

LABORATORY 6 new

Aerobic and Anaerobic Cellular Respiration

Cells can obtain the chemical energy stored in sugars by breaking the sugar molecules apart in a series of enzyme-mediated reactions. Energy is extracted most efficiently in the presence of oxygen via the process known as **aerobic respiration**. Under conditions where oxygen is scarce or absent, some cells are still able to split glucose to obtain energy via the process of **fermentation**—but far less energy per glucose molecule is extracted, because glucose cannot be fully broken down without oxygen.

In today's lab, you will work in teams of four and cooperatively design two experiments to study anaerobic and aerobic metabolism. Although the members of a team may divide up the work of designing and performing the two experiments as they choose, each team member will be responsible for understanding and explaining both experiments (and both types of metabolism) for presentations, exams and quizzes.

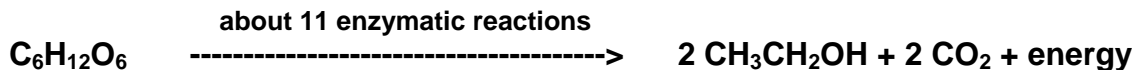
Objectives

After doing this lab, you should be able to:

- Design and perform an experiment to test the relative ability of yeast to ferment various potential nutrients.
- Design and perform an experiment to find the relative rates of respiration of various organisms under various conditions.
- Using graphing software such as Excel, graph and analyze rate curves to obtain fermentation or respiration rates, and then graph and compare these rates.
- Explain, discuss, and synthesize the results of the fermentation and respiration experiments, on both the evolutionary and molecular levels.

I. Fermentation: anaerobic metabolism

Certain organisms can produce ATP by utilizing metabolic pathways that do not require molecular oxygen. Usually, these are referred to as **fermentation** or **anaerobic glycolysis**. In general terms, these pathways involve the sequential conversion of a carbohydrate into partially oxidized end product(s). Energy is released during these reactions, and stored as the bonds of ATP. One species of free-living (*i.e.*, non-parasitic) unicellular fungus, commonly known as Brewer's yeast (*Saccharomyces cerevisiae*), can "ferment" glucose to yield ethanol and carbon dioxide:



We can estimate the rate of this pathway by measuring the yield of CO₂ over time using *fermentation tubes* (Figure 6-1). With the ability to measure the rate of fermentation, we can ask many interesting questions. For example, can yeast ferment other sugars or organic compounds, besides glucose? If so, which ones and how well? Here's where the creative process begins. Consider where yeast are found in nature. HINT: what's that "bloom" on the grape (Figure 2, and see Rossini *et al.* 1982)? How do

you suppose prehistoric people discovered fermented fruit (a.k.a. wine) and fermented grain porridge (a.k.a. beer)? See Legras *et al.* (2007) for more about the history of the human-yeast symbiosis. Now consider whether the various organic compounds available in the lab (which may include monosaccharides, disaccharides, amino acids, and simple organic acids) could be found in the microhabitat of yeast. If a potential nutrient is not encountered by an organism often, the organism may not evolve the metabolic machinery to utilize it. Even if a potential nutrient can be utilized, consider how it might have to be modified before it can enter the cell, and then enter the glycolysis pathway. Compare the molecular structures of the compounds—size, shape, bonding, etc. Think about it, ask questions, share ideas... and design an experiment! Each group should choose a distinct question to explore. Be sure to run your design by your TA, who will be happy to provide helpful suggestions.

Question: _____

H₀ _____

H_A _____

rationale for H_A _____

A. Experimental procedure

Available at the back bench in the lab are 0.5M solutions of sugars and other simple organic compounds, and a beaker of live yeast suspended in water. Before your lab period, we incubated the yeast aerobically so that they would use up all their carbohydrate reserves. Why do you suppose this was necessary?

1. Try to select the same size and style of fermentation tubes for all of your treatments.
2. Because our tubes are hand-blown and unmarked, you must calibrate each of them before beginning your experiment. Use either of the methods below:
 - a. With a syringe, introduce water into the tube, 0.5 mL at a time, and tip the tube so that the water enters the sealed end of the tube. Each time you add water, mark the meniscus with a wax pencil (Figure 6-1).
 - b. *Alternatively, to save time*, you may assume that the straight part of the tube has a constant bore (inside diameter). Add 1.0 mL of water, 0.5 mL at a time as above, to calibrate the rounded end of the tube. Then, add 10.0 mL of water all at once (less for a very small tube). Mark the meniscus, measure the length from the previous mark, and calculate the mL/cm. You can now convert length measurements along the straight part of the tube to volumes.
3. Add 2 mL of yeast suspension and 10 mL of a potential nutrient solution to each tube—this should provide the yeast with a fine feast to last for a couple of hours!

4. Tilt the tube so that the liquid fills the tip and no air bubbles remain (Figure 6-1). Some tubes may require more total liquid than 12 mL to completely fill them—if yours is one of these, add more liquid (but keep the *proportion* of yeast to potential nutrient solution the same!).
5. Vegetable oil is provided, should you want to make a (mostly) airtight seal on top of the yeast + nutrient suspension. Use a Pasteur pipet to apply a *very thin film* of oil.

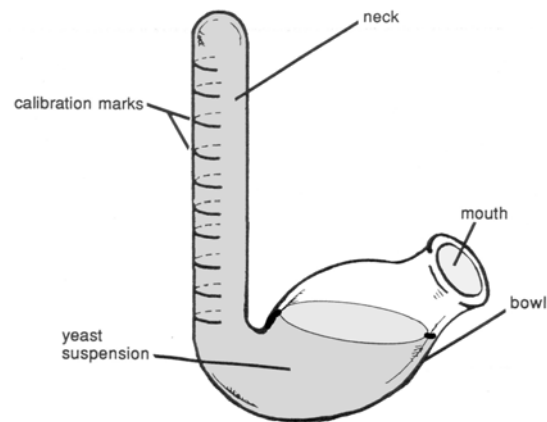


Figure 6-1. A fermentation tube showing calibration marks every 0.5 cc (= 0.5 mL). Yeast suspension + a potential nutrient solution have just been added—note that the tube is filled with liquid to the tip!



Figure 6-2. Ripening Syrah grapes showing the characteristic powdery white coating, or 'bloom', on their surface (Hass 2008).

Use the table below to record your experimental set up. You needn't use all the rows.

Table 6-1. Experimental conditions used to test the relative fermentation rates of _____ by yeast

Tube #	Name of organic compound	mL yeast suspension	mL of 0.5 M organic compound	Temp. (°C)	

B. Data collection

Use Table 6-2 below to record your experimental results. Write an appropriate legend. (NOTE: It may take about 30 minutes before you observe any generation of CO₂.)

Table 6-2. _____

Time (min)	Tube 1 (mL CO ₂)	Tube 2 (mL CO ₂)	Tube 3 (mL CO ₂)	Tube 4 (mL CO ₂)	Tube 5 (mL CO ₂)
0					
10					
20					
30					
40					
50					
60					
70					
80					
90					
100					
110					
120					

C. Data analysis

1. Enter your data from Table 6-2 into a graphing program such as Excel, and make a graph with time on the x-axis and CO₂ production on the y-axis. Plot the data from all treatments on the same graph for ease of comparison.
2. Calculate the fermentation rate of each substance by yeast, in mL CO₂/minute. To do this, find the stable, linear (or mostly so) portion of the rate curve for each treatment. Add a best fit line *for the linear portion only* using the software and do a

regression analysis to get the slope—that's your fermentation rate! You should also know how to obtain the slope by hand by calculating $\Delta y/\Delta x$. Your TA will show you how to do the analysis, and is happy to give you extra help, but please ask ASAP.

3. Convert your fermentation rates from mL CO₂/minute to mL CO₂/hour.
4. Correct the fermentation rates to control for the weight of the yeast. To estimate the weight of the yeast, find the proportional weight of yeast you used in each tube, knowing that the stock suspension contains 18 g of dry yeast/100 mL of water. Express your adjusted rate as mL CO₂/g/hour.
5. Make a new figure showing only the adjusted fermentation rates on the y-axis vs. the potential nutrient (independent variable) on the x-axis. Because the independent variable consists of attribute data, you should use a bar graph. See [How to Create Figures and Tables](#).

C. Discussion

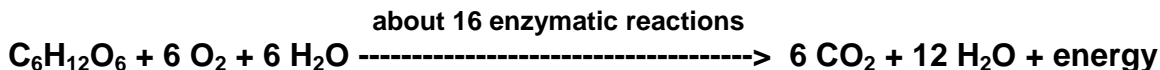
Consider the following questions and integrate them, if relevant, into your presentation *in a logical fashion*.

1. Compare the adjusted fermentation rates for your different treatments. Was your hypothesis supported?
2. We used equivalent molarities of the organic substances, instead of equivalent percentages of weight/volume. Was this the best method for your experiment?
3. To what degree might the yeast cell membrane be permeable to each of these substances? How might you test this?
4. Even if a substance passes through the cell membrane, it may not be fermented. Why might this be?
5. In using the fermentation tube, did you quantify all the CO₂ being generated? Was fermentation the only metabolic pathway going on? (HINT: one end of the tube is open! D'oh!) Might this affect your results?
6. Was CO₂ the only component of the gas produced in your experiment? What else might be there? How might this affect your results? Are there any other variables that may have been uncontrolled in your experiment?
7. Can you state with confidence that the fermentation rates among your treatments are significantly different? Why or why not? How could you determine whether the rates are significantly different? How might you modify the experiment to gain confidence in your results?

II. Respiration: aerobic metabolism

Almost all eukaryotic and many prokaryotic organisms are dependent upon a continuous supply of oxygen in order to sustain life. Ultimately, molecular oxygen taken into the organism is used as the terminal electron acceptor in the electron transport chain, and appears as part of a water molecule at the end of the reaction. At the same time, just as hydrogen atoms are removed from Krebs Cycle intermediates and

ultimately transferred to oxygen, carbon dioxide is removed by decarboxylation reactions of the Krebs Cycle. Most of this carbon dioxide is released as a waste product (unless it is being recycled for photosynthesis in an organism that has this ability), ultimately to be expelled by the organism into its environment. The breakdown of carbohydrates to produce ATP requires a long series of enzyme mediated reactions. The *overall* process may be expressed as follows:



The rate of aerobic metabolism differs among various organisms. Body size, the amount of metabolic energy used to regulate body temperature, life stage, physiological state (e.g. dormant vs. actively growing), and activity level are just some of the factors that may influence the rate of respiration.

To find the rate of respiration, one could theoretically measure an organism's intake of any of the reactants, or its output of any of the products (including energy), over time. In practice, however, some measurements are easier to perform than others. Often, researchers measure either O₂ consumption or CO₂ production over time. You will use an electrochemical O₂ gas sensor (Vernier Software, www.vernier.com) to measure the consumption of O₂ gas in a closed chamber that we will today call a *respirometer*.

There are several types of small, respiring organisms available, which may include:

- Animals such as insects
- Germinating and dormant seeds
- Potatoes or other plant storage organs; fruits, etc.
- Yeast suspension
- Organic soil or compost
- Living plant parts or invertebrate animals obtainable outside

Some of the tools you will have available include:

- Ice, hotplates, thermometers, a heated waterbath, and large beakers for making additional waterbaths of various temperatures
- Foil for darkening the reaction chamber
- Solutions of simple organic compounds (e.g. sugars, amino acids, Krebs cycle intermediates) for use with *yeast only*

Examine the variety of these specimens and potential environmental conditions. Consider whether your organisms are immature (undergoing relatively rapid mitosis), mature, or dormant. What is each organism using for a nutrient/energy source? How might this affect its rate of oxygen consumption? Might the presence or absence of light affect the rate of respiration, by causing a metabolic or behavioral change?

Consider metabolic modes. Is your organism endothermic (obtains its body heat metabolically) or ectothermic (obtains its body heat from the environment)? Is it homeothermic (regulates its own body temperature) or poikilothermic (body temperature is regulated by the environment)? Is your organism undergoing any other metabolic reactions that might affect the amount of oxygen in your experimental system? Consider these questions carefully, but don't *assume* anything. If you're not sure, ask

Follow the basic procedures below, but be prepared to modify them based on the organisms you are using and the conditions you are setting up. Use common sense!

- **If you are using yeast suspension**, follow these steps:
 1. Label a test tube for each organic substance you are testing.
 2. Into test tube #1, add 2 mL of a potential nutrient, and 2 mL of yeast suspension. Mix by covering and inverting the test tube 2x.
 3. Incubate tube #1 at 38°C for 10 minutes.
 4. After 10 minutes, pour the contents of the tube into the plastic respirometer chamber (Figure 6-3), taking care not to splash. Measure the rate of respiration as described below.
 5. Repeat steps 1-4 for the next tube.
 6. To estimate the weight of the yeast, calculate the proportional weight of yeast you used in each tube, knowing that the stock suspension contains 18 g of dry yeast/100 mL of water.
- **If you are using macroscopic organisms**, follow these steps:
 1. Decide how many individuals to test. Do a trial run with 10-20 individuals and modify if necessary (*e.g.* if little or no O₂ consumption is measured).
 2. Weigh the organism(s) you will test. If they are wet, gently pat them dry with a tissue before weighing.
 3. Keep organisms in a covered beaker while they are awaiting testing.
 4. Carefully place the weighed organism(s) in the respirometer chamber (Figure 6-3) and measure their rate of respiration as described below.
- **If you are using a soil sample** follow these steps:
 1. Decide how much soil to test. Do a trial run with about 1" of soil in the chamber and modify if necessary (*e.g.* if little or no O₂ consumption is measured). **Do not allow the soil or any moisture to touch the O₂ sensor.**
 2. Weigh the soil sample you will test.
 3. Place the soil in the respirometer chamber (Figure 6-3) using a wide neck (or paper) funnel, and measure the rate of respiration as described below.

More helpful hints:

- If testing the effects of temperature, incubate each organism for 10 minutes beforehand in an open container, at the desired temperature. Maintain the respirometer chamber at that temperature as well. **Do not exceed 38°C.**
- If testing the effects of light, incubate the organisms for 10 minutes beforehand under the appropriate conditions.

- Because each group has only one respirometer, you will have to perform your treatments sequentially. Plan your incubations accordingly! Complete one set of treatments before starting any replicate trials.
- Keep the organisms as unstressed as possible while they are waiting to be tested.

B. Setting up the O₂ gas sensor and data logger software

One team member with a PC or Mac laptop computer will kindly volunteer it for data collection.

1. Holding it only by the edges, place the Logger Lite CD in the CD drive and follow the installation directions that appear. If you've already installed the program, skip this step. All students in BIL 151 are entitled to install Logger Lite on their computers.
2. *Only after you have installed the software*, connect the O₂ gas sensor and interface to the USB port on your computer. **Keep the O₂ sensor upright at all times. Treat it with extreme care or you may damage it and will be penalized!**
3. Start the Logger Lite 1.4 software by clicking on the icon on the desktop.
4. The software will detect the sensor and load a data table and graph.
5. On the "**Experiment**" menu, click on "**Data Collection.**" Change "**length**" to **10 minutes** and "**sampling rate**" to **10 samples/minute** (= 6 seconds/sample). You can change these settings again at any time.

C. Collecting data

1. Place the organism(s) in the respirometer chamber as detailed above.
2. Be sure the neck of the respirometer chamber is clean and dry.
3. **Carefully and gently** place the O₂ gas sensor into the plastic respirometer chamber as shown in figure 6-3. Gently push the sensor down until it stops.

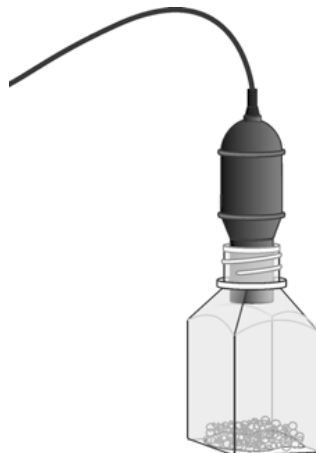


Figure 6-3. O₂ sensor and respirometer ready to collect data.

4. *Wait one minute* for the system to stabilize, then click “**Collect**” on the toolbar at the top of the Logger Lite window. The sensor will now start measuring and displaying, 10x per minute, the O₂ concentration (as %O₂) of the air in the chamber.
5. Measure or describe variables relevant to your experiment (e.g. temperature near the respirometer, pH of nutrient solutions, activity level of organisms)
6. If O₂ readings start to plateau, or you are sure you have found the stable linear portion of the rate curve, you may click “**Stop**.”
7. Click “**Store**” to store this data run. **Save** the file.
8. Gently remove the O₂ sensor and place it upright in its dry 250 mL beaker.
9. Remove the organism(s) from the respirometer chamber; keep them for now in a beaker. *Only yeast* may be poured down the drain! *Thoroughly* rinse the chamber with tap water 3x. Shake out excess water and dry the neck of the chamber.
10. Repeat steps 1-9 as needed to perform one trial of each treatment.
11. Do additional trials of the treatments if time permits. You can open a fresh data table and graph by clicking “**New**” on the top toolbar (don’t forget to save the old graph first!).

C. Data analysis

We can now use the software to calculate a “raw” rate of respiration for each treatment. Examine the curves on your graph. Each curve shows the time course of O₂ consumption. At first there may be several minutes of inconsistent readings, as the organism adjusts to the conditions and the system equilibrates. Then, there should be a steadily falling linear portion of the curve that represents the rate of respiration. After finding this “raw” rate of respiration, we will correct it for:

- Weight of the organisms
- Volume of O₂ (instead of %O₂) consumed
- Standard temperature and pressure (optional)

Follow the steps below for each curve.

1. Highlight the linear (or mostly so) portion of the curve using the mouse.
2. Click on “**Analyze**” on the top menu, then “**Linear Fit**.” In the dialog box that appears, be sure to check the curve you’ve just highlighted. A best fit linear regression line will now appear for your highlighted points, along with a floating box containing the equation of the line. The *correlation* statistic (*r*) shows how well your actual data points fit the line, with a correlation of 1.0 showing a perfect fit. Using the mouse, you can grab and move the brackets to change the highlighted points, to see if there is a better fit—the line, correlation, and equation will automatically update.
3. Look at the equation of the best fit line. The slope, or *m*, shows the change in %O₂ over the change in time—in other words the raw (uncorrected) rate of respiration in %O₂/minute.

4. Convert %O₂/minute to %O₂/hour.
5. Now divide the raw rate of respiration by the actual weight of the organism(s) used in this trial to get %O₂/g/hour.
6. Repeat steps 1-5 for all the replicate trials, if any, for this same treatment.
7. Average the raw rates of respiration for all replicate trials of this treatment to get a mean %O₂/g/hour.
8. The working volume of the respiration chamber, with the O₂ gas sensor inserted, is estimated to be 280 mL. Now you can calculate the mean mL O₂/g/hour respired by the organism as a percentage of the total working volume. Calculate the standard deviation too, if you had 3 or more trials for a treatment.
9. *Optional:* If your TA requires it, correct for standard temperature and pressure using the formula:

$$V_2 = \frac{P_1 V_1 T_2}{P_2 T_1}$$

Where V₂ is the corrected volume of oxygen consumed under STP and

P₁ = atmospheric pressure of the lab (in mm Hg, from the **barometer** at the front of the lab)

V₁ = oxygen consumed under experimental conditions; just use the mL O₂/g/hour

T₁ = temperature of the respirometer (in degrees Kelvin; °K = °C + 273)

P₂ = 760 mm Hg (standard pressure)

T₂ = 273°K (standard temperature)

Note that the units of V₂ will be expressed as mL O₂. Once you obtain this value you can simply use it as the corrected mean mL O₂/g/hour.

10. Repeat steps 1-9 for each treatment.
11. Compare the corrected rates of respiration from your different treatments, results from other groups, and any relevant results e.g. from table 6-4.
12. Create a new figure on Excel or other graphing software, showing *only* the mean rates of respiration on the y-axis (in mL O₂/g/hour) vs. your treatment (independent) variable on the x-axis. If your independent variable consists of attribute data, you must use a bar graph. If it consists of continuous numerical data, you may use a line graph. See [How to Create Figures and Tables](#).
13. If appropriate, add a best fit line for your data points and perform a linear regression (or other statistical analysis).

14. *Optional:* If you did three or more replicate trials for your treatments, show experimental variation for each of your points using error bars. Your TA will show you how to make standard error bars on Excel, if she or he would like you to do this.

D. Discussion

In your presentation, consider the questions posed throughout this lab chapter. Also consider the following issues, if relevant to your experiment, and integrate them into your presentation or report *in a logical fashion*:

1. Do your results support any of the hypothesized effects of size, life stage, metabolic mode and body temperature, physiological state, or activity level of an organism on its rate of respiration?
2. Explain the utilization of different organic substances for respiration in yeast, and compare to fermentation. What is the advantage to yeast of being able to use both pathways? How do we know the yeast was respiring in the respirometer, and fermenting in the fermentation tube?
3. Does soil respire? Explain.
4. Although you may consider the weight of your organisms to be the total weight of living, metabolizing tissue, this may not be the case. What else might you be weighing—besides metabolizing tissue—in each organism? How might this affect your results? Are there any other variables that may have been uncontrolled in your experiment?
5. Can you state with confidence that the oxygen consumption by your different species, or under your different conditions, was significantly different? Why or why not? How might you modify your experiment to gain this confidence?

References

- Florey, E. 1967. *An Introduction to General and Comparative Physiology*. W.B. Saunders Company, Philadelphia.
- Haas, J. 2008. Harvest, week of September 15th: Watching and waiting. Blog Tablas Creek, Tablas Creek Vinyard.
<http://tablascreek.typepad.com/tablas/2008/09/harvest-week--1.html>.
- Legras, J-L, D. Merdinoglu, J-M. Cornuet, and F. Karst 2007. Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. *Molecular Ecology* 16: 2091-2102.
- Rossini, G., F. Federici, and A. Martini 1982. Yeast flora of grape berries during ripening. *Microbial Ecology* 8(1): 83-89.

Table 6-4. Oxygen consumption of various species at rest (in ml O₂/g/hour).
Data from Florey (1967).

Phylum	common name	Genus	ml O ₂ /g/hour
Ciliophora	paramecium	<i>Paramecium</i>	1.00
Rhizopoda	amoeba	<i>Amoeba</i>	0.20
Porifera	sponge	<i>Suberites</i>	0.24
Cnidaria	sea anemone	<i>Anemonia</i>	0.013
	jellyfish	<i>Aurelia</i>	0.0034
Nematoda	hookworm	<i>Ascaris</i>	0.50
	roundworm	<i>Rotylenchus</i>	0.66
Mollusca	clam	<i>Anodonta</i>	0.002
	mussel	<i>Mytilus</i>	0.055
	octopus	<i>Octopus</i>	0.09
Annelida	lugworm	<i>Arenicola</i>	0.031
	tube worm	<i>Chaetopterus</i>	0.008
Arthropoda	crab	<i>Uca</i>	0.005
	lobster	<i>Homarus</i>	0.50
	crayfish	<i>Cambarus</i>	0.10
	butterfly	<i>Vanessa</i>	0.60
Echinodermata	sea cucumber	<i>Holothuria</i>	0.01
	sea star	<i>Asterias</i>	0.03
Chordata	goldfish	<i>Carrasius auratus</i>	0.07
	trout	<i>Salmo trutta</i>	0.22
	rat	<i>Rattus norvegicus</i>	0.95
	cat	<i>Felis cattus</i>	0.44
	human	<i>Homo sapiens</i>	0.20

KINGDOM PLANTAE, Division Anthophyta (flowering plants)

Plant name	type of structure	condition	ml O ₂ /g/hour
alfalfa	seed	dormant	0.38
	seed	germinating	1.06
barley	seed	germinating	0.715
	endosperm	nutrient supply	0.076
carrot	root	mature	0.025
	leaf	mature	0.440
spinach	leaf	mature	0.515
sweet potato	root	mature	0.96
ash tree	cambium	mitotic	0.233
	phloem	mature	0.154
	sapwood	conducting	0.047
red maple	cambium	mitotic	0.220
	phloem	mature	0.170
apple	flesh	ripe	0.030
	skin	ripe	0.095