PRE-LAB FOR YEAST RESPIRATION AND FERMENTATION

PURPOSE:
To identify the products of yeast cultures grown under aerobic and anaerobic conditions

STUDENTS' ENTERING COMPETENCIES:
Before doing this lab, students should understand:

Safety
The term: Cellular Respiration
The products of yeast cultures grown under aerobic and anaerobic conditions
Differences between cellular respiration and fermentation
Distillation
Yeast cultures
Principles of Gas Chromatography
How to use an analytical balance
Indicators
How to use a centrifuge

TOPS YEAST EXPERIMENT EXTENSION ACTIVITIES

1. Yeast/H₂O₂ with watch glass and glowing splint
2. Bread/pretzel making
3. Taste test: matzo bread
4. Yeast and sugar in Erlenmeyer flask will blow up balloon over mouth of flask
5. Yeast reproduction: budding
6. Use straw to expire into bromthymol blue
7. Indicators and pH
8. Use centrifuge to separate oil, water, and food coloring
9. Use microscope to observe/identify yeast cells
10. Graphing activity: put 5 mL of EtOH and 3 mL of H₂O in a 25 mm diameter test tube. Place a #4, two hole rubber stopper in the test tube with a thermometer in one hole and a glass bend in the other. Slowly boil. Record temperature at 3 seconds intervals. Graphing of data will display plateaus of boiling points of liquids.

REAL LIFE EXAMPLES

1. STEROID TESTING: - Ben Johnson, a member of the Canadian Olympic track team, was disqualified based on positive test results for anabolic steroid use as concluded by the use of gas chromatography.
2. CRIME LAB - GC is often used to test for drug and alcohol content in the bloodstream.

   - The World Trade Center Bombing: GC was used to identify compounds found at the bomb site. These findings were consistent with compounds found in the terrorists' underground lab.

3. DRUG THERAPY - GC is used to analyze blood content of chemicals used in chemotherapy.

4. METEORS - When meteors enter our atmosphere, scientists are able to isolate the chemical components in the gas pockets of the meteor using GC.

SAFETY CONCERNS/LAB TECHNIQUES

1. A cold hot plate looks just like a hot hot plate. Be careful.
2. Be careful of the distillation tube because it also gets hot.
3. It is important to secure both the flask and the thermometer to the ring stand with clamps.
4. Place your thermometer properly.

5. Verify the yeast sample you are going to use (aerobic or anaerobic) and write it in your lab book.
6. Weigh your centrifuge tube with the cap on prior to adding the yeast sample. Do not forget to write down the mass in your lab book. You will need this number to calculate the mass of the yeast cells that remain.
7. Be sure to stir yeast well prior to pouring into flask or centrifuge tube.
8. Label your centrifuge tube before placing in the centrifuge so you can find it when it is finished.
9. Be sure you have screwed the centrifuge cap on firmly.
10. All centrifuge tubes must be balanced. To balance your tube, place a classmate's tube, of the same volume, opposite yours.
11. Open the centrifuge only after it has stopped.
12. Do not stop the centrifuge with your fingers.
13. Decant as much water as possible before weighing the tube. You want to know the mass of the yeast, not the water.

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**PRELAB ACTIVITY**

Answer the following questions based on the information in your textbook, the lab handouts you have been given and classroom discussions of glycolysis, fermentation, and aerobic respiration.

1. An example of an organism that goes through fermentation.

2. Where in your body might you find fermentation taking place?

3. What commercial products take advantage of this naturally occurring process. Give three examples.

4. Which process is more efficient at converting carbohydrates (ex. glucose) into energy (i.e ATP)? Explain briefly.
5. If you were an anaerobic organism, would you prefer to live in a sealed flask or in one with aeration? Why?

Why would the opposite environment be of no benefit to you?

6. What is a gaseous by-product of the type of fermentation we will be doing in this lab? ________________
   In champagne, what evidence is there of this gas?

PROCEDURE QUESTIONS:

7. There are three large flasks that will be prepared in advance. They are labeled A, B, C. What is inside each? What preparations were made to each one?

A. ____________________ B. ____________________ C. ____________________
   ____________________ ____________________ ____________________
   ____________________ ____________________ ____________________

8. What temperature should you heat the culture to before turning off the hot plate? _________
   What temperature is too high? ____________

9. Why should the temperature of the culture stay away from 90°C and above? What happens at these temperatures?
10. Based on your vast sum of stored knowledge, which flask (A, B, or C) would you expect to give off more alcohol (as shown by gas chromatograph)? Why? Why not for the other two flasks?

Yeast Fermentation Minilabs
DIRECTIONS

1. Bromthymol blue, oxygen, and carbon dioxide.
What causes the change in a bromthymol blue solution? Design and carry out an experiment to determine the color change and its cause.

Materials that can be used are straws, flasks, water, yeast culture, aquarium air bubbler, weak acid solution (HCl, hydrochloric acid), and weak base solution (sodium bicarbonate, NaHCO₃).

2. Yeast culture and gas formation.
What gas is being formed in an active yeast culture? Design an experiment to determine the nature of the gas.

Materials: (see above).

3. Yeast Cells
What do yeast cells look like and how big are they? Design an experiment to illustrate the shape and size of yeast cells.

Materials: Yeast culture, pipet, microscope, microscope slide, coverslip, various vital stains.

4. Build models of organic molecules involved in Fermentation and Glycolysis. Submit the model and an accurate drawing to get credit.

5. Research the question - "Why Beer is Bubbly". Write down 5 facts you learn from this research.
Gas Chromatograph

The GC is a machine that will help us to separate compounds found in the samples A, B, and C. Gas chromatography is similar to the TLC we have used in the past. This time, however, we are going to use the gas phase of the sample. When looking at the graph you receive, you should notice the retention time. This tells how fast each substance moved through the machine. For example, water is a very small molecule and moves very quickly through the machine. The larger the molecule, the slower it moves through the machine. Also, the graph will show the relative amounts of substances. The larger the area under the curve, the more substance there is.

In your samples, you will hopefully see a difference between samples A, B, and C. In sample A, oxygen was added to the flask. Cellular respiration was predominantly performed by the yeast. In the presence of oxygen, aerobic cellular respiration will occur. The products are carbon dioxide and water along with energy in the form of ATP. However, in some areas of the flask, (as well as in our own bodies), oxygen may not have been available in the quantities necessary to perform cellular respiration. So fermentation could occur, but not in large quantities.

Without oxygen present, fermentation occurs. The product of fermentation is a smaller amount of ATP and alcohol (ethanol is a form of alcohol). You can see the products in the graph of sample B. Sample B was not given oxygen (anaerobic) and therefore performed fermentation.

In sample C, no yeast was added. This is the control of the experiment. Neither cellular respiration nor fermentation occurred because there was no organism to perform these processes.

You should be able to determine the substances that made up your sample compound using the retention times of each. You must use the known standards to compare your results to identify the specific substances.
Pre-Lab Questions: Yeast Respiration & Fermentation

1. What is cellular respiration?

2. What are the two stages of cellular respiration?

3. What is fermentation?

4. What is ATP? Why is ATP important?

5. How do the products differ between respiration and fermentation?

6. In which situation is air present - during respiration or fermentation?

7. How many stages are there in fermentation? Which stage that is present in cellular respiration is not present in fermentation?

8. In which process, respiration or fermentation, are carbohydrates not completely broken down? How does this affect the amount of ATP released?

9. Why is fermentation important for yeast?
10. What is gas chromatography used for?

11. What does "elute" mean? What might affect elution rates?

12. How can we use gas chromatography to identify substances found in a mixture?

13. What is the purpose of this lab?

14. What is the difference between cultures A, B, C? (We will discuss this.)

15. What temperature should be maintained in order to get the best results?

16. How are you going to maintain the temperature? (We will discuss.)

17. What should be done when the water reaches a temperature of 70 C? Why?

18. Why is it important not to let water drip from the paper towel in the collection vial?

19. What is a distillate?