Lab 5. Alcoholic Fermentation in Yeast

Prelab Assignment

Before coming to lab, read carefully the introduction and the procedures of this experiment, and then answer the prelab questions at the end of this lab handout. In addition to lab 5, you will also start Lab 6! Hand in the prelab assignments for both Lab 5 *and* Lab 6 just *before* the start of your scheduled lab period.

Goals of this Lab

After completing this lab exercise you should be able to...

- Describe alcoholic fermentation and aerobic respiration, noting the reactants and products, and the relative energy efficiency of each.
- Describe the roles of the following in anaerobic and/or aerobic respiration: oxidation/reduction reactions, NAD^+, NADH, FAD, FADH, O_2, glycolysis, Kreb's cycle, Electron Transport Chain
- Use a Biology gas pressure sensor to determine which sugar, sucrose or lactose is best metabolized anaerobically by yeast.

Introduction

Anaerobic energy production in yeast will be studied in this lab investigation. In this lab you will determine which sugar, ***sucrose*** or ***lactose***, is best metabolized by yeast, while in **Part II** you will design an experiment to determine the effect ethanol has on the rate of fermentation.

Cultures around the world have for millennia used yeast fermentation to produce bread and alcoholic beverages. Yeast is able to metabolize some foods, but not others. In order for an organism to make use of a potential source of food, it must be capable of transporting the food into its cells. It must also have the proper enzymes capable of breaking the food's chemical bonds in a useful way. Sugars are vital to all living organisms. Yeast is capable of using some, but not all sugars as a food source. Yeast can metabolize sugar in two ways, ***aerobically,*** with the aid of oxygen, or ***anaerobically***, without oxygen.

Although the ***aerobic fermentation*** of sugars is energetically much more efficient, in this experiment we will set the conditions so that yeast carries out anaerobic respiration—i.e. Alcoholic fermentation. When the yeast respire glucose aerobically, oxygen gas is consumed at the same rate that CO_2 gas is produced—there would be no change in the gas pressure in the test tube. The net equation for the more than two dozen steps involved in the ***aerobic respiration*** of glucose is:

$$\underset{\text{glucose}}{C_6H_{12}O_{6\,(aq)}} + \underset{\text{oxygen}}{6\,O_{2\,(g)}} \xrightarrow{\text{Enzymes}} \underset{\text{water}}{6\,H_2O_{\,(l)}} + \underset{\text{carbon dioxide}}{6\,CO_{2\,(g)}} + \textbf{energy (36-38 ATP + Heat)}$$

When yeast ferments the sugars anaerobically, however, CO_2 production will cause a change in the pressure of a closed test tube, since no oxygen is being consumed. We can use this pressure change to monitor the rate of respiration and metabolic activity of the organism. A gas pressure sensor will be used to monitor the fermentation of sugar.

Adapted from Experiment 12B in *Biology with Computers*

The ***alcoholic fermentation*** of glucose is described by the following net equation:

$$\underset{\text{glucose}}{C_6H_{12}O_{6\,(aq)}} \xrightarrow{\text{Enzymes}} \underset{\text{ethanol}}{2\ CH_3CH_2OH_{(aq)}} + \underset{\text{carbon dioxide}}{2\ CO_{2\,(g)}} + \textbf{energy}\ \ \textbf{(2 ATP + heat)}$$

Both alcoholic fermentation and aerobic respiration are multi-step processes that involve the transfer of energy stored in the chemical bonds of glucose to bonds in ***adenosine triphosphate***, ***ATP.*** The energy stored in ATP can then be used to perform cellular work: provide energy for biosynthetic reactions (e.g. growth and repair processes, active transport, etc.). All organisms (i.e. monerans, protists, fungi, plants, and animals) utilize aerobic respiration and/or fermentation (anaerobic respiration) to produce ATP to power their cellular processes.

Note that ***ethanol*** is a by-product of alcoholic fermentation (figure 1). Ethanol, a 2-carbon alcohol, is also known as ethyl alcohol and, less correctly, simply as "alcohol". Since yeast do not have the enzymes needed to metabolize ethanol (i.e. ethanol dehydrogenase and acetaldehyde dehydrogenase), much of the energy stored in the molecules of glucose is trapped in the molecules of ethanol and is unavailable for use by yeast cells. The complete break down of glucose to carbon dioxide and water in aerobic respiration yields much more energy than does alcoholic fermentation: 36-38 ATP, versus only 2 ATP molecules produced by anaerobic respiration. Ethanol molecules produced by alcoholic fermentation diffuse from yeast cells into the surrounding aqueous environment. Since ethanol is harmful to cellular membranes yeast cells will die if ethanol concentrations reach a critical level.

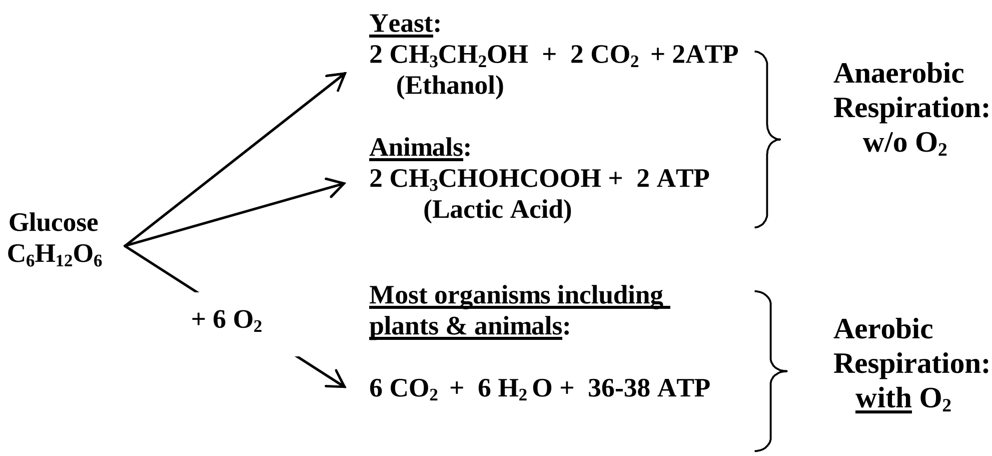


Figure 1. Summary of three of the many possible fates of the 6-carbon sugar glucose under anaerobic and aerobic conditions.

When anaerobic respiration occurs in animals (figure 1) it is known as ***lactic acid fermentation*** since lactic acid, a 3-carbon organic acid is the end product. Like alcoholic fermentation, lactic acid fermentation produces only 2 ATP, but lactic acid is the byproduct, not ethanol. Perhaps if ethanol was produced anaerobically in animals more people would take up anaerobic sports such as sprinting and weight lifting!! Since lactic acid production is toxic to cells, anaerobic respiration can only occur for short periods of time in animals. In the presence of oxygen each lactic acid molecule can be broken down to carbon dioxide and water.

Aerobic Respiration

Aerobic respiration (Figure 2 on page 4) occurs in three stages: ***glycolysis*** (involves soluble enzymes in the cytoplasm), ***Kreb's cycle*** (uses soluble enzymes in the matrix of mitochondria), and the ***electron transport chain*** (a chain of proteins found on the inner membrane of the mitochondria). Both alcoholic and lactic acid fermentation involve *only* glycolysis. Since both the Kreb's cycle and the electron transport chain require oxygen to function, neither process can occur under anaerobic conditions.

Fermentation and aerobic respiration involve ***oxidation-reduction reactions***. ***"Redox"*** reactions involve electron transfers: ***oxidation*** is the loss of electrons from a substance, while ***reduction*** is the gain of electrons. In cellular respiration, two hydrogen atoms at a time are enzymatically removed from glucose (oxidation) and transferred to the co-enzyme ***NAD*** $^+$ (Nicotinamide Adenine Dinucleotide; ***Niacin***, a common vitamin supplement, is a component of NAD), reducing it to ***NADH + H***$^+$. Since each hydrogen atom consists of one proton and one electron, think of this redox reaction involving the transfer of two electrons, one from each hydrogen atom, and one proton to NAD^+, with the second proton, H^+, released into the solution within the cell. Under aerobic conditions NADH transfers the two electrons to the electron transport chain. The transfer of electrons from one E.T.C. protein to another releases energy. Roughly 40% of this energy is used to produce ATP (usually 3 ATP per NADH), while the other 60% is released as heat. The final electron acceptor of the E.T.C. is ***oxygen***. One molecule of water is produced for every two electrons and two hydrogen ions transferred to each oxygen atom by the electron transport chain.

$$\mathbf{2\,H^+ + 2\,e^- + 1/2\,O_2 \xrightarrow{\text{E.T.C.}} H_2O}$$

Some animals (e.g. the kangaroo rat of eastern Washington) generate enough "metabolic" water by aerobic respiration so that they can live a lifetime without drinking a single drop of water!

During ***glycolysis*** (figure 2 on page 4), the 6-carbon glucose molecule is converted via a series of enzyme-controlled reactions into two molecules of ***pyruvic acid,*** a 3-carbon organic acid along with a net gain of 2 ATP and 2 NADH + H^+. In the presence of oxygen (aerobic respiration), pyruvate enters the mitochondria and another series of enzyme-mediated reactions called the ***Kreb's cycle***. The Kreb's cycle converts pyruvate molecules into carbon dioxide and water. This is accomplished by a series of reactions that generate ATP, NADH + H^+, and ***FADH₂*** (Flavine Adenine Dinucleotide; ***Vitamin B₂*** or "***riboflavin***", common vitamin supplement, is a component of FAD). FAD is a hydrogen carrier that generates 2 ATP for each pair of electrons it transfers to the E.T.C.

Anaerobic Respiration

For glycolysis to function there must be a ***continuous supply of NAD***$^+$. Under anaerobic conditions NADH is unable to release its cargo of electrons to the E.T.C. since oxygen is ***not*** available to be the final electron acceptor. To keep glycolysis operating under anaerobic conditions, animal cells regenerate NAD^+ by NADH transferring its cargo to pyruvate to produce lactic acid.

Lactic Acid Fermentation in animal cells

Glycolysis: Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 Pyruvic Acid + 2 ATP + 2 NADH + 2 H^+
Reduction of Pyruvate: 2 Pyruvic Acid + 2 NADH + 2 H^+ → 2 Lactic Acid + 2 NAD^+

Net reaction: **Glucose + 2 ADP + 2 P_i → 2 Lactic Acid + 2 ATP**

On the other hand, yeast first ***decarboxylate*** (i.e. remove CO_2) pyruvate to produce ***acetaldehyde,*** a two carbon compound. Acetaldehyde is then reduced by NADH to form ethanol.

Alcoholic Fermentation in Yeast

Glycolysis:	Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 Pyruvic Acid + 2 ATP + 2 NADH + 2 H^+
Decarboxylation of Pyruvate:	2 Pyruvic Acid → 2 Acetaldehyde + 2 CO_2
Reduction of Acetaldehyde:	2 Acetaldehyde + 2 NADH + 2 H^+ → 2 Ethanol + 2 NAD^+

Net reaction: **Glucose + 2 ADP + 2 P_i → 2 Ethanol + 2 CO_2 + 2 ATP**

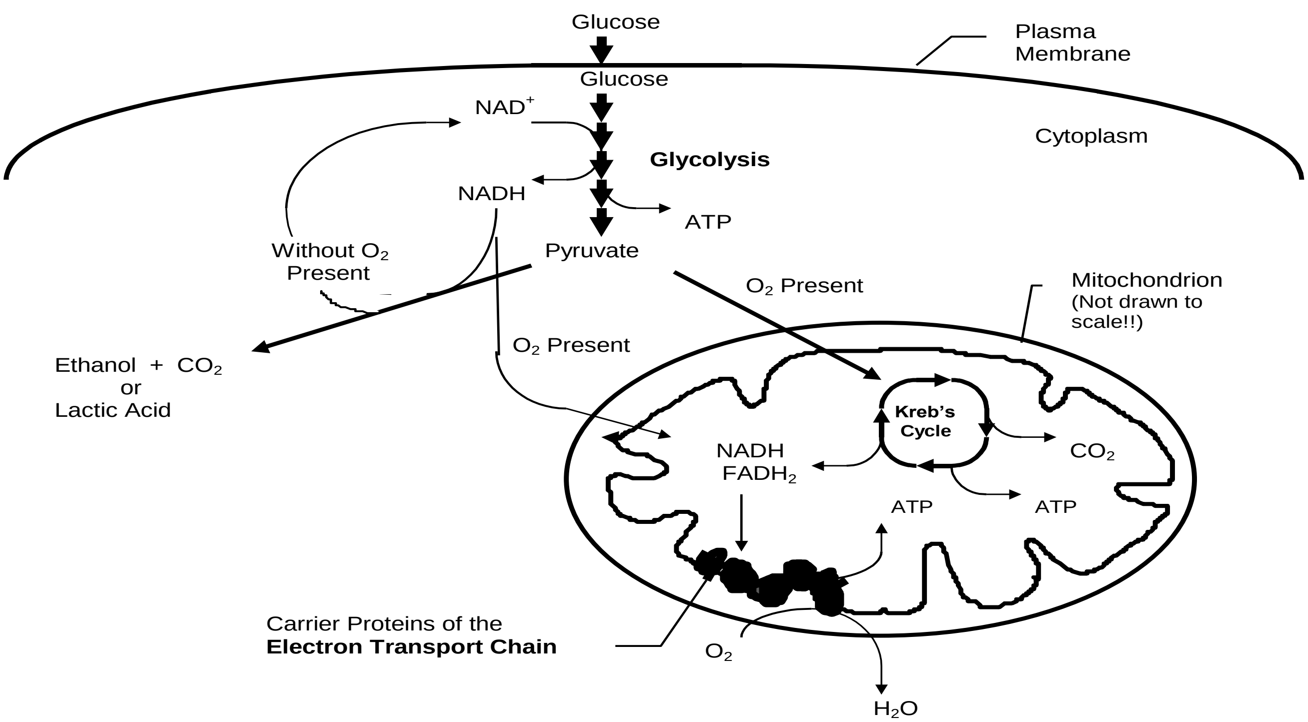


Figure 2. Aerobic cellular respiration consists of glycolysis, Kreb's cycle, and the electron transport chain. Anaerobic respiration involves only glycolysis and regenerates NAD^+ by either reducing pyruvate to produce lactic acid (animals), or by decarboxylating pyruvate to produce acetaldehyde (not shown) and then reducing acetaldehyde to produce ethanol. Under aerobic conditions the NADH produced by glycolysis enters the mitochondria of the cell where it becomes oxidized to regenerate NAD^+ by donating electrons to the electron transport chain, which results in the production of nearly 90% of the 36-38 ATP molecules produced per glucose molecule metabolized aerobically.

Sugar Fermentation in Yeast

Materials

Per Group of 2 Students:

- Computer
- Serial Box Interface
- Vernier Biology Gas Pressure Sensor
- Stopper assembly fitted with tubing
- 18 x 150 mm test tube
- 1-L beaker (for water bath)
- Basting bulb
- Thermometer
- Test tube rack

Per Class:

- Thermostatically controlled water bath (set at 37°C)
- Yeast suspension (7g baker's yeast per 100 ml of warm water)
- Dropper bottles of:
  5.0 % (w/v) Sucrose
  5.0 % (w/v) Lactose

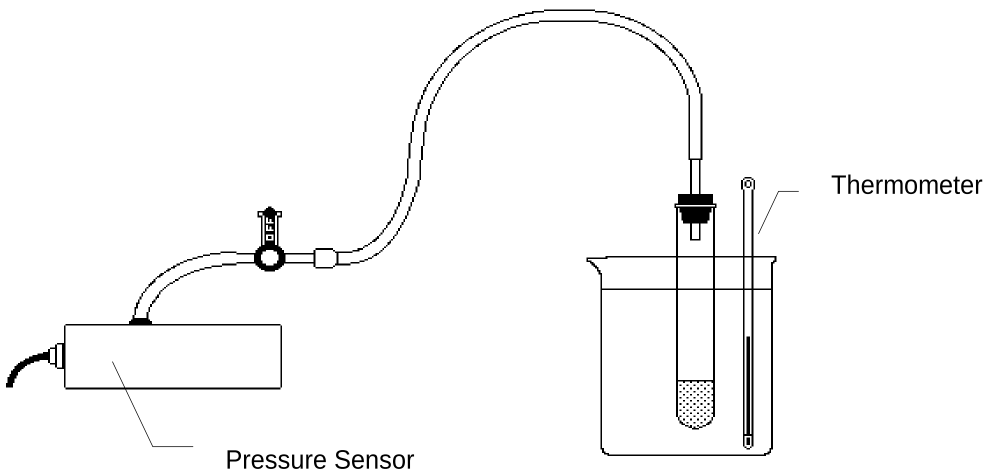


Figure 3. Experimental Set-up with reaction vessel in a water bath maintained at a constant temperature.

Important considerations:

i.) To ensure an accurate measurement of pressure during alcoholic fermentation, the test tube must be <u>*swirled at a steady rate*</u> to facilitate the liberation of CO_2 gas from the solution.

ii.) The reaction vessel should be submerged in the water bath as far as possible to keep the entire contents at the temperature of the water bath.

iii.) There is a valve attached to the rubber stopper (not shown above) that should be open while attaching the assembly to the test tube, and then closed just before the start of data collection—see figures 5 and 6.

Procedure

Preparation of the computer for Data Collection

1. Prepare the computer for data collection as follows:
   a. Connect the Gas Pressure Sensor to the Go-Link.
   b. Open the *Biology with Computers* software as follows: Go to my computer → click on classes on madrona → Science → Classes → *Logger Pro* → Experiments → Biology with Computers → EXP 12B Fermentation (Press)
   c. Verify that the vertical axis has pressure scaled from ~90 to ~130 kPa, the horizontal axis has time scaled from 0 to 15 minutes and that "Sugar Fermentation in Yeast" is the caption at the top of the chart.
   d. Check the accuracy of the pressure sensor: A pressure reading of ~ 99 to ~103 kPa should appear when the sensor is open to the atmosphere.
2. Connect the stopper assembly to the pressure sensor, making sure all connections are "finger tight" and do not leak!
3. **Adjusting the Valve to the Pressure Sensor.**
   Open the valve on the rubber stopper assembly so that it is *open to the atmosphere*. Do this by turning the handle of valve on the rubber stopper so that it is in a vertical position as in figure 6, below.

Figure 5. Open to the atmosphere. Any gas generated in the tube will be vented to the air

Figure 6. Closed to the atmosphere. Any gas generated in the tube will be contained and monitored by the pressure sensor.

Preparation of the Water Bath and Sucrose/Yeast Mixture

4. Obtain two test tubes and label them 1 and 2.
5. **Your team will first investigate the fermentation of sucrose by carrying out the following procedure, and then repeating these steps with the second sugar, lactose.**
6. Use a graduated cylinder to put 2.5 mL of 5.0% sucrose in test tube 1.
7. Obtain the yeast suspension. Gently swirl the yeast suspension to mix the yeast that has settled to the bottom. Use a graduated cylinder to put 2.5 mL of yeast into Test Tube 1. Swirl the tube to mix the yeast into the sugar solution.
8. Incubate the test tube containing the sugar/yeast mixture for 10 minutes at about 37°C in the thermostatically controlled water bath.

9. Prepare a water bath (see figure 3) for the yeast to ensure that the yeast will remain at a constant and controlled temperature when collecting data, steps 11-13. To prepare the water bath combine warm and cool water in a 1-liter beaker until it reaches 38 – 39°C. Fill the beaker with water until the beaker is almost full, but won't spill over when the test tube containing the yeast and sugar is placed in it. Make sure to keep the water temperature constant at about 37°C.

10. After 10 min. of incubation in the thermostatically controlled water bath place the rubber stopper assembly firmly into Test Tube 1. The stopper is connected to a plastic tube that goes to the pressure sensor. Check that all connections are tight and then place test tube into your water bath. Be sure to keep the temperature of the water bath constant. If you need to add more hot or cold water, first remove about as much water as you will be adding or the beaker may overflow. Use a basting bulb to remove excess water.

Collection of Data

11. **Close the system to the atmosphere**: Close the valve on the rubber stopper assembly so the carbon dioxide produced by fermentation cannot escape. Do this by turning the handle of valve on the rubber stopper assembly to a horizontal position as in figure 6. The pressure sensor will now be measuring the pressure within the test tube. **After data collection has started do not tamper with the fittings, as this will alter the pressure of the system**.

12. Begin collecting data by clicking the Start button. **Important**: Gently **swirl** the test tube continuously while collecting data (this helps to liberate the carbon dioxide gas from the solution and helps to keep the contents mixed well). **Monitor the temperature of the water bath. Be sure that it does not change by more than one degree.**

13. Collect data until you are certain that there is a linear relationship between the pressure and time. Depending on the activity of the yeast, this usually takes 1 to 3 minutes. If the pressure exceeds 130 kPa, stop the computer by clicking the Stop button. Open the air valve on the pressure sensor to avoid it popping off!

14. **Determination of the rate of fermentation.** The slope of the line on the monitor is equal to the rate of fermentation. Use the computer to calculate the slope as follows:

    a. Move the cursor to the point where the pressure values *begin* to have a linear relationship. Hold down the mouse button. Drag the cursor to the *end* of the linear section of the curve (as in fig. 7 on the next page) and release the mouse button.

    b. Click the Linear Fit button, , to perform a linear regression. A floating box will appear with the formula for a best fit line. Click on the box and set the number of decimal places to give the slope 3 significant figures.

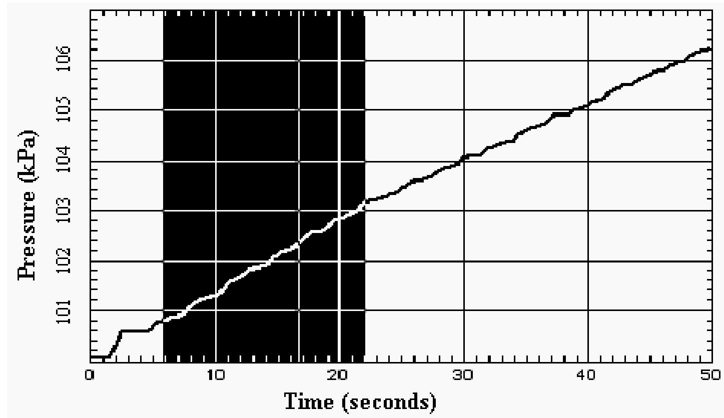
    Note: The equation for the line displayed on the monitor is **y = mx + b,** where…

    - **y** is the variable on the y-axis, pressure (in kPa)
    - **x** is the variable on the x-axis, time (in minutes)
    - **m** is the slope of the line = rate of reaction (in kPa/min)
    - **b** is the y-intercept (the pressure at t = 0; i.e. atmospheric pressure in kPa)

    c. Record to *3 significant figures* the slope of the line, *m*, as the rate of fermentation (kPa/min) in Table 1 of the report sheet.

    d. Share your group's data with the class by recording the sugar type and the rate of respiration on the class data sheet provided by your instructor.

Figure 7. Determination of the rate of fermentation from a plot of Pressure vs. Time. The data in the graph is fictitious and *not* intended to represent data obtained in this experiment!!



Fermentation of Lactose

15. Use a graduated cylinder to put 2.5 mL of 5.0% lactose in test tube 2.
16. Obtain the yeast suspension. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Put 2.5 mL of yeast into Test Tube 2. Mix the yeast into the sugar solution.
17. Repeat Steps 8 – 14 using Test Tube 2.
18. Record the data obtained by the other teams in Table 1 on the report sheet.

Control

19. Use a graduated cylinder to put 2.5 mL of DI water in test tube 3.
20. Obtain the yeast suspension. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Put 2.5 mL of yeast into Test Tube 3. Mix the yeast into the DI water.
21. Repeat Steps 8 – 14 using Test Tube 3.
22. Record the data obtained by the other teams in Table 1 on the report sheet.

Lab 5 Report Sheet
Alcoholic Fermentation in Yeast
Biol 211

Name ______________________________________
Group Number _________ **Date** ______________

Results

Table 1. Class Data: Rate of alcoholic fermentation of various sugars by yeast.

Group No.	Group Members	Rate of Alcoholic Fermentation (kPa/min.)		
		Sucrose	Lactose	Control (no sugar used)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
	Average Rate of Alcoholic Fermentation (kPa/min.)			

Analysis of Results

1. Using *Excel* or by hand, use the class data to make a graph of Average rate of alcoholic fermentation vs. sugar type. Include the control treatment on the graph. Label the graph fully (including units & correct significant figures) and *give it a proper title*.

2. Considering the results in this experiment, can yeast utilize lactose and sucrose equally well? What do the results from the control tell indicate? *Quote specific results* to support you explanations.

3. Use your knowledge of cell transport mechanisms and cellular respiration to explain the results obtained in part 1. That is, if the sugars sucrose and lactose were not metabolized equally well by yeast, then explain why.

Applying your Knowledge

4. To save time in performing this experiment no control trials were performed. If this were a novel experiment that you attempted to publish in a scientific journal, your methodology and results would be harshly criticized since the procedure did not include any control trials, and thus there is no guarantee that the yeast are responsible for the results obtained.

   Suppose an experiment is performed to compare yeast's ability to ferment glucose and pyruvate under anaerobic conditions. Four control tubes would be required. Design the four control tubes by making check marks in the appropriate columns to indicate the contents of each control tube, and note the purpose of each tube. The contents of experimental tubes: (All solutions made with DI water)

   Tube #1E: 2.5 mL yeast suspension + 2.5 mL 5% Glucose
   Tube #2E: 2.5 mL yeast suspension + 2.5 mL 5% Pyruvate

Control Tube	Contents of Control Tube				Purpose of Control Tube
	Glucose Solution	Pyruvate Solution	Yeast	DI Water	
1C					
2C					
3C					
4C					

5. What kind of sugar is sucrose? Monosaccharide, disaccharide, or polysaccharide (circle one)

6. What must happen to sucrose before it can enter glycolysis? <u>*Explain*</u>.

7. Suppose that an organism could metabolize sucrose. How many ATP molecules would be produced per sucrose molecule if it were metabolized aerobically and anaerobically? <u>*Explain your reasoning*</u>.
   a. Aerobic respiration of sucrose yields: _____ ATP

   b. Anaerobic respiration of sucrose yields: _____ ATP

8. Could a Vernier biology gas pressure sensor be used to monitor the following processes? Briefly explain why or why not.

   a. Alcoholic fermentation: Yes or No ? (Circle one)

   b. Lactic Acid Fermentation: Yes or No ? (Circle one)

9. Suppose for a prolonged period of time your diet is deficient in niacin, an essential vitamin that the human body cannot make. How would this deficiency influence each of the following. Circle the correct response and briefly explain your reasoning.

   a. Glycolysis would:
      i. increase in activity. ii. decrease in activity. iii. be unaffected.

   b. The Kreb's cycle would:
      i. increase in activity. ii. decrease in activity. iii. be unaffected.

   c. The electron transport chain would:
      i. increase in activity. ii. decrease in activity. iii. be unaffected.

Science Cartoons of Sidney Harris

available at http://www.sciencecartoonsplus.com/pages/gallery.php

"IF HE PRESSES THE FIRST LEVER, SOME FOOD COMES OUT. IF HE PRESSES THE SECOND LEVER, SOME CANDY COMES OUT. IF HE PRESSES THE THIRD LEVER A FEMALE MOUSE COMES OUT. AFTER A WEEK OF THIS HE'S LOOKING FOR ANOTHER LEVER."

"HE'S BEEN AT IT TOO LONG. NOW, WHEN THE BELL RINGS, DR. PAVLOV SALIVATES."

Lab 5 Prelab Questions
Alcoholic Fermentation in Yeast
Biol 211

Name ___________________________________
Group Number ________ **Date** ______________

Instructions: Do the Prelab reading at the beginning of this lab handout *before* attempting to answer the questions that follow! Hand in this assignment just *before* the start of your scheduled lab period.

1. Write the overall balanced chemical equation for the *aerobic respiration of glucose* by yeast, indicating the number of ATP molecules produced.

2. Write the overall balanced equation for the *anaerobic respiration* of glucose by yeast, indicating the number of ATP molecules produced.

3. Why are there different numbers of ATP produced when yeast metabolize glucose aerobically vs. anaerobically?

4. Just before fermentation begins, what is the concentration (% w/v) of sugar in the yeast/sugar mixture? Explain your reasoning. (Hint: Don't overlook the fact that the sugar solution is being diluted by the yeast suspension!)

5. Could a Vernier biology gas pressure sensor be used to monitor *aerobic* respiration in yeast? Yes or No ? (Circle one) Briefly explain why or why not