Aerobic and Anaerobic Respiration

a) Introduction

Cellular respiration refers to the process of cells converting the chemical energy stored in organic molecules (such as glucose) into ATP, which is the high energy molecule that is used by proteins inside cells. The largest amount of ATP energy is obtained from glucose when glucose is oxidized completely in the presence of oxygen:

$$C_6H_{12}O_6 + 6O_2 \text{ --------------> } 6CO_2 + 6H_2O$$

This reaction is called cellular aerobic respiration, and it provides cells with 32 ATP per molecule of glucose.

In human beings, our cells primarily utilize cellular aerobic respiration to meet their energy requirements when we are resting. When beginning moderate exercise, however, our muscle cells switch to using a mixture of aerobic and anaerobic respiration (see below for details about anaerobic respiration). If the moderate exercise lasts more than a minute or two, our muscle cells return to using primarily cellular aerobic respiration, but at a much higher rate than under resting condition.

Yeast are unicellular eukaryotic organisms in the same kingdom as mushrooms. They are able to utilize many types of sugar (but not all types) as energy sources in order to make ATP. Why can't yeast use all types of sugars as energy sources? In order for any organism to make use of a potential source of food, it must be capable of transporting the molecules of that food into its cells. This requires membrane transport proteins, and each membrane transport protein is very specific for binding to and transporting just one particular type of molecule. The yeast therefore may simply lack the correct membrane transport protein to import a particular type of sugar across their cell membrane. Furthermore, an organism's cells must also have the proper enzyme proteins capable of breaking the food's chemical bonds in a useful way. Just like transport proteins, each enzyme has an active site specific for just one particular molecule. The yeast may therefore simply lack the correct enzymes necessary to break down a particular sugar type.

For which types of sugars do yeast have the proper membrane transport proteins and enzymes? You will investigate this question in activity C, but here are some clues. We expect yeast to have membrane transport proteins and enzymes for sugars that they often encounter in natural environments. Fruit are a major source of sugars in natural environments. The exact amounts and types of sugars vary from fruit to fruit, but in general, the three most abundant sugars in fruits are the two monosaccharides glucose and fructose, and the disaccharide sucrose (which is composed of one glucose linked to one fructose). In contrast, there is no lactose in fruit. Lactose (which is a disaccharide made of one glucose linked to one galactose) is only found in milk, which is not readily available to yeast in natural environments.

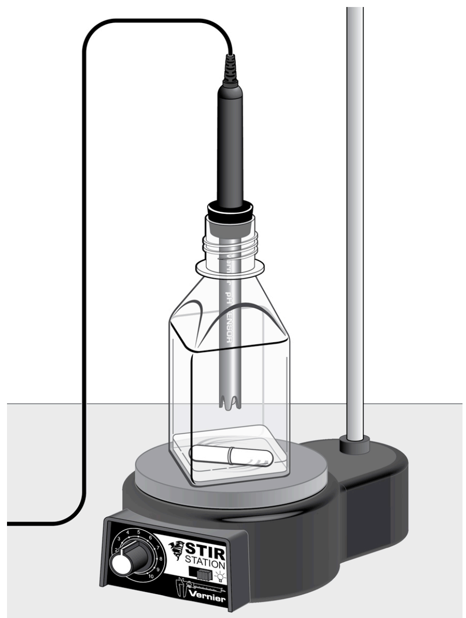
Yeast can metabolize sugar in two ways, *aerobically,* with the aid of oxygen, or *anaerobically*, without oxygen. When yeast metabolizes a sugar under anaerobic conditions, ethanol (CH_3CH_2OH) and carbon dioxide (CO_2) gas are produced. This process is called ethanol fermentation. An equation for the fermentation of the simple sugar glucose ($C_6H_{12}O_6$) is:

$$C_6H_{12}O_6 \text{ --------------> } 2C_2H_6O + 2CO_2$$

The above reaction is an example of cellular anaerobic respiration, and it provides cells with 2 ATP per molecule of glucose. When humans do anaerobic respiration, the glucose is split into two molecules of lactic acid ($C_3H_6O_3$) instead of alcohol and CO_2.

If sugars are readily available, baker's yeast (*Saccharomyces cerevisiae*) prefers to metabolize glucose and other sugars anaerobically, through fermentation, even when there is sufficient oxygen present to perform aerobic respiration (this preference is called the Crabtree effect). The Crabtree effect is somewhat surprising because the yeast obtain much fewer ATP per glucose.

The anaerobic metabolic activity of yeast can be determined by the measuring the yeast's rate of ethanol production using an ethanol sensor inside a fermentation vessel, as is shown on the next page.



Today’s lab has two activities: (1) Comparing the resting and exercising rates of cellular aerobic respiration in a volunteer from your group, and (2) Comparing the rates of yeast anaerobic respiration of a variety of sugars.

b) Aerobic respiration in human beings experimental procedure

1) Obtain the following materials: A plastic bottle and a straw.

2) Add 30 ml of water from the flask on the front desk to your plastic bottle.

3) Add 1 drop of phenolphthalein pH indicator to the water in your bottle. Lastly, add 30 drops of 1M NaOH (a strong base) to the water in your bottle. The NaOH should cause the indicator to become pink. The pink color of the indicates that the solution is basic.

4) Select a volunteer from your group. The volunteer should be someone who is physically fit. The volunteer should be seated comfortably in a chair at a lab bench.

5) Using a timer or the wall clock, start timing from the moment that the volunteer begins to blow bubbles through the water using the straw. The volunteer should keep blowing until the solution becomes clear (all the pink color should be completely gone. It helps to hold the bottle over white paper to make sure all the pink is gone). Stop timing when the solution is clear.

The change in solution color from pink to clear indicates that the NaOH base in the solution has been neutralized. The NaOH in the solution was neutralized by H^+ that were generated from the CO_2 in the volunteer's breath reacting with water molecules in the solution. The chemical equation for the production of H^+ from the reaction of CO_2 with water is this:

$$CO_2 \quad + \quad H_2O \quad \text{--------->} \quad H^+ \quad + \quad HCO_3^-$$

CO_2 in breath comes primarily from aerobic respiration occurring the cells. Therefore the time it takes the volunteer's breath to fully neutralize the NaOH in the solution can be used as a rough estimation for the rate of aerobic respiration in the volunteer's cells.

6) In the results table of this handout, record the time that it took the resting volunteer to neutralize the NaOH in the solution.

7) Wash out the bottle with water from the sink and dry it.

8) Repeat steps 2 – 5. The only change is that that the volunteer should now do the bubble blowing *while walking up and down the stairs*. Start the timer when the volunteer begins to walk up the stairs while blowing bubbles. Stop the timer when the solution becomes completely clear.

9) In the results table of this handout, record the time that it took the exercising volunteer to neutralize the NaOH in the solution.

10) Wash out the bottle with water from the sink and dry it. Return it to where you obtained it.

c) Anaerobic respiration in yeast experimental procedure

1) Obtain the following materials: A laptop computer, an ethanol sensor, an interface box with cables, a fermentation flask with a split rubber stopper, a green pipette device, a magnetic stir bar, and a magnetic stir plate. The computer user name is ChemBio. The password is SC13NC3.

2) Set up the ethanol sensor for data collection.

   a) Connect the ethanol sensor to the data-collection interface.

   b) Start Logger *Pro* and choose New from the File menu.

   c) Find a screen button with a tiny clock icon. Click this then set the duration to 10 minutes and the rate to 10 samples/minute.

   d) Find a screen button with a tiny A icon. This is the autoscale feature. Click this just after you begin each experimental run to make the Y-axis size proportional to your result values.

3) Bring your group's fermentation flask and green pipette device to the front desk, where you will find a yeast culture suspension stirring on a magnetic stir plate. Pipet 5.0 ml of the yeast suspension into your fermentation flask. **Important**: The yeast suspension must be removed from the middle of a yeast source that is being stirred by the magnetic stirrer at a constant stirring speed.

5) Add 5.0 ml of the glucose solution to your fermentation flask then return to your desk.

6) Add a stir bar to the fermentation flask. Place the fermentation flask on your stir plate at slow speed. Smooth steady slow stirring works best. Once the stir bar is spinning, find the slowest speed that keeps it stirring.

7) Quickly place the ethanol sensor into the fermentation chamber, twisting the stopper snugly into the mouth of the chamber. If the sensor does not make an airtight seal alcohol vapor will escape, giving a falsely low rate of respiration.

The tip of the ethanol sensor should be in the air above the sample. **Important:** The ethanol sensor is a gas sensor–do **not** submerge the sensor into the liquid.

8) Begin measuring ethanol concentration by clicking ▶ Collect. Data will be collected for 10 minutes. Click the Autoscale button (the button with the A) as soon as you start each experimental run.

9) When data collection has finished, determine the rate of fermentation:

a) Using the mouse, select the final 3 minutes of the run, where the ethanol concentration was smoothly increasing in a straight line. Do not select the start of the run where the ethanol concentration slope was changing rapidly.

b) Click the Linear Fit button, , which has a tint R on it. A floating box will appear with the formula for a best fit line. This is the slope of the curve, which represents the rate of ethanol production by the yeast.

c) Record the slope of the line, *m*, as the fermentation rate in the data table.

d) Close the best fit floating box.

e) Show your instructor your first data before continuing.

10) Prepare the flask for testing the next sugar as follows: Remove the ethanol sensor from the fermentation chamber. Set the sensor aside and allow it to equilibrate in room air for 5 minutes. Fill the fermentation chamber with tap water and empty it into the sink. Repeat this rinsing process two more times. Make sure that all yeast have been removed. Thoroughly dry the inside of the chamber with a paper towel.

11) Repeat Steps 4–10 using sucrose, lactose, and distilled water (the negative control).

d) Data Tables

(1) Aerobic respiration in the volunteer

Time for the resting volunteer to neutralize the NaOH solution: ______________

Time for the exercising volunteer to neutralize the NaOH solution: ______________

Based on these results, roughly how many times higher was the volunteer's rate of cellular aerobic respiration during exercise, compared to their rate of cellular aerobic respiration during rest? ___________

(2) Anaerobic respiration in yeast

Sugar:	Rate of fermentation	Class average
Glucose	______________	___________
Sucrose	______________	___________
Lactose	______________	___________
Water	______________	___________

e) Review questions A

1) Write the full and balanced chemical equation for cellular aerobic respiration.

2) Write the full and balanced chemical equation for cellular anaerobic respiration in yeast. Also write the equation for cellular anaerobic respiration in in humans.

3) The cells perform cellular respiration to replenish their _______ (a molecule) levels.

4) Bicarbonate ion (HCO_3^-) and hydrogen ion (H+) are made when ______ reacts with water.

   A) oxygen
   B) hydrogen
   C) carbon dioxide
   D) carbon monoxide

5) Write out the full and balanced chemical equation for the production of H^+ in aqueous solution when CO_2 enters the solution.

6) A person is resting, then the person gets up and begins to exercise. As they start to exercise, the amount of H^+ in their blood will increase/decrease/not change (←circle one of the three answers). Justify the answer you circled.

7) Considering the results of yeast part of today's experiment, do yeast equally utilize all sugars as energy sources? Explain.

8) Name two types of types of proteins (discussed in the prelab lecture) in yeast cells that can affect the yeast's respiration rate for a given sugar.

9) Write the complete molecular formula for glucose monosaccharide.

10) What two monosaccharides make up a sucrose disaccharide molecule?

11) What two monosaccharides make up a lactose disaccharide molecule?

12) What is the Crabtree effect?

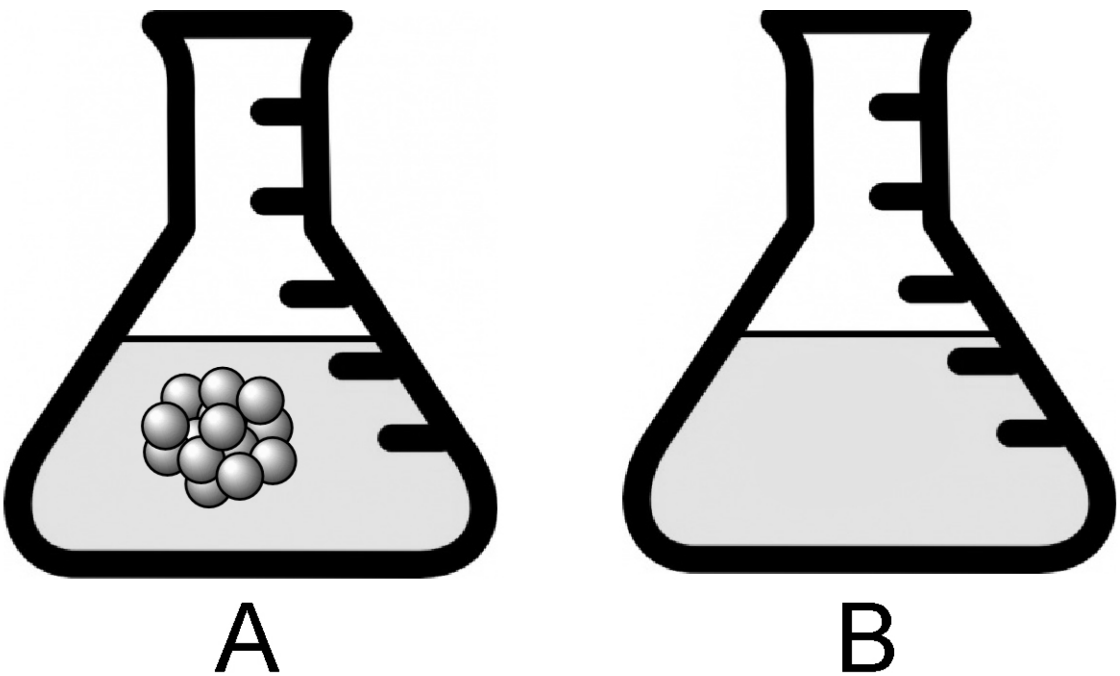
13) Name the molecule in the breath that is responsible for the pH change when your group's volunteer breathed into water: __________. Write the chemical equation that shows how this molecule in the breath leads to a pH change in the water.

f) Review questions B

14) The Crabtree effect at first seems disadvantageous to the yeast. This is because, even in the presence of oxygen, the yeast only obtain ____ (a number) of ATP from each glucose molecule. If the yeast used the available oxygen to perform aerobic respiration, the yeast would obtain _____ (a number) of ATPs from each glucose molecule.

15) Some evolutionary biologists have theorized that the Crabtree effect offers an advantage to yeast that outweighs the disadvantage of lower ATP production. The hypothetical advantage is related to their production of ethanol (but *not* to the use of ethanol in alcoholic beverages by human beings). Speculate why it might be advantageous to the yeast to produce alcohol:

Flasks A and B contain an unbuffered solution that is pH 7.0. A sample of living tissue is placed in flask A. Flask B contains no cells (See figure below).



The cells in flask A perform cellular aerobic respiration for several minutes. Based on the concepts you learned performing the pH and respiration laboratory activity, answer the following questions.

16) The solution in flask A above will become acidic/basic/remain neutral (←circle one of the three answers).

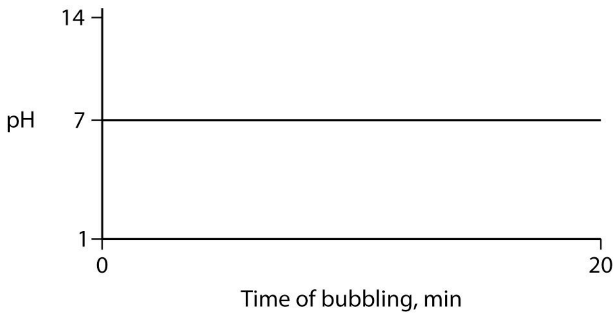
17) Justify the answer you circled in question (16)

18) The solution in flask B will become… acidic/basic/remain neutral (←circle one of the three answers).

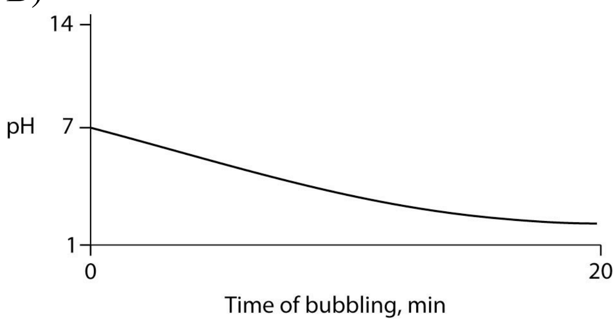
19) Justify the answer you circled in question (18)

20) All the disaccharides used in this experiment were made by a dehydration synthesis between two six-carbon monosaccharides. Write the molecular formula for a disaccharide made from two six-carbon monosaccharides:

21) If a person bubbles their breath through a straw into a glass of pure water, which of the following graphs accurately shows the effect of their breath on the water's pH?



B)



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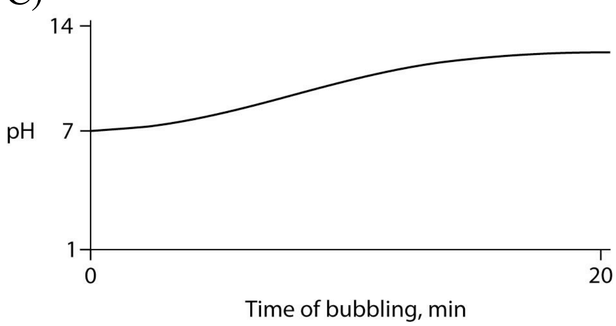

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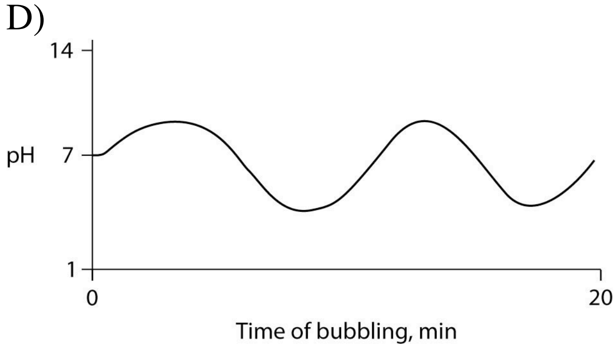

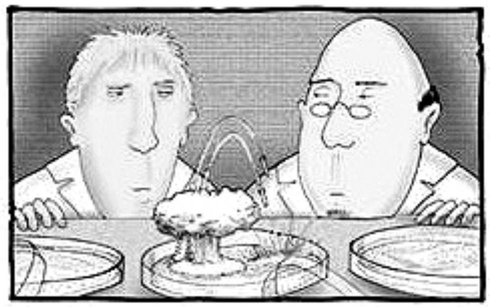

Once again, war breaks out in the middle yeast.