Investigating Anaerobic Cellular Respiration (yeast cell fermentation)

SC Academic Standards: 5.L.4B; 6.L.4A; 6.L.4B; 6.L.5; 7.L.3A; 7.L.3B; H.B.3;

NGSS DCI: 5-LS2.A; 5-LS2.B; MS-LS1.C; MS-PS3.D; HS-LS1.C

Science and Engineering Practices: S.1A.1; S.1A.3; S.1A.4; S.1A.6; S.1A.7

Crosscutting Concepts: Cause and Effect: Mechanism and Explanation; Energy and Matter; Stability and Change.

Focus Question(s): What are the reactants and products of anaerobic cellular respiration in yeast cells?

Conceptual Understanding: All organisms need energy to live and grow. Energy is obtained from food. The role an organism serves in an ecosystem can be described by the way in which it gets its energy. Energy is transferred within an ecosystem as organisms produce, consume, or decompose food. A healthy ecosystem is one in which a diversity of life forms are able to meet their needs in a relatively stable web of life.

Life is the quality that differentiates living things (organisms) from nonliving objects or those that were once living. All organisms are made up of cells, need food and water, a way to dispose of waste, and an environment in which they can live.

The Fungi Kingdom consists of organisms that do not make their own food (heterotrophs) but obtain their nutrition through external absorption. Fungi can be grouped by their growth habit or fruiting structure and respond to changes in the environmental stimuli similar to plants.

Cells transform energy that organisms need to perform essential life functions through a complex sequence of reactions in which chemical energy is transferred from one system of interacting molecules to another.

Background: Perhaps the most fundamental characteristic of living organisms is the ability to harness energy to do work. Cell division, growth, metabolic reactions, absorption of nutrients and movement of muscles are but a few of the many biological processes cells achieve by harnessing energy and doing work.

The vast majority of living things on Earth depend directly upon the energy of sunlight because plants, animals, fungi (including yeasts), protists and many bacteria use photosynthetically produced sugars as the source of energy for biological work. During a series of complex chemical reactions the energy stored in a sugar molecule, such as glucose ($C_6H_{12}O_6$) is released in the form of electrons. The energy in these electrons is then harnessed to synthesize **adeonosine triphosphate (ATP)** which is used to do the work in a cell.

During **aerobic** cell respiration, **oxygen** is needed to release the stored energy of glucose. Oxygen is the final electron acceptor in the electron transport chain reactions that release energy from glucose in the form of electrons. The reaction for aerobic cell respiration is shown here:

$C_6H_{12}O_6 + O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + 36 or 38 ATP chemical energy + heat Energy

Part of the energy released is conserved in the chemical form of ATP, which is used to do the work of a cell, and the remainder is converted and released in the form of **heat**, which dissipates into the environment. A net yield of 36 ATP molecules are produced per each glucose molecule by eukaryotic organisms (prokaryotes make 38 ATP per glucose).

Aerobic cell respiration is of course more complicated than the above equation hints at. There are four parts to aerobic cell respiration (glycolysis, where glucose is broken down into pyruvate in a series of enzyme mediated steps; pyruvate conversion into acetyl coenzyme A; the electron transport chain, or ETC, where oxygen is essential in the transport of electrons and where a hydrogen (H^+) gradient set up across the mitochondrial membrane; and chemiosmotic oxidative phosphorylation, where the H^+ gradient is released through ATP synthase (a protein embedded in the mitochondrial membrane at the end of the ETC), and ADP is phosphorylated to ATP.

However, sometimes oxygen is not readily available, or easily absorbed, as in the case of yeast cells in water, and so an alternate metabolic pathway must be used to form ATP. Yes, there is oxygen in water, but not nearly as much as in air (maybe 5% if you are lucky and have cold clear well aerated water, compared to 21% in air) and yeast cells, which can use oxygen when available, aren't very good at uptaking it from water. Thus yeast are termed **facultative anaerobes** because they can survive and do cell respiration when they have effectively no oxygen. This type of anaerobic cell respiration only goes through step one (glycolysis) of the fore-mentioned 4 step process of aerobic cellular respiration and so doesn't make as much ATP as the full four step aerobic process.

The process of **fermentation** is one type of **anaerobic** cellular respiration, respiration where oxygen is not needed to release the stored energy in glucose. Energy is still released from glucose in the form of electrons, but a molecule other than oxygen is the final electron acceptor in the fermentation reactions. Anaerobic

fermentation is relatively inefficient because glucose doesn't get broken down completely into water and carbon dioxide and all the stored energy is not released. Consequently, there is a net yield of only 2 ATP per glucose versus the more efficient release of 36 ATP per glucose of aerobic respiration.

In fermentation, the glucose is only partially broken down. Much of the energy originally available in glucose remains in the products produced. Plant and fungal cells produce alcohol as a result of fermentation and animal cells produce lactic acid. The equation for alcohol fermentation is below, and compared to aerobic respiration:

Anaerobic:

$C_6H_{12}O_6$ + zymase → 2 C_2H_5OH + 2 CO_2 + 2 ATP + heat energy

(glucose + yeast enzymes → ethanol + carbon dioxide + energy)

Aerobic:

$C_6H_{12}O_6$ + O_2 → 6 CO_2 + 6 H_2O + 36 or 38 ATP chemical energy + heat Energy

Such a limited amount of ATP as produced in yeast fermentation, or by some of the prokaryotic bacteria, is sufficient for a single celled yeast to perform its biological work; it would not be enough to sustain the high energy demands of a large multicellular eukaryote (such as a human) for very long. Humans do have one type of fermentation reaction that can be tolerated for short times – this occurs in overworked muscle cells that have used up the available oxygen and will ferment glucose into lactic acid, the buildup of which causes muscle cramps. When oxygen becomes available again (deep breathing) the lactic acid is converted back to a form that can be aerobically broken down to water and carbon dioxide. Lactic acid fermentation is also used by many bacteria and is involved in the formation of yogurt and sauerkraut. Another type of fermentation, called alcoholic fermentation, involves the release of ethanol and carbon dioxide by-products. Eukaryotic yeasts will produce beer and wine in this way.

Today in lab we try to determine the necessary ingredients for yeast fermentation of sugar, and as an extension we can also determine the rate of fermentation in yeast cells. We will measure the rate of cellular respiration using either distilled water or one of two different sugar sources, glucose (a monosaccharide) or sucrose (a disaccharide). You can modify this lab by adding starch (a polysaccharide).

In order to be able to measure whether or not fermentation is occurring you need to be able to measure either how fast your reactants (to the left of the arrow) are disappearing (being used up) **<u>or</u>** how fast your products (to the right of the arrow) are being formed.

Materials: *per group:* 30 ml live yeast solution and 10 ml boiled yeast solution, 30 ml 5% glucose solution, 60 ml 5% sucrose solution, water, 10 ml graduated cylinders, 4 fermentation vials vials (each vial has 1 small and 1 large component), timer, ruler, lab notebook and pencil.

Carolina Biological: Replacement Vials – Basic Fermentation Biokit RE-202200

Small = C70659 @ $0.50 each

Large = C70538 @ $0.50 each (indicate 'Price per Missy Hodges' on P.O.)

Previous Knowledge: (mathematical and computational thinking): Remind the students that when displayed graphically, a line with a steeper slope has a faster **rate** of reaction than a line with a slope that is more gradual and not as steep.

Previous Knowledge: (biology): **Enzymes** are protein catalysts, meaning they make exergonic reactions occur more quickly without being changed, used up, or destroyed – they do this by lowering the energy of activation needed to change the reactants (or substrate) to product. Heat and acid can both denature proteins (and thus enzymes). When an enzyme is denatured, it unfolds or unravels, and loses its three-dimensional shape – so the active site isn't present and the enzyme will not function. There are about 11 enzymatic reactions that convert glucose to alcohol and CO_2 anaerobically.

Procedure:

In groups of 4, assign each student one treatment to set up. After each of the 4 treatments in the group are set up, place all right side up next to each other, in order. At the same time each student will flip his fermentation vial upside down, and at this point you will start to time the reactions.

Every group will perform all four of the treatments once, but as a class we will have replicates (as many replicates as we have groups). Remember, replication is a part of science – when a large number of trials, or replicates, have consistent results you become increasingly confident that your results are "true".

The following table shows the four treatments that each group will do.

Treatment Group	Yeast added to Vial	Solution added to Vial
A	10 ml live yeast	5% glucose
B	10 ml live yeast	5% sucrose
C	10 ml boiled (killed) yeast	5% sucrose
D	10 ml live yeast	water

(**TEACHER**: take 4 packets of yeast and mix with about 400 ml of warm water about 10 minutes before beginning lab. Then, remove about 80 ml and microwave it or use a hot plate to boil it for 2-3 minutes. When microwaving, be careful to watch so that it doesn't boil over and spill! About 1 minute in 20 second bursts is usually fine). You need 10 ml boiled yeast and 30 ml live yeast for EACH group).

1. Swirl the baker's yeast (*Saccharomyces cerevisea)* suspension well and pour into a graduated cylinder to the 10 ml mark.

2. Pour your yeast suspensions into the small plastic fermentation vial. You need 10 ml of either the live yeast of the heat-killed yeast.

3. Start the reaction by filling the small vial <u>to the brim</u> with water or sucrose as indicated in the table, and immediately lower the larger plastic vial upside down on top of the smaller vial to cover it.

4. When <u>all 4 treatments</u> are ready, holding the vials together with your finger and thumb, invert the vials three times to mix the yeast solution. Place the vials <u>upside down</u> so that the smaller one is now resting upside down inside within the larger one (as pictured). Make sure they are in a well-protected area of your desk so that you won't knock it over.

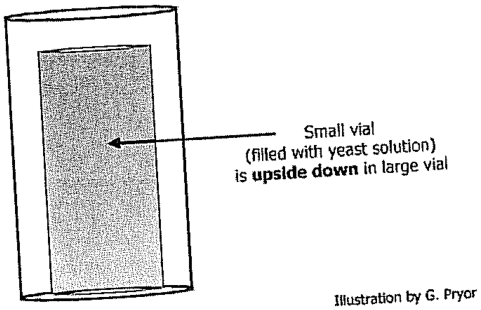


Illustration by G. Pryor

5. Start timing your reaction as soon as you set the vials upside down on the desk. If fermentation occurs, a carbon dioxide gas bubble (or many small bubbles) will appear at the top (really, the bottom) of the small vial. The reaction mix will be displaced, and pushed out into the larger vial. If foaming makes it hard to see the inner vial, move the smaller vial against the wall of the larger one (don't press down on it though!)

6. Using your ruler, measure (in mm) the length of the gas bubble as time progresses. Measure once every 5 minutes for 30 minutes.

7. The data from each group should be listed in a table constructed on the blackboard, averaged, and then plotted as a line graph.

Data Analysis:

You are measuring the size of the fermentation bubble (in mm) at each 5 minute interval, for thirty minutes total. You have 4 different treatment groups. See the sample data table.

Time (min)	Tr. Group A Trials 1 2 3 4	Avg	Tr. Group B Trials 1 2 3 4	Avg	Tr. Group C Trials 1 2 3 4	Avg	Tr. Group D Trials 1 2 3 4	Avg
0								
5								
10								
15								
20								
25								
30								

Table 1. Size of fermentation bubble for each treatment (Tr.) group.

Graph the average SIZE of the fermentation bubble vs. TIME for each of the 4 treatment groups. We have two options here. Each individual group can record and graph their own results, in which case there are no replicates (or trials). However, the better idea is to include class data (**replication** gives you more confidence in your results). Draw a LINE graph with each line representing a different treatment group - so there will be four lines total on the graph.

What goes on the X-axis? In this case, TIME (min) is on the X-axis and the lines are the different treatments (what the experimenter manipulated).

What goes on the Y-axis? (What did you measure? The SIZE of the fermentation bubble (mm)).

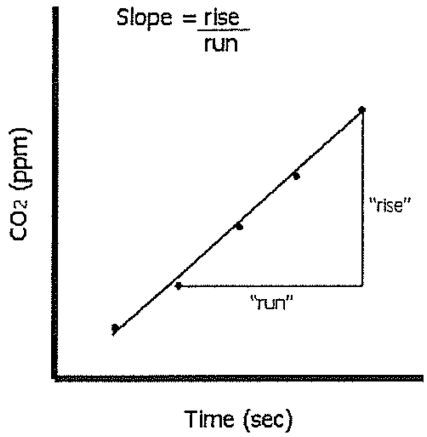
What are some variables that should be **Controlled** for? (amount of yeast solution, temperature the yeast solution incubated at, amount of sugar or water solution added, size of the vial, etc).

What can be considered our **Control** (or comparison) experiment? (The experiment with just yeast solution and water, and the experiment with boiled yeast solution and sugar). In neither case do we expect fermentation bubbles to form, because one of the necessary ingredients in absent. If bubbles do form, then we can conclude we did something wrong!

Looking at your graph, which treatments indicated fermentation was occurring?

Last, determine the RATE of fermentation in the vials that had a reaction using slope = rise / run. The slope is the rate of the fermentation reaction.

Remind the students that when displayed graphically, a line with a steeper slope has a faster rate of reaction than a line with a slope that is more gradual and not as steep.



Reflection Questions:

1. **What gas was produced by the yeast in the experiment?** (CO_2).

2. **Remember that sucrose is a disaccharide, a combination of 1 glucose plus 1 fructose molecule. To ferment sucrose, it must first be broken down into glucose and fructose (a different yeast enzyme does this).**

    a) **Which sugar, sucrose or glucose, appears to be broken down faster by yeast cells?** (glucose).
    b) **Explain this in your own words:** (it is a monsaccharide).

3. **Looking at the graph, how can you tell which sugar was broken down faster? (had the fastest rate of reaction)?** (glucose).

4. **What was the purpose of using a treatment with boiled yeast and a sucrose solution?** (control – enzymes denatured, what will sugar do on its own?).

5. **What was the purpose of using a treatment with yeast and water (instead of sugar)?** (control – no sugar. what will yeast do on its own, will it make CO_2?)

6. **After about 20 minutes of incubation, the gas production rate often slows down. What happens to the slope of the curve when the rate slows down? What might be an explanation for this reduction in rate?** (the slope decreases, flattens. Perhaps the yeast cells make toxic wastes and so are less healthy, do less respire., perhaps the use up the sugar, perhaps they are overcrowded as they grow, etc).

Models and Explanations: This experiment looks at the factors needed for anaerobic fermentation of sugar by yeast cells, and at the rates of fermentation of mono vs. disaccharides. For fermentation to occur, you need to have all of the following: a live cell (containing enzymes to break down, or ferment, the sugar), and a sugar source. Heat-killed cells have had their enzymes denatured by the high temperature, and so these enzymes don't work. No sugar means no source of carbon for the live cell to ferment, or break down. **A student who demonstrates understanding** of these concepts can explain that the heat-killed cells have been denatured and so vials containing boiled yeast will not ferment, nor will vials that lack a carbon source as food for the yeast. To have successful anaerobic fermentation you don't need oxygen but you do need a sugar source and the enzymes required to break that sugar down. Last, sucrose is expected to produce CO_2 at a slower rate because it is a disaccharide and must first be converted to glucose by the cell, before it can be broken down.

Bibliography:

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Student Worksheet:

Cells can obtain the 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 **anaerobic respiration**, also known as **fermentation**--but far less energy per glucose is extracted, since glucose cannot be fully broken down without oxygen. In this lab we will look at how baker's yeast, the fungus *Saccharomyces cereviseae*, anaerobically breaks down sugar into alcohol, CO2 and ATP: $C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + 2$ ATP. Using appropriate equipment, we can estimate the **rate** of this pathway for any given sugar by determining the yield of CO_2 over time.

Question: What do we need in order to see a fermentation reaction? Or, which of the following situations will produce a fermentation reaction?

Treatment Group	Yeast added to Vial (do this first)	Solution added to Vial (fill to brim)	Prediction: Reaction will occur – yes or no?
A	10 ml live yeast	5% glucose	
B	10 ml live yeast	5% sucrose	
C	10 ml boiled (killed) yeast	5% sucrose	
D	10 ml live yeast	water	

Hypothesis: If fermentation occurs, then carbon dioxide gas will be produced.

Predict: which of the following reactions (A, B, C, D) will produce a fermentation reaction?

What will be the **independent** variable?

What will be the **dependent** variable?

What variables should be **controlled** for?

What treatment(s) can be considered the **control** (or comparison) experiment(s)?

Why did we do many trials and not just one?

Data:
You are measuring the size of the fermentation bubble (in mm) at each 5 minute interval, for thirty minutes total. You have 4 different treatment groups.

Time (min)	Tr. Group A Trials 1 2 3 4				Avg	Tr. Group B Trials 1 2 3 4				Avg	Tr. Group C Trials 1 2 3 4				Avg	Tr. Group D Trials 1 2 3 4				Avg
0																				
5																				
10																				
15																				
20																				
25																				
30																				

Table 1. Size of fermentation bubble for each treatment (Tr.) group.

What was the conclusion of your experiment?

Was your hypothesis supported or rejected?

Graph your data.

Reflection:

1. What gas was produced by the yeast in the experiment?
2. Remember that sucrose is a disaccharide, a combination of 1 glucose plus 1 fructose molecule. To ferment sucrose, it must first be broken down into glucose and fructose (a different yeast enzyme does this). Which sugar, sucrose or glucose, appears to be broken down faster by yeast cells? Why?
3. Looking at the graph, how can you tell which sugar was broken down faster? (had the fastest rate of reaction)?
4. What was the purpose of using a treatment with boiled yeast and a sucrose solution?
5. What was the purpose of using a treatment with yeast and water (instead of sugar)?
6. After about 20 minutes of incubation, the gas production rate often slows down. What happens to the slope of the curve when the rate slows down? What might be an explanation for this reduction in rate?