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Introduction

Why this topic?

Before going to high school, there was a market that my family and I would go to every Saturday morning. In this market, I would see artisans of all kinds making their specialties but my favorite station was the bakery. There would always be a baker making his own bread fresh in the morning and I loved watching as he would knead the dough and then leave it to rise before putting it in the oven to cook and rise more. I was fascinated with this process that had been passed down and specialized throughout his family for years so when looking for a bio IA topic I decided to see some of the variables that bakers like him might encounter. Hence, I chose the question "How does the concentration of O_2 in water affect the respiration rate of yeast?".

Approach

Once I arrived at my question it was necessary to decide how to approach this with an experiment. I decided to use an aerator to increase the oxygen concentration in the water to certain amounts for my experiment. Then I would choose 4 different concentrations and run four different trials for each of them. The concentrations that I chose were 7mg/L, 8mg/L, 8.5mg/L, 9mg/L. I would control other variables by keeping a constant water temperature, using the same type of yeast, and using the same amount of yeast. The respiration rate of yeast will be measured by attaching a balloon to the container with the yeast and solution and measuring the increase in circumference of the balloon. As the yeast undergoes cell respiration, it will release carbon dioxide gas, blowing up the balloon as given by the chemical formula below.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

So, an increased respiration rate will lead to a larger balloon circumference and a decreased respiration rate will lead to a smaller balloon circumference.

Research Question

How does the concentration of O2 in water affect the respiration rate of yeast?

Hypothesis

The hypothesis created for this experiment is that a higher concentration of O2 in the water will cause a higher respiration rate for the yeast. This is because the oxygen in the water will be able to diffuse across the membrane of the yeast and allow it to undergo aerobic respiration producing CO2 gas. At a higher concentration, there will be a greater differential and oxygen will diffuse more rapidly through osmosis than it would at a lower concentration. Hence, at higher O2 concentrations, yeast will undergo more cellular respiration and therefore have a higher respiration rate.

For the statistical analysis of this experiment: there will be two hypotheses:
Null Hypothesis: There is no effect of dissolved oxygen concentration on yeast's respiration rate.
Alternative Hypothesis: Differences in dissolved oxygen concentrations will cause a change in the respiration rate of yeast.

Materials

- Erlenmeyer Flask
- Distilled Water
- Pure white sugar
- Balloons (any type)
- Rubber bands
- Paper towels
- *Saccharomyces cerevisiae* (Yeast)
- Aerator
- Plastic wrap
- Tape
- Marker
- O2 sensor
- String
- Ruler

Methodology

Changing O2 Concentration

For the solution with a concentration of 7DO/L, 400mL of heated water was added to the Erlenmeyer flask. The O2 sensor was placed in the water and measurements began. As the water temperature dropped, O2 concentration rose until it was measured at 7DO/L. The O2 sensor was removed and the Flask was sealed with plastic wrap and tape until the water reached room temperature to ensure that the concentration would stay the same. For the solution with 8DO/L, 400mL of lukewarm water was added to the Erlenmeyer flask. The O2 sensor was used to ensure that the oxygen concentration was at 8 and the flask was sealed with tape and plastic wrap. For the solution with a concentration of 8.5DO/L, 400mL of water was added to the Erlenmeyer flask. The aerator was placed into the flask with the O2 sensor and allowed to run until the sensor measured the right concentration. The flask was sealed with plastic wrap and tape. For the solution with a concentration of 9DO/L, 400mL of chilled water was placed in the Erlenmeyer flask. The aerator was put in the water with the O2 sensor and allowed to run until the sensor measured the oxygen concentration of 9DO/L. The sensor and aerator were removed and the flask was sealed with plastic wrap and tape. The water was set aside until it came to room temperature.

Preparing Solution

Once the oxygen concentration and temperature were correct, the plastic wrap and tape were removed and 28.350 grams of sucrose was added to the water. The solution was stirred until the sucrose was completely dissolved. 14.175 grams of *S. cerevisiae* were added and a balloon was stretched over the end of the Erlenmeyer flask to create a seal. This solution was set aside for 30 minutes to undergo respiration.

Pictures #1 and #2

These pictures show the balloon gas collection method that was used for the experiment. The gas is created by the yeast in the Erlenmeyer Flask and collected by the expanding balloon. Once the trail is over the balloon is taken off and tied to prevent any gas from escaping.

Measurements

After 30 minutes, the balloon was twisted to prevent any gas from entering or escaping while it was removed from the flask. The end of the balloon was removed from the flask and tied off to seal the balloon. The balloon was labeled according to its trial number and conditions and set aside. Once this process was repeated for all 16 trials, a piece of string was wrapped around the widest part of the balloon vertically. A marker was used to draw a line on the string to mark the circumference of the balloon. A ruler was used to measure this circumference and the data was recorded in a table. This process was repeated for all 16 trials.

Safety and Ethical Considerations:

There were no ethical considerations to take into considerations during this experiment. Additionally, the yeast used poses no significant risks so it was sufficient for the experimenter to thoroughly wash hands before and after conducting trials.

Data and Calculations

Table #1

Trial	1	2	3	4	Average
O2 Conc (mg/L)					
7	19.47cm	18.99cm	22.53cm	21.97cm	20.74cm
8	19.50cm	23.29cm	21.01cm	22.97cm	21.70cm
8.5	20.36cm	22.52cm	21.00cm	20.89cm	21.19cm
9	20.12cm	19.46cm	20.67cm	22.43cm	20.67cm

Calculating Standard Error

This process is shown for only one of the groups but is repeated for all four

$$Mean = 20.74$$

$$|19.47 - 20.70| = 1.23$$

$$|18.99 - 20.70| = 1.71$$

$$|22.53 - 20.70| = 1.83$$

$$|20.97 - 20.70| = 0.27$$

$$\sum(deviations)^2 = (1.23)^2 + (1.71)^2 + (1.83)^2 + (0.27)^2 = 7.8588$$

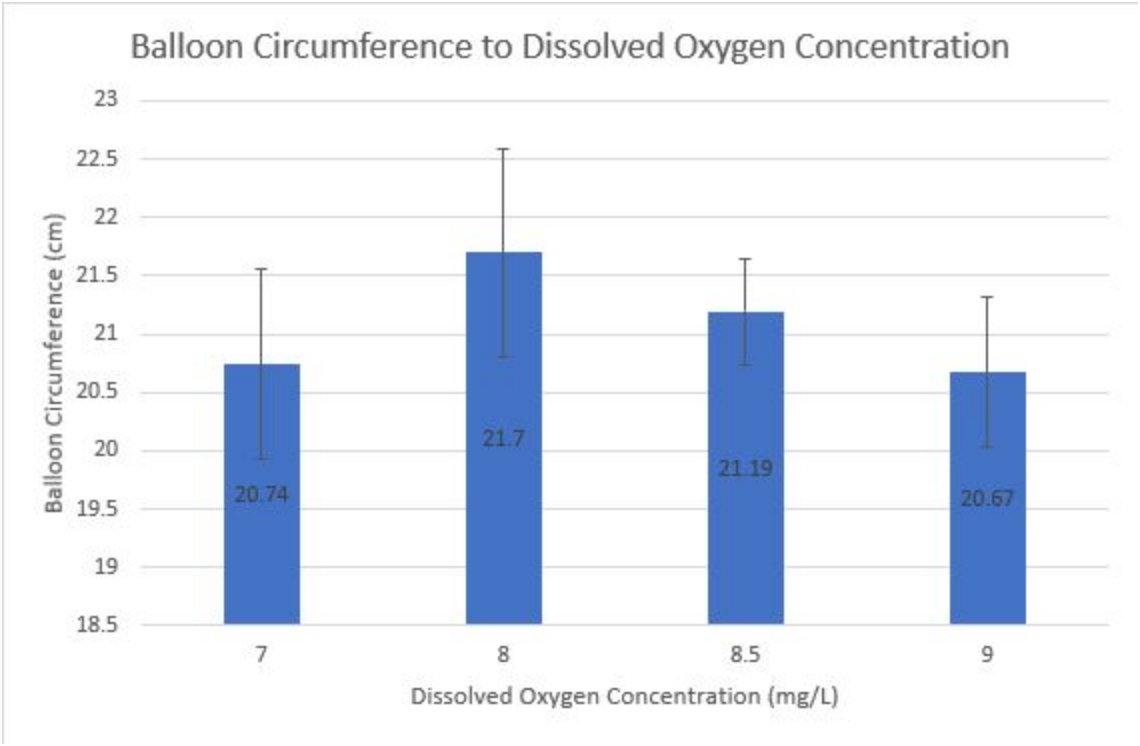
$$7.8588/(4-1) = 2.6196$$

$$\frac{\sqrt{2.6196}}{\sqrt{4}} = 0.81 = Standard\ Error\ (SE)$$

Table #2

Conc. of Oxygen (mg/L)	Standard Error (cm)
7	0.81
8	0.89
8.5	0.46
9	0.64

Graph #1



Analysis

Statistics

In Graph #1 it is shown that the data values collected from this experiment are not significantly different from one another. This is because all of their error bars overlap at a certain range of points. Hence this experiment fails to reject the null hypothesis and conclude that there is no provable effect that dissolved oxygen has on the respiration rate of yeast.

Hypothesis

The data from this experiment was very unexpected. No significant change was found between the separate groups. This means that the alternative hypothesis that O2 concentration has any effect on the respiration rate of *S. cerevisiae* cannot be supported by the experiment.. There are three main possible causes for this result. The first is that the oxygen levels were too close together to produce significantly different results. With limited equipment, there was only so much that could be done to change dissolved oxygen levels. Heating and sealing the water would only successfully drop levels to about 6.5 mg/L and the aerator could only bring them up to around 9.25mg/L. This meant that the amount of change that could be made to the independent variable was limited and as a result, could have limited the data. The second possibility involves the allosteric regulation of the cellular respiration process by ATP. This regulation means that a cell fixes the rate at which it produces ATP so that it does not burn through all of its resources at once. This regulation could cause the oxygen and sugar to be consumed at a constant rate regardless of concentrations. This would consequently result in CO2 being produced at a constant rate. The final possibility is due to the Crabtree Effect. This is the phenomenon that causes the yeast to undergo fermentation in aerobic conditions with a

high glucose saturation. If the Crabtree effect took place, this would cause the concentration of oxygen to be irrelevant because the yeast cells would be using anaerobic fermentation, therefore nullifying differences caused by oxygen. Any of these three possibilities could cause the lack of significant difference between the groups in the experiment and the failure to reject the null hypothesis.

Conclusion

Hypothesis

In conclusion, I failed to reject the null hypothesis that the concentration of dissolved oxygen has no significant effect on the respiration rate of yeast. This means that my original hypothesis that an increased dissolved oxygen concentration would create increased respiration rates was not able to be proved by this experiment. This can be attributed to one of the reasons mentioned in my analysis or to a combination of them.

Comparison

During my research I found various different experiments with yeast similar to mine that achieved different results than my experiment. One experiment conducted by Marcelo Orellana and others concluded that the dissolved oxygen levels in their experiment had significant impacts on metabolic pathways in yeast cells. However, their experiment was testing the effects of oxygen levels that were much lower than the ones in my experiment. As a result, those researchers were finding differences associated with changing from anaerobic respiration to aerobic respiration while I was looking for differences that were caused in just aerobic respiration. Additionally, these researchers found that once the yeast switched to cellular respiration using aerobic pathways, the oxygen-uptake-rate or OUR seemed to flatten. This is consistent with my results that once yeast is undergoing aerobic respiration, the amount of oxygen present in the environment does not affect the rate of respiration.

Challenges

Over the course of this experiment I encountered many different challenges. The first was to figure out how to lower the dissolved oxygen concentration. Because my aerator could only raise the dissolved oxygen concentration, I did not have a way to get a level below what I started at. Eventually I figured out that by starting off with hot water, that is not able to dissolve as much oxygen, and letting it cool I would be able to get a lower oxygen level while maintaining temperature. Another challenge that I encountered during this experiment was with the balloons. There were multiple instances in which after tying the end of a balloon to seal the gas inside, it would leak and deflate. I eventually found that adding a layer of tape to the end of the balloon for extra sealant would solve this problem but as a result of this I had to repeat four of my trials.

Sources of Error

There were multiple sources of error during my experiment that could have influenced my results. The first and main source of error was room temperature. This experiment took multiple days and the most reliable way for me to maintain a constant temperature for my experiment was to do my room temperature. However, one of the days of my experiment was significantly colder than the rest. This occurred on the day that I did the trials for the 8.5 group.

Because I measured the balloons after completing the trials there were two different ways that this temperature difference could have had an effect on my experiment. Firstly, yeast is known to respirate more slowly at lower temperatures and in low enough temperatures to not respirate at all. Secondly, the lower temperatures could have caused the gas in the balloons to contract giving falsely low measurements. Both of these would cause the data values to be lower than they should have been. This could suggest that there is a spike around 8.5mg/L oxygen concentration in respiration rates which would show an optimal oxygen concentration for yeast respiration. However, more experimentation with a better control over this source of error would need to be done. This source of error could be controlled by measuring all of the balloons on the same day at the end of the experiment. This would ensure that their volumes are not being comparatively affected by temperature. There would also need to be a way to ensure that the solutions were being kept at a stable temperature. A hot plate with a stirrer could be easily used for this. A second source of error that is possible could be the spoiling of the yeast. Yeast can go bad if exposed to air, moisture, or heat. Because this experiment was done over the course of multiple days, it is possible that these could have caused the yeast to begin going bad because it was not stored in a sealed or refrigerated container. The order in which I ran my trials was the 8mg/L group, the 8.5mg/L group, the 9mg/L group, and the 7mg/L group. If the yeast began to spoil, over the course of the experiment, less of it would be viable meaning that the measurement for the first group would be accurate but the measurements for following groups would be lowered for each day after that their trials were run. This would mean that the 8mg/L group and the 8.5mg/L group would be fairly accurate while the 7mg/L group and the 9mg/L group may have lower readings than they should. This source of error mostly cancels the previous one because this would lower most of the rest of the groups with the 8.5mg/L group that was affected. This second source of error could be counteracted by storing the yeast in a refrigerated and sealed container.

Further Questions

This experiment raises two main questions. The first question is “Does the concentration of oxygen have an effect on the rate at which yeast produces ethanol?”. This is an important question because during my research I found that yeast does not purely use aerobic respiration even under aerobic conditions. The cells use both cellular respiration and fermentation which produces ethanol. So, the question is if a higher oxygen concentration will make the yeast undergo less fermentation and therefore produce less ethanol. The second question is “Does the concentration of oxygen have an effect on the viability of yeast cells after an extended period of time?”. This question arises because during my research I found that while yeast can survive in certain conditions it is not always preferable and many of the cells can die. This made me wonder if certain levels of oxygen allowed yeast to survive for some time but eventually began killing the cells. This proposes my second questions and also questions if there is an optimal concentration which the second extension would find.

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