Effect of Sugars on Respiration in Yeast
Biology 171L FA17

Lab 5: Effect of Sugars on Respiration in Yeast

Student Learning Outcomes
1. Learn to create a culture medium for a simple unicellular organism, yeast.
2. Calculate the rate of CO₂ production and compare the rates of cellular respiration using various types of sugar as the source of food.

Relevant Readings
Campbell Biology, Chapter 9, especially pp. 162-176
A short Guide to Writing about Biology, Chapter 9, especially pp. 196 – 203

Homework Synopsis (see pages 5-9 & 5-10 for full description)
- Part I – Mastering Biology
- Part II – Science Communication – Discussion exercises
- Part III – Data Analysis – Short Answer
- Using the Scientific Literature Assignment

INTRODUCTION
All living cells need energy to grow and function. All chemical activity in a cell or organism is called metabolism. A significant part of metabolism is decomposition reactions that involve breaking down more complex molecules into simpler units or molecules. This process releases energy in the forms of heat and chemical energy. Cell respiration is a decomposition reaction involving several steps and energy is generated along the way by breaking down the hydrocarbon molecules.

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy (38 ATP)} \]

Aerobic respiration breaks down a single molecule of glucose (contains six carbon molecules) into six carbon dioxide molecules (each have just one carbon atom). This pathway is composed of several steps and products produced along the way are used to generate even more chemical energy in the form of ATP molecules. Simple sugars are broken down very quickly and are an immediate source for energy. Molecules larger than glucose are also used, but require additional decomposition. Cellular respiration is used to break down starches, lipids, and proteins, but simple sugars are the first and easiest food choice.

Carbohydrates consist of sugars and the more complex molecules, starches. Sugar molecules are made up of carbon, hydrogen, and oxygen atoms in a 1:2:1 ratio. The simplest sugars, monosaccharides, all have the same number and types of atoms but can form slightly different structures when the atoms bond together. Three common monosaccharides are glucose, fructose, and galactose. You’ll be studying glucose in today’s lab.
Table 1: Steps in aerobic respiration. Aerobic respiration of one glucose molecule produces a total of 38 molecules of ATP.

<table>
<thead>
<tr>
<th>Location in Cell</th>
<th>Starting Materials:</th>
<th>End Product</th>
<th>Net Energy Production</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STEP 1: GLYCOLYSIS</strong></td>
<td>Glucose</td>
<td>2 Pyruvate</td>
<td>+2 ATP</td>
</tr>
<tr>
<td>Cell Cytoplasm</td>
<td>6 carbon sugar chain (usually cyclic)</td>
<td>pyruvate</td>
<td>NADH</td>
</tr>
<tr>
<td><strong>STEP 2: Prep &amp; KREB’S CYCLE</strong></td>
<td>Pyruvate</td>
<td>6 CO₂</td>
<td>+2 ATP</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>pyruvate</td>
<td></td>
<td>NADH FADH₂</td>
</tr>
<tr>
<td><strong>STEP 3: ELECTRON TRANSPORT SYSTEM/CHAIN</strong></td>
<td>NADH and FADH₂ (produced in glycolysis and Kreb’s Cycle)</td>
<td>OXYGEN</td>
<td>+34 ATP</td>
</tr>
<tr>
<td>Mitochondrial Inner Membrane</td>
<td>OXYGEN</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glucose, C₆H₁₂O₆, is a monosaccharide containing six carbon atoms and is the primary “fuel” for cellular respiration. It is found in human blood and is commonly referred to as “blood sugar.” Glucose is distributed to all the cells in our body by our circulatory system. High levels of blood sugar trigger the production of a protein, insulin, which increases the permeability of cell membranes by opening up glucose channels. This speeds up the intake of glucose into each cell, lowering the blood sugar level back to a normal range and providing cells with a supply of glucose for energy. Diabetic individuals either do not produce adequate insulin or their cells stop responding to insulin. Cells do not absorb enough glucose out of the bloodstream. High blood sugar levels over time can create serious health problems. It causes damage to small vessels, especially in the eyes, and can lead to blindness and nerve damage.

Sugars become more complex as sugar units bond together to form chains. Disaccharides are formed when two simple sugars are bonded together. Sucrose, C₁₂H₂₂O₁₁ is a disaccharide made...
from the bonding of two monosaccharides, a glucose molecule and a fructose molecule. Sucrose occurs naturally in every fruit and vegetable and is a product of photosynthesis. It’s commonly known as table sugar and more than likely, you have a jar of sucrose sitting on your kitchen counter next to a jar of flour.

Lactose, C$_{12}$H$_{22}$O$_{11}$, is a disaccharide formed by the bonding of two monosaccharides, galactose and glucose. Lactose is most commonly found in milk, and thus is called milk sugar. Disaccharides and other polysaccharides must be broken down into monosaccharides by specific enzymes in order to be easily used. Mammals secrete an enzyme called lactase or beta-D-galactosidase to break down lactose into its monosaccharide base units, glucose and galactose. The monosaccharides are more suited for cell absorption. Most mammals produce less of the lactase enzyme as they mature and naturally consume less milk. Individuals who are lactose intolerant are unable to break down the lactose. The lactose continues to journey through the digestive tract and becomes a ripe food source for bacteria in the intestines that produce gas as a by-product. This presents itself in symptoms such as bloating and flatulence. People who are lactose intolerant can minimize symptoms by taking lactase pills.

Monosaccharides bond together to form chains of polysaccharides. Glucose molecules can form long chains known as glycogen that is used by animals as a short-term food storage molecule.

YEAST
Yeasts are unicellular, eukaryotic fungi. Yeasts grow rapidly and have simple nutritional requirements. Yeast can respire aerobically but is also able to undergo anaerobic respiration, also known as fermentation, when oxygen is insufficient. There are several species of yeast and today’s experiment will be using Saccharomyces cerevisiae, better known as baker’s yeast or brewer’s yeast. Early usage of this species may have been the result of isolating it from grape skins (found in the white film sometimes covering the skin).

Yeasts are capable of using some, but not all, sugars as a food source. Today’s laboratory will be studying the rate of cellular respiration of yeasts in an aerobic environment looking at various types of food sources. In order to do so, the production of carbon dioxide gas will be measured to indicate the rate of respiration. You will be comparing glucose, sucrose, lactose, an artificial sweetener, e.g., Splenda™, and water and their ability to act as an energy source in aerobic respiration.

TREATMENT CONTROLS
Today, you will be using a treatment control. A treatment control provides a baseline against which you can compare all your treatments. The treatment control is subject to all of the same conditions as the treatments. In today’s lab, the treatments are the various sugars and the distilled water provides the treatment control. Specifically the water is called a negative control, because you will not be feeding the yeast a source of food. This will allow you to gauge the effect of your treatments, i.e., the different sources of food, against a null condition, no food.
Preparation for Lab
1. Read through Introduction
2. Research topics and terms that you are not familiar with or do not fully understand; e.g.,
   a. Cellular Respiration: Aerobic and Anaerobic
   b. Simple Carbohydrates
   c. Yeast
3. Read through the Experiment Procedure
4. Prepare your notebooks (e.g., write out protocols, prepare data tables, etc.)

EXPERIMENT PROCEDURE

Overview:
In order to compare how yeast uses different sugars, you will be incubating mixtures of yeast and sugar solutions together and measuring respiration at the end of the incubation period. Each sugar/yeast solution will incubate in a water bath for ten minutes. At the end of the incubation period, you will use a CO₂ gas sensor to measure the amount of CO₂ produced by the yeast in a fixed time, which will allow you to calculate the respiration rate.

Set up water bath:
1. You will be sharing a water bath with other lab groups. Your TA will instruct you on how to use the water baths. Your TA may have already turned on the water bath, but you still need to monitor that the water bath has adequate water to cover your solution and that the water has reached the appropriate temperature. Do not use until the water has reached a temperature of least 38°C. If the bath is not on, turn it on and wait for the water to warm up.
2. Check often to ensure that the temperature remains in the right range, i.e., 38–40°C. When using a thermometer to check the water temperature, make sure that the thermometer is NOT touching the metal sides or bottom of the water bath. This could alter the temperature reading.
3. If water bath is too warm, bring the temperature down by adding a small amount of distilled water and/or adjusting the temperature controls.

Set up sensor software:
4. Set up your laptop, Vernier data logger (LabPro), and the CO₂ gas sensor (see laminated card).
5. Start up the laptop and wait a few minutes before booting the LoggerPro Program (icon found on desktop). Sometimes the computer needs to load the driver to recognize the Vernier probes before you open the program.
6. Check CO₂ gas sensor. If it has a switch, ensure that it is set to “LOW” (0-10,000 ppm).
7. When LoggerPro opens up, check the window at the bottom left corner of the screen to
check that your probe is registering values for CO₂.

8. Probe should be allowed to warm up for **2 minutes**. Ideally, in an open environment (*like outside*), it should read between 380 and 440 ppm for carbon dioxide. It may be calibrated by inserting a paper clip in to the CAL port on the probe. Ask your TA or TI for help if the probe needs calibration.

9. In the **Experiment** heading in the main menu, find and select **Data Collection**. When the **Data Collection** window pops up, make sure that the mode is **Time Based** and change the length to 240 seconds (4 minutes).

10. Rinse out the respiration chamber with a little bit of distilled water and dry with tissue/paper towel.

![Fig. 1: Set-up for Respiration Chamber](image)

**Preparing yeast cultures:**

11. Either on your desk or in the classroom, you should obtain a set of seven (7) falcon tubes. If they are not already labeled, you should label them.

<table>
<thead>
<tr>
<th>Medium</th>
<th>2.5%</th>
<th>5%</th>
<th>10%</th>
<th>5%</th>
<th>5%</th>
<th>5%</th>
<th>Pure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label</td>
<td>Glucose</td>
<td>Glucose</td>
<td>Glucose</td>
<td>Sucrose</td>
<td>Lactose</td>
<td>Splenda™</td>
<td>dH₂O</td>
</tr>
<tr>
<td>Color</td>
<td>2.5%GLU</td>
<td>5% GLU</td>
<td>10%GLU</td>
<td>5% SUC</td>
<td>5% LAC</td>
<td>5%SPL</td>
<td>De-I H₂O</td>
</tr>
</tbody>
</table>

12. Since you’ll be sharing water baths with other groups, make sure you know which tubes are yours.

13. Take a solution one at a time from the shared class area.

14. Fill each tube with 3 mL of the corresponding sugar solution.

15. To keep consistency between the various cultures, you’re going to stagger each test medium (type of sugar) to allow for adequate test time. One partner needs to be responsible for keeping time to ensure that the time elapsed between the addition of yeast and the start of measuring carbon dioxide production is constant between all samples.

16. Perform a test-run using 1 treatment medium from start to finish. Pick a medium, e.g., 2.5% Glucose, to test first.
17. In the appropriately labeled glass test tube, obtain 3 mL of a yeast culture from your TA or TI.

18. When you are ready to begin the incubation, pour the sugar into the yeast.

19. Swirl the tube until the contents are well mixed and place into test tube racks in the water baths. **Do not cap tubes; you want them open.** Tubes may float a little because of their buoyancy so make sure they are not in danger of tipping into water.

20. **Immediately begin timing incubation period.** Start incubation time: ________________

21. Check the water bath every time you put in or take out a test tube to ensure that the temperature is remaining at 38-40°C.

22. Let your culture sit in the warm water bath for ten minutes, while you set up your CO₂ gas sensor.

**Observing Respiration in Yeast Cultures:**

23. When the first yeast culture has incubated for ten minutes, you can begin data collection.

24. Record the temp of the water bath, and the time, as you take the 1st tube out of the water bath.

25. Temperature: ________________ Stop Incubation Time: ________________

26. Swirl the test tube as the yeast may have settled to the bottom and quickly dump all of the contents into the respiration chamber.

27. Quickly close the respiration chamber by inserting the CO₂ gas sensor. Check that the chamber is sealed properly. **A bad seal (see Fig. 1) will mean inaccurate readings!!!.**

28. Press the green **Collect** button to start data collection. It will stop after 4 minutes of data collection. If data collection does not stop, stop it manually. You have not entered the “Time Based” function (step 9) properly.

29. Record the total respiration after 4 minutes.

30. Examine the final graph to identify the time period where the CO₂ values begin to increase. Holding the left mouse button, highlight the graph from that point until the end of the data.

31. Click on the **Linear Fit** button at the top [small icon with “R=”].

32. A box will appear with the equation for the linear fit graph. The slope of the line “m” represents the rate of respiration (rate of carbon dioxide production).

33. Uncap the respiration chamber and dispose of the tested yeast solutions into a waste container in the class.

34. Rinse out respiration chamber well with water and dry the inside thoroughly with tissue/paper towel.

35. Calculate the total incubation time: the time from addition of yeast to the dumping into
the respiration chamber. You will need to aim for the same consistent timeframe for the remaining media cultures. **Total incubation time:**

36. **Prepare the CO₂ sensor for the next measurements by using a notebook or paper to fan air across the openings in the CO₂ Gas Sensor Probe until the readings stop decreasing.**

**Testing the remaining treatment media:**

37. With your partner, discuss how you will test the remaining treatment media and still ensure that the incubation times remain consistent (steps 20, 25 & 34). You can either test them one by one or develop an effective staggering method.

38. Repeat the steps above to obtain respiration rates for all test samples. LoggerPro may ask you if it is okay to clear previous data trials. Press **OK.**

39. Share your data with the TA who will compile class averages and post on Laulima for comparison in your homework.

**Individual Group Data:**

<table>
<thead>
<tr>
<th>Sugar Tested</th>
<th>Temperature, °C</th>
<th>Total Respiration in 4 minutes (ppm)</th>
<th>Respiration Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5% Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Sucrose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Lactose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Splenda™</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Class Data:**

<table>
<thead>
<tr>
<th>Sugar Tested</th>
<th>Total Respiration in 4 minutes (ppm)</th>
<th>Respiration Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5% Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Lactose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Splenda™</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lab 5 Homework – Due Week of October 2, 2017

Format is important. All written homework should be typed, double-spaced, Times New Roman 12-pt font, with 1-inch margins. Remember to include your name, section and the name of your TA.

Read about best practices when writing the Discussion (pp. 196-203) section of a lab report in “A Short Guide to Writing About Biology”, and any other resources you find helpful (e.g., http://writingcenter.unc.edu/handouts/scientific-reports/ or https://labwrite.ncsu.edu/).

Part 1 – Mastering Biology (36 points):
A. Answer the questions in the assignment entitled “6. Catalase ” on the Mastering Biology site. You have until the night before lab at 11:59pm to complete these questions.

Part 2 – Science Communication (35 points):
A. Writing the Discussion Section of a Lab Report (10 pts)
   a. Describe of the elements of a well-written ‘Discussion’ section in one or two paragraphs. Include such elements as the purpose, correct amount of detail, proper tense and voice, reference to past studies, and any other information you feel is important.
   b. Why is the Discussion section important? How does it contribute to the construction of knowledge in biology?

B. Writing the Discussion Section of the Respiration in Yeast Lab (20 pts)
   Write the Discussion section for this lab. Use the guidelines you developed in your answer above, and the information you reviewed in the Discussion section in “A Short Guide to Writing About Biology”, pages 196-203, and http://writingcenter.unc.edu/handouts/scientific-reports/ or https://labwrite.ncsu.edu/.
   Find one article from the primary literature to use in your discussion to support one of your conclusions. Be sure to cite this article appropriately. Grades will be based on the Universal Grading Rubric that can be found at the end of the manual and on Laulima.

C. Reflection (5 pts)
   Evaluate your Discussion section for the respiration lab (question 2.B.). Describe how well your Discussion section conforms to the guidelines you outlined in question 2.A. Use the rubric provided at the end of this manual to help you. Do you need to make any changes to improve your Discussion section? In your answer, outline the steps you will take. Make these changes now to get maximum points for your answer in part 2.B.
Part 3 – Data Analysis (30 points)

Answer the following questions:

1. Figures and tables are used to summarize data. Choose the most appropriate method to summarize both your data and the class data and submit any relevant statistics, charts, etc. Be sure to include appropriate captions. (6 pts)

2. How do your data compare to the class average? Explain your hypotheses for any discrepancies. (2 pts)

3. Thoroughly discuss each sugar, including Splenda™, as a food source for yeast. What made that medium an ideal or less than ideal source for energy? Which medium had the highest rate of carbon dioxide production? Why were some sugars metabolized while others were not? Use the evidence you collected in your experiments to support your answers. (10 pts)

4. Describe the purpose of a control and use today’s control as an example. What factor(s) will remain constant from trial to trial? (3 pts)

5. How did concentration play into the rate of respiration? Explain how it met or differed from expectations. (2 pts)

6. What could you have done with this lab to force the yeast to switch to anaerobic fermentation? Think realistically about how this might be accomplished. (2 pt)

7. What are some problems with our analysis of Splenda™? (2 pts)

8. Baker’s Yeast is also known as Brewer’s Yeast because it is used to ferment sugar sources and produce alcohol in an environment void of oxygen. Because it utilizes anaerobic fermentation to ferment sugars from grapes, the level of sugars present drop as they are broken down until a certain point where all anaerobic fermentation ceases completely, despite the fact that sufficient levels of sugar may still remain. Suggest an explanation for why this might happen. (3 pts)
<table>
<thead>
<tr>
<th>Criteria</th>
<th>Not addressed (0-2)</th>
<th>Novice (3)</th>
<th>Intermediate (4)</th>
<th>Expert (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discussion: Conclusions based on data selected</strong></td>
<td>• Conclusions have little or no basis in data provided.</td>
<td>• Conclusions have some direct basis in the data, but may contain some gaps in logic or data or are overly broad.</td>
<td>• Conclusions are clearly and logically drawn from and bounded by the data provided with no gaps in logic.</td>
<td>• Conclusions are completely justified by data.</td>
</tr>
<tr>
<td></td>
<td>• Connections between hypothesis, data and conclusion are non-existent, limited,</td>
<td>• Connections between hypothesis, data and conclusions are present but weak.</td>
<td>• A reasonable and clear chain of logic from hypothesis to data to conclusions is made.</td>
<td>• Connections address and logically refute or explain conflicting data</td>
</tr>
<tr>
<td></td>
<td>vague or otherwise insufficient to allow reasonable evaluation of their merit.</td>
<td>• Conflicting or missing data are poorly addressed.</td>
<td>• Conclusions attempt to discuss or explain conflicting or missing data.</td>
<td>• Synthesis of data in conclusion may generate new insights.</td>
</tr>
<tr>
<td></td>
<td>• Conflicting data are not addressed.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Discussion: Alternative explanations</strong></td>
<td>• are not provided</td>
<td>• are provided in the discussion only</td>
<td>• Some alternative explanations are tested as hypotheses; those not tested are reasonably evaluated in the discussion.</td>
<td>• have become a suite of interrelated hypotheses that are explicitly tested with data.</td>
</tr>
<tr>
<td></td>
<td>• are trivial or irrelevant</td>
<td>• may include some trivial or irrelevant alternatives.</td>
<td>• Discussion of alternatives is reasonably complete, uses data where possible and results in at least some alternatives being persuasively dismissed.</td>
<td>• Discussion and analysis of alternatives is based on data, complete and persuasive with a single clearly supported explanation remaining by the end of the discussion.</td>
</tr>
<tr>
<td></td>
<td>• are mentioned but not discussed or eliminated.</td>
<td>• Discussion addresses some but not all of the alternatives in a reasonable way.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternative explanations are considered and clearly eliminated by data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in a persuasive discussion.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criteria</td>
<td>Not addressed (0-2)</td>
<td>Novice (3)</td>
<td>Intermediate (4)</td>
<td>Expert (5)</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------</td>
<td>------------</td>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Discussion: Limitations of design</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limitations of the data and/or experimental design and corresponding implications discussed.</td>
<td>• are not discussed.</td>
<td>• are discussed in a trivial way (e.g. “human error” is the major limitation invoked).</td>
<td>• are relevant, but not addressed in a comprehensive way • Conclusions fail to address or overstep the bounds indicated by the limitations.</td>
<td>• are presented as factors modifying the author’s conclusions. • Conclusions take these limitations into account.</td>
</tr>
<tr>
<td>Use of Primary Literature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relevant and reasonably complete discussion of how this research project relates to others’ work in the field (scientific context provided).</td>
<td>• Primary literature references are not included.</td>
<td>• Primary literature references are limited (only one or two primary references in the whole paper) • References to the textbook, lab manual, or websites may occur. • Citations are at least partially correctly formatted. Note that proper format includes a one-to-one correspondence between in-text and end of text references (no references at end that are not in text and vice versa) as well as any citation style currently in use by a relevant biology journal.</td>
<td>• Primary literature references are more extensive (at least one citation for each major concept) • Literature cited is predominantly (&gt; 90%) primary literatures. • Primary literature references are used primarily to provide background information and context for conclusions • Primary literature references</td>
<td>• Primary literature references indicate an extensive literature search was performed. • Primary literature references frame the question in the introduction by indicating the gaps in current knowledge of the field. • Primary literature references are used in the discussion to make the connections between the writer’s work and other research in the field clear • Primary literature references are properly and accurately cited</td>
</tr>
</tbody>
</table>

**Biol 171L - FA17**

**Effect of Sugars on Respiration in Yeast**