then later compared to the control sample left on the ground by testing the amount of CO$_2$ that was produced from each group through the process of counting CO$_2$ bubbles.

**Materials and Methods**

**The Space Grant Consortium Project**

The balloon satellite project experiment on *Saccharomyces Cerevisiae* was part of the Colorado Space Grant Consortium, which is a program funded by NASA that involves nineteen different colleges throughout the state of Colorado and provides students the means to partake in different hands-on space related projects, courses, and outreach activities [1]. This project required a group of students to participate in the conceptualization, design, and implementation of a payload and experiment that could be attached to a balloon satellite for ascent and descent to and from the stratosphere.

The main payload in this project was designed to be large enough to house two experiments, which would take this trip to the stratosphere together. The experiments within this payload were transported separately and brought to the launch site, in Limon, Colorado, where on the morning of launch everything was assembled. The payload was designed to be light, large enough so that both experiments could be places inside, sealed to keep the environment inside less exposed to the elements outside, and durable enough to survive a fall back to Earth.

**The Payload**

During this project there was a weight limit of 850 grams because of the participation of other students who also had their payloads attached to the balloon. Since there was a weight limit, the entire payload
was made out of thin polystyrene extruded foam that was laminated between two sheets of paper. Aluminum foil ventilation tape was used to seal the box as well as hold everything together. Certain parts of the box were connected using 2-part cement epoxy and the interior items were held together with Ethylene Vinyl-Acetate, which is also known as hot glue. An Arduino unit was placed within the payload with sensors that could measure atmospheric pressure, temperature, and the orientation of the payload. The entire Arduino unit, connected to the lid of the payload with Velcro tabs, was powered by a 12v rechargeable battery pack, which was connected to the side of the box. Through the center of the payload, a plastic tube was inserted with a washer at the top of the payload and a washer at the bottom of the payload, which would later be used to feed a rope through the center so that it could be strung up to the balloon on launch day.

*Saccharomyces Cerevisiae*

The organism of primary focus during this experiment was *Saccharomyces Cerevisiae*, which is a common form of yeast that is used in a variety of applications, such as baking, brewing, and other productions of wines and alcohols. This species of yeast has been studied, by NASA, on different occasions, which focused on multiple genetic variations of the genus, as well as other experiments that focused on responses in genes due to the species being placed in microgravity [9][12].

Three packets of *S. Cerevisiae* were connected to the interior of the payload using Velcro tabs. The packets of yeast in the experiment were found online and had to be packaged properly, which meant that they had to be air tight and dehydrated. The packets had to be air tight because of the large amounts of pressure change the yeast would be subjected to during the ascent and descent to and from the stratosphere.

**Determining CO₂ Production**

Data pertaining to the production of CO₂ was procured using a procedure, which focused on counting CO₂ bubbles to determine the CO₂ production. During this procedure, six fermentation tubes were washed and sterilized (three were used for the variable group and three were used for the control group) and an incubator was heated up to 40°C. Solutions of 10% glucose and 10% sucrose were prepared and set aside. With weight boats, a digital scale was utilized to weigh and measure 0.5 grams of *Saccharomyces Cerevisiae*. 15 milliliters of each solution above were measured using a graduated cylinder and added to separate 100 milliliter beakers. The 0.5 grams of *S. Cerevisiae* was added to each beaker of solution and then was stirred until fully dissolved. Each solution in the beakers were poured into the fermentation tubes then sealed with parafilm and placed into the incubator for a total of 15 minutes. Initial measurements at the end of this procedure were recorded and the after each 15 minute interval the fermentations tubes were removed from the incubator and the CO₂ levels were measured again for each 15 minute interval until 45 minutes has elapsed. After finishing the procedure, 95% confidence intervals were calculated for the results pertaining to the variable groups.

**Temperature**

A huge factor during this experiment that related to a stressor was temperature change. During the ascent through the various layers of atmosphere, temperatures vary drastically. As a payload climbs in the troposphere, which extends from a height of approximately 6 to 20 kilometers high, the
temperatures drop because the atmosphere becomes thinner. As a payload gains altitude the temperature drops from approximately 17°C to -51°C at the tropopause, which is located right near the start of the stratosphere [7]. After reaching the stratosphere, which extends from approximately 16 to 48.3 kilometers high, temperatures increase as the payload climbs. From the edge of the tropopause to the edge of the stratopause the temperatures change from -51°C to -15°C, which is caused because of the absorption, due to the ozone, of the sun’s ultraviolet radiation. During the ascent of the payload, these extreme temperature changes can be detrimental to biological systems [6][7]. To circumvent drastic changes in temperature, the payload is sealed so that heat from the batteries can counter some of the effects.

**Results**

The results show that, after determining the CO₂ productions of the control and variable, the CO₂ output continues to rise after each 15 minute interval of the procedure.

![Figure 1](image1.png)

*Figure 1*. A scatter plot that illustrates the levels of CO₂ production rates of *Saccharomyces Cerevisiae* at time intervals 15, 30, and 45. The bars represent 95% confidence intervals, where n=3 each variable instance.

As shown in figure 1, the calculated 95% confidence intervals (C.I.) of the measured CO₂ productions in the variable

![Figure 2](image2.png)

*Figure 2*. A bar graph that illustrates the levels of CO₂ production rates of *Saccharomyces Cerevisiae*. This is noticed in Figure 2, where the bar graph seems to be increasing at a large rate for each 15 minute interval. In the initial 15 minute interval the rates of CO₂ production remained pretty equal, but after the next 15 minutes the rates for the variable experiment increased extensively. By the time the 45 minute period elapsed the variable group had surpassed the control group by a large amount. Looking at the data on Figure 2, the amount of CO₂ production in the sucrose variable increases by 62.5% on the 15 minute interval compared to the sucrose control, an increase of 77.8% on the 30 minute interval and, an 86.3% increase on the 45 minute interval. The production of CO₂ in the glucose variable increases similarly, at 40.1% increase at 15 minutes, 65% increase at 30 minutes, and 70% increase at 45 minutes.
glucose and the variable sucrose overlap at all points throughout the entire 45 minute time interval (glucose C.I. variable is $C.I. = 0.185 < \mu < 13.595$, and the sucrose C.I. variable is $C.I. = -2.199 < \mu < 24.199$). This indicates that the variable values of CO$_2$ production levels of the sucrose and glucose show no significant differences throughout the interval, but looking closer it is shown that none of the controls in the experiment are overlapped by the 95% confidence interval. Since the controls are not overlapped by the 95% confidence interval of the variables it shows that it does not fall on the population parameter of the variable portion of this experiment, which shows that there is a huge change in data in the variable compared to the control.

The following calculations were done to formulate the confidence intervals of the variable group in the experiment:

- **Mean:**
  - $\mu = \frac{\sum x_i}{n}$
  - Where $n = 3$
    - Sucrose mean = 11
    - Glucose mean = 8.89

- **Standard Deviation:**
  - $\sigma = \sqrt{\frac{\sum (\mu-x_i)^2}{n-1}}$
    - Sucrose $\sigma = 11.6636$
    - Glucose $\sigma = 5.92496$

- **95% Confidence Interval:**
  - 95% $C.I. = \mu \pm \frac{z\sigma}{\sqrt{n}}$
  - Where $z = 1.96$
    - Sucrose
      - $C.I. = 11 \pm 13.2$
    - Glucose
      - $C.I. = 8.89 \pm 6.7$

**Discussion**

During this experiment the results indicated that the variable group had some type of alteration that caused it to output more CO$_2$ than the control experiment. Groupings of the controls show that the CO$_2$ outputs are pretty accurate because of very close results that were found for the sucrose and glucose over the different periods of time during the experiment. The large difference between the control and variable groups was not foreseen initially. Originally, it was thought that the trip to the stratosphere would make CO$_2$ productions lower compared to the control. This final output of evidence did not support the initial hypothesis so the hypothesis had to be thrown-out.

In other experiments done on the productions of CO$_2$ the trend where the amount of CO$_2$ production increased from the initial values were persistent in multiple experiments [3][5]. This trend is the same as the trend noticed through the stratosphere experiment results.

There are many things that could have affected the output of CO$_2$ in *S. Cerevisiae* such as the possibility of an increased reproduction rate on the way to and from the stratosphere, which would increase the amount of *S. Cerevisiae* available to produce CO$_2$, and transposable elements, or jumping genes, in different cells are a known factor that can cause genetic alterations, which in turn change certain inner cell workings, such as the production of CO$_2$. Transposition can cause inactive genes to become active due to extreme levels of stress. Not only does transposition cause inactive genes to become active, it also causes active genes to become inactive. This movement of genes can cause a variety of mutations [11]. The detection of transposable genes is done through computer programs with algorithms that are designed to detect them. There is a large list of programs available that can be used to detect repeats in genetic materials as well as
transposon elements [8]. This process would be necessary to further investigate this claims as well as to acquire more data that can be utilized to understand these outcomes more in depth.

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References


