Exercise 4 - Biology 105

Respiration

Purpose:
- Introduce concepts of respiration
- Reinforce planning of controlled experiments
- Reinforce statistical analysis, graphing, and interpretation and discussion.
- Reinforce use of measurements

This lab begins a two week sequence:
1. This week we will ask a question about respiration. We will design an experiment to explore that question and learn how to use an apparatus for measuring the process. We will collect some data and analyze it.
2. Next week you will work in groups to ask a follow-up question about respiration that can be answered with the same apparatus. Your group will design and carry out the experiment.

CELLULAR RESPIRATION[1]

Cellular respiration is not breathing, but rather a process by which a cell transfers energy from food in order to run its life processes, such as reproduction, growth, and development. Just as you burn fuel in your car to turn its wheels and produce waste (exhaust) in the process, so yeast cells burn fuel to release energy for running its machinery, producing waste in the process. The fuel in cellular respiration is glucose. The yeast we will be using is brewer's yeast (***Saccharomyces cerevisiae***), a single-celled fungus.

If yeast cells are given a source of sugar (fuel) in an anaerobic (oxygen-lacking) environment, the cells' waste products will be ethyl alcohol and carbon dioxide.

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow 2\ \underset{\text{ethyl alcohol}}{C_2H_5OH} + 2\ \underset{\text{carbon dioxide}}{CO_2} + \text{energy trapped in ATP + Heat}$$

Reaction 1

This process of **alcoholic fermentation**[1] has been used by humans for millennia to produce a wide variety of intoxicating beverages. But the yeast, of course, does this process, not to produce ethyl alcohol, but to transfer energy to ATPs (a chemical containing energy readily available to the cell). The alcohol would kill the yeast if it did not get diluted in the water around the yeast cells.

We also use yeast in this fashion to cause bread and other baked goods to rise before baking. We grow yeast in the dough, and as these cells respire in order to make ATPs, the sticky dough traps their CO_2 waste product and the dough rises.

If yeast cells are given sugar in an aerobic (oxygen-containing) environment, the yeast cells change their means of respiration somewhat and use a more efficient process involving **aerobic respiration**[1], which releases carbon dioxide as waste, but not alcohol.

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 6\ \underset{\text{oxygen gas}}{O_2} \longrightarrow 6\ \underset{\text{carbon dioxide}}{CO_2} + 6\ \underset{\text{water}}{H_2O} + \text{energy trapped in ATP + Heat}$$

Reaction 2

[1] "Cellular respiration" is generally synonymous with aerobic respiration, but here we use it (or just respiration) as a general term for any process that captures ATP by breaking molecules apart. Thus, "alcoholic fermentation" and "aerobic respiration" become kinds of cellular respiration.

I. Introduction

A. The Problem

For centuries, people have been harvesting ripe grapes, smashing them and letting the juice ferment into wine. People did not know exactly what was happening at the molecular level, but they knew that the juice started out sweet then changed to alcohol and a lot of gas (carbon dioxide). If the gas is not allowed to escape, it makes the bubbles of champagne.

Grape juice (sweet) → alcohol (not sweet) + gas

Reaction 3

Occasionally, the wine did not ferment properly and the wine producers ended up with vinegar or something even worse. Getting a batch of vinegar (or worse) instead of a batch of wine was a costly problem.

In 1856, some French wine producers asked Louis Pasteur, a young chemist at a nearby university, to help them with this problem. Microscopes had been invented in the 1600s, so Pasteur took samples of grape juice that had properly fermented into wine and looked at them under the microscope. He found cells of yeast. But in the vats that had turned to vinegar he always found something else. He always found cells of bacteria, as well as yeast.

That finding raised an hypothesis in Pasteur's mind about what exactly was causing fermentation to occur.

B. Alternative hypotheses

Pasteur's hypothesis was that a particular species of yeast (which we now call 'brewer's yeast) was causing fermentation. That is, the brewer's yeast was somehow turning sugar into alcohol and gas, and not the sour acids that are in vinegar. It was an outrageous hypothesis for its time. People thought cells were really simple--not much more than crystals. Looking at yeast with the microscopes you don't see anything inside. So people were skeptical of Pasteur's hypothesis. He had to test his idea and try to <u>persuade</u> people that he was right. He had to collect evidence that supported his hypothesis.

What was Pasteur's hypothesis?

_____

_____

What are other possible hypotheses explaining the change of the sugary mixture to alcohol and gas?

_____

_____

_____

_____

_____

_____

II. Methods

We can study this question with a controlled experiment. A controlled experiment manipulates and simplifies nature to make it easier to isolate the effects of one factor at a time.

A. Independent variable

What is the independent variable? That is, what is the thing whose effect we want to see? (Recheck Pasteur's hypothesis)

_____

B. Dependent variable:

The dependent variable is what we are going to measure. Our problem is we can't SEE respiration happening inside the living yeast cells! If we can't see it, how can we convince the skeptics that something invisible is happening there? The answer is actually simple. If fermentation is really happening the way Pasteur suspected, we should be able to measure the EFFECTS of the process. This is called an indirect measurement.

Look at Reaction 1. What kinds of things could we measure? HINT: As the reaction occurs, what is being used up and what is being produced?

_____

_____

_____

_____

Indirect measurement is used a lot in science. We can't directly see many of the things we want to study, like molecules or chemical reactions. So we have to decide if they are there by saying what effects they should have that we are able to measure. If we find those predicted effects, perhaps we understand what is happening.

In this case, the easiest dependent variable to measure accurately is the amount of carbon dioxide produced. No chemical test is needed. Here's how to do it, using a device you will construct called a respirometer.

To prepare a respirometer. (Do not put these together until you have filled out 'Methods II: A - I.' The description is given here to aid you in addressing questions posed in II: C - I.)

1. Fill a small glass test tube to the brim with fluid. Practice this first with plain water, but in the experiment you will use combinations of sugar solution and yeast suspension as described later.

2. Carefully place the open end of a larger test tube over the small test tube, and using the eraser end of a pencil, push the small test tube to the top of the larger one until the two are flush against one another (see Figure 1A).

3. Holding the pencil firmly against the small test tube, quickly invert them (Figure 1B). If done correctly only a few drops of liquid will have escaped from the small test tube. There will be an air bubble in the top of the respirometer as shown in Fig 1B. Obviously, at this stage it's not a product of fermentation. So when you set up your respirometers, it's important to record the size of any bubble at the beginning. If an air bubble is more than 4 mm long, try again.

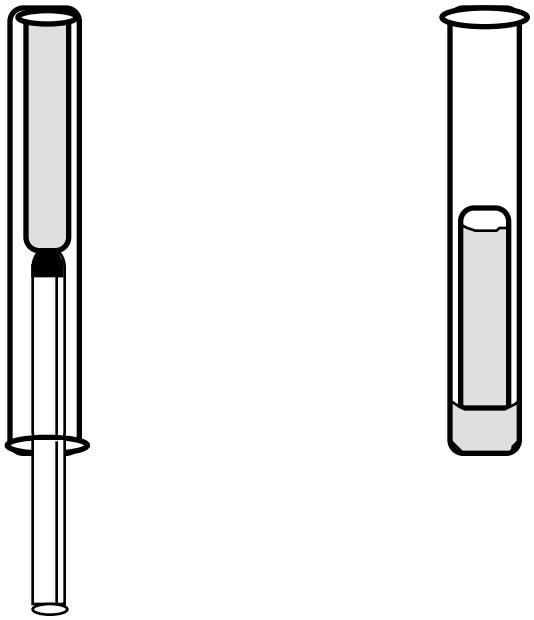

Figure 1. Preparation of respirometers.

4. We will let the yeast incubate 10 to 20 minutes. If yeast are producing carbon dioxide, what should happen to the bubble?

_____

5. So our dependent variable is the change in bubble length, that is, the difference in bubble length before and after incubating the respirometers.. As a group or as a class decide on exactly how you will all measure bubble length--from where to where exactly.

_____

_____

C. Experimental Treatment

Now that we know our hypotheses and apparatus, we have to decide what to put into the small tubes of the respirometers in order to test the hypotheses.

The experimental treatment tubes will represent the highly simplified version of the wine-making vats. Instead of sweet grapes, we will use a 1% sucrose solution (1g of sucrose filled to 100 ml of distilled water). Instead of the yeast on the surface of the grape, we will use a different species, commercially prepared baker's yeast (2 g of dried yeast in 40ml of distilled water). This is a suspension, not a solution, because the yeast does not dissolve; it will settle to the bottom. So you will have to stir well before putting yeast into each respirometer.

For your experimental respirometer, take the small test tube and:
• fill it half full of 1% sucrose solution (already made up) , then
• fill the small tube with well stirred yeast suspension.
• incubate in a warm water bath at body temperature (37°C) for about 20 minutes.

If Pasteur's hypothesis is right, what should happen?

D. Control treatments
But if the bubble in the experimental treatment tube increases, we can't automatically conclude yeast broke down the sugar to produce the gas.

1. How can we know that the yeast alone won't produce gas? What should we put in a control respirometer to see if yeast doesn't need sugar to produce the gas. Note: You have to keep the amount of yeast the same as in the experimental respirometer.

2. How do we know that yeast is really necessary? Perhaps the sugar solution alone or some other organism in the water is using sugar and producing gas. What should we put in another control respirometer to see if the yeast is necessary to produce gas? Note: you have to keep the amount of sugar the same as in the experimental respirometer.

E. Controlled variables: What other factors besides amount of yeast and amount of sugar need to be kept the same for all 3 treatments ?

F. Replicates.
If we have a sample size of just one, the results in that single sample may not be typical. So scientists always replicate experiments. They do the experiment multiple times to see if they get similar results each time.

In this class, each group will set up 1 experimental and 2 control tubes. If there are 6 groups in the class, that will give us a sample size of 6 for each treatment. We will enter the data into a spreadsheet to calculate averages and statistical comparisons of the treatments.

G. Protocol
A protocol is the series of steps you will follow in setting up and carrying out your study. Write out those steps. Explain how you will keep constant variables the same for all 3 respirometers. Specify who in the group will do what.

H. Predictions.
Now that we know the alternative hypotheses and methods, let's make sure that our methods can produce distinct results for each hypothesis. Predictions are not saying what we think will happen. Predictions are deductions about what results we should see IF each hypothesis is correct. To have a good test, the prediction from each hypothesis must be different.

What was Pasteur's hypothesis? _____
If that hypothesis is correct, how should the change in length of the bubbles compare in the experimental and control respirometers?

Experimental (Yeast + sucrose solution) _____

Control 1 (Yeast + water) _____

Control 2 (Sucrose solution + water) _____

What was another hypothesis? _____
If that hypothesis is correct, how should the change in length of the bubbles compare ?

Experimental (Yeast + sucrose solution) _____

Control 1 (Yeast + water) _____

Control 2 (Sucrose solution + water) _____

What was another hypothesis? _____
If that hypothesis is correct, how should the change in length of the bubbles compare ?

Experimental (Yeast + sucrose solution)_____

Control 1 (Yeast + water) _____

Control 2 (Sucrose solution + water)_____

I. Assumptions. It is difficult to test everything in any experiment, so each experiment usually begins with a scientist making some assumptions. It's normal to make assumptions, but it's important to know what one's assumptions are! Some of the assumptions made in today's experiment are:

1. The gas producing the bubble increase is carbon dioxide.

2. That our artificial system resembles what goes on in nature -- an assumption of all experiments.

3. That our experimental tubes are the same size as our control tubes.

List others.

_____

_____

_____

_____

III. Results

Enter your data in the class data table. Copy the completed class data table.

Enter the data in the margin of error template available in class or on the Biology Department's statistics web site (www.radford.edu/~biol-web/stats.html). Go to the section on 'Standard Error Bars.' Study the example for entering data in the spreadsheet template. Open the template and enter your data as instructed.

Graph your results. On the same graph, include for each treatment a) the mean change in bubble length, and b) the margin of error.

Describe how the means compare. Which kind of tube has the largest mean increase in bubble size. ? How did this compare to the other tubes ?

Look at the margins of error of each mean. Do the margins of error for one kind of tube overlap those of the other kinds?

IV. Conclusions and Discussion

Here you say if the results support the hypothesis, the alternative hypothesis or neither. First you need to use the margin of error statistic to conclude if there is a significant difference among the treatments. Return to the Biology Statistics site and read the page on "How to Use and Interpret the Margin of Error Statistic." Study the example and apply the same logic and language to your results. Tell which treatments were significantly different. Compare those significant differences to what your earlier predictions and explain what hypothesis, if any, was supported.

Often, we can see new, unanticipated discoveries in our results, which should be discussed. What results did we get that were not part of your predictions? What might be possible explanations?

The results and conclusions of any scientific paper may or may not be right. The work needs to be repeated, often with improved methods, to see if we get consistent results.
if repeating this experiment, how could it be done more carefully or accurately? What are problems to avoid?

The results of one scientific study are just a small step in a long process of investigation. One study generates new, related questions to explore. Next week, different groups will explore their own follow-up questions from this experiment. List as many questions as you can that could use respirometers to explore further the process of fermentation and what affects it.