Investigating Bean Plant Respiration

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-LS1.C; 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): Do plants undergo aerobic cellular respiration?

Conceptual Understanding: Cells are the most basic unit of any living organism. All organisms are composed of one (unicellular) or many cells (multicellular) and require food and water, a way to dispose of waste, and an environment in which they can live in order to survive.

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.

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.

The Plant Kingdom consists of organisms that primarily make their own food (autotrophs) and are commonly classified based on internal structures that function in the transport of food and water. Plants have structural and behavioral adaptations that increase the chances of reproduction and survival in changing environments.

Background: We will measure the rate of aerobic cellular respiration in germinating bean seeds (a plant) by measuring the volume of O_2 consumed.

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.

Living things need energy to carry on most of their processes. 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. These reactions are collectively called "cellular respiration".

ALL living things (plants AND animals) undergo cellular respiration – that is, they break down food into ATP. The ATP is a smaller form of chemical energy than food energy, such as glucose, and so is more useable (imagine ATP as a $1 bill (and you have 100 of them!) and imagine that you have 1 glucose sugar (symbolized as a single $100 bill) it's the same amount of money (or energy), but which will be more useful at a Coke machine? The goal of aerobic cellular respiration is to take the higher energy food molecules (like glucose, or fat, or protein) and convert them into many, but lower energy, ATP molecules. Both food (like sugars, starches, proteins, or fats) and ATP are chemical forms of energy, versus light energy or heat, but they differ in size. We store energy in our bodies as the larger starches and fats; we use energy as the smaller ATP's. Aerobic cellular respiration in the mitochondria takes the larger energy rich food molecules and converts them to smaller ATP molecules. So ALL living things – plants, animals, bacteria, protists, fungi – need ATP and they get ATP by breaking down food in a complex process. Some organisms do this anaerobically (some bacteria and yeasts, for example, undergo alcoholic fermentation when they, in the absence of oxygen, break down sugar into alcohol and CO_2) but most living things require oxygen for cellular respiration. The cells of germinating seeds, for example, oxidize carbohydrates (sugars) to produce the energy needed for growth of the seedling.

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 glucose by eukaryotic organisms (prokaryotes make 38).

Eukaryotic cells have a special organelle, the mitochondria, in which ATP energy is made aerobically in a 4 step process. Aerobic cell respiration is of course more complicated than the above equation hints at. The four parts to aerobic cell respiration are: 1. glycolysis, where glucose is broken down into pyruvate in a series of enzyme mediated steps; 2. pyruvate conversion into acetyl coenzyme A; 3. 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 4. 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.

The important thing to remember for younger students is that ALL living things need energy, and ALL living things process food into ATP by a process called cellular respiration. So – both plants and animals do cellular respiration, and both plants and animals have organelles called mitochondria in which this processing of food takes place. Plants and animals both do respiration; but plants alone do photosynthesis (well, also photosynthetic bacteria and protists, but again, for younger students, you can leave it at plants versus animals). Plants and Animals both have mitochondria, but only plants (not animals) have chloroplasts (the organelle of photosynthesis).

SO, just like animals, plants NEED oxygen too (its true plants produce oxygen in photosynthesis – much of this is released through the stomata in plant leaves. Much of the oxygen that plants need comes from the soil – in through the roots. This is why when you overwater a plant, and the soil becomes waterlogged, a plant may die - if water fills all the air spaces in the soil (instead of air which has 21% oxygen) then oxygen content of the soil decreases as water doesn't hold as much oxygen as air does (maybe 5%). So to prove that plants need oxygen, and use it in a process called aerobic respiration, a respirometer is set up.

Materials: *per group:* three test tubes with two-holed rubber stoppers set us as a respirometer (with a bent glass pipette in one hole and a tube that can be clamped shut in the other), cotton balls, 15% KOH I a dropper bottle, germinated bean seeds (seeds that have been soaked under damp paper towels for 4 days), dry bean seeds (not germinated), a large glass beaker, water, timer, lab book and pencil, marker dye (food coloring in water), a plastic pipette, and safety glasses.

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): The important thing to remember for younger students is that ALL living things need energy, and ALL living things process food into ATP by a process called cellular respiration. So – both plants and animals do cellular respiration, and both plants and animals have organelles called mitochondria in which this processing of food takes place. Plants and animals both do respiration; but plants alone do photosynthesis (well, also photosynthetic bacteria and protists, but again, for younger students, you can leave it at plants versus animals). Plants and Animals both have mitochondria, but only plants (not animals) have chloroplasts (the organelle of photosynthesis).

Previous Knowledge: (chemistry / physical science): Temperature will also affect the measurement of gas consumption because gasses expand when they warm and contract when they cool.

Procedure: In groups of 4, gather materials for three respirometers. Every group will perform all three 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	Seeds added to Vial
A	None
B	Half full with germinated seeds
C	Half full with dry (ungerminated) seeds

Temperature will also affect the measurement of gas consumption because gasses expand when they warm and contract when they cool. This effect will be minimized by keeping the respirometer tubes immersed in water at room temperature. Water temperature changes slowly, so the water will minimize temperature fluctuations inside the tubes.

1. Set up the three respirometers as follows: Place two cotton balls in the bottom of each tube. The cotton should occupy approximately 2 cm of space on the bottom of the tube.

2. Use a dropper to add 15% KOH solution to the cotton in each tube. Use enough KOH to saturate the cotton but not enough to pour out of the test tube. Use the same amount of KOH in each tube. Be careful not to let KOH come in contact with the sides of the test tube because it will kill the bean seeds.

3. Push a small wad of dry cotton on top of the KOH-saturated cotton in each tube. This will prevent KOH from coming in contact with the bean seeds and killing them.

4. Fill one of the tubes half full with germinated bean seeds and record the number of bean seeds used.

5. Fill the second tube half full with ungerminated (dry) bean seeds – use the same number of seeds as you used in tube 1 (this is a controlled variable).

6. The third tube will remain empty to measure the effect of temperature changes.

7. Assemble each apparatus and place the tubes in the water tank. Be sure that the rubber stopper is inserted tightly into the test tube. The valve in the stopper of each tube ***should be kept open so that air can move through the stopper*** and into or out of the tube.

8. Before adding the marker dye, double-check to see that the valve on top of the stopper is open and that air can pass through. Sometimes the rubber tube that passes through the valve remains squeezed shut even though the valve is open. Be sure that you can see an opening through the tube.

9. After the respirometer tube is inserted into the water tank, use a dropper to push a drop of a colored liquid into the tip of each of the graduated pipettes. Try to force the marker into the region past the tip where its position can be read using the calibrations on the pipette.

10. Squeeze the plastic bulb to push the liquid marker dye into the glass pipette as shown below. The glass pipette is marked with milliliter marks but the narrow tip does not have any marks. The dye should be pushed far enough into the pipette that it is in the area where there are milliliter marks. This will enable you to read how far the dye moves from the beginning of the experiment to the end.

11. Record the time that the respirometer is set up and the dye is in place. You will be ready to begin the experiment after the tubes have been in the water for 5 minutes.

12. Before beginning the experiment, be sure that the dye in the pipette is in a region of the pipette that has calibration marks. This is important because you will measure the amount of movement of the dye. Also, check the rubber stoppers to be sure that they are firmly inserted into the test tubes.

13. After the respirometer has been idle for 5 minutes, ***close the valve in the top of each of the tubes***. After the valves are closed, any air movement into the tubes will cause the dye in the pipette to move inward. After the valves are closed, record the position of the dye and continue to record its position every 5 minutes for a total of twenty five minutes.

14. While you wait you will use the respirometer that does not contain bean seeds to test the effects of temperature (here, with no living cells, cellular respiration is not happening). Create hypotheses regarding the movement of the liquid in the

respirometer tube when it is placed in cold water and when it is warmed. Record these hypotheses in your notebook.

- **Hypothesis**: If the gasses are warmed they will expand.
- **Prediction**: when the respirometer is in cold water, the dye will move towards the test tube (circle one) ___slowly / quickly___ Which direction does it move? ____________________
- **Prediction**: when the respirometer is in warm water, the dye will move (circle one) ___slowly / quickly___.

  Which direction does it move? ____________________.

15. Place the respirometer in a beaker of ice water to observe movement of the fluid in the pipette. Remove the tube from the icewater before the marker dye moves all the way into the tube. Next, warm the tube by placing it in hot water. What happened in each case? Record your observations in your notebook.

Data Analysis:

You are measuring the respiration of the germinated bean seeds by measuring the gas volume inside the respirometer. As the seeds respire, they will take in oxygen from the air in the test tube. As O_2 gas is used up in aerobic cellular respiration, gas volume in the respirometer will decrease. This causes the marker dye to travel in the graduated pipette toward the test tube. However, all living things are also giving off CO_2 during respiration, and that pushes the marker dye AWAY from the test tube. Any change in gas volume will be due to both O_2 consumption and CO_2 production. In order to minimize the confounding effect of CO_2, KOH will be added to the tubes. It reacts with CO_2 to form solid potassium carbonate
($CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$). The solid will not have a measurable increase in the volume inside the tubes.

Temperature will also affect the measurement of gas consumption because gasses expand when they warm and contract when they cool. This effect will be minimized by keeping the respirometer tubes immersed in water at room temperature. Water temperature changes slowly, so the water will minimize temperature fluctuations inside the tubes.

TIME (min)	Germinated Bean Seeds							Ungerminated Bean Seeds						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average (mm)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average (mm)
0														
5														
10														
15														
20														
25														

Table 1. Distance Dye Moved in Respirometer (mm)

Graph the average distance the dye moved in the pipette. You will make a line graph, with two lines (use a key). What goes on the X-axis? In this case, Time (min).

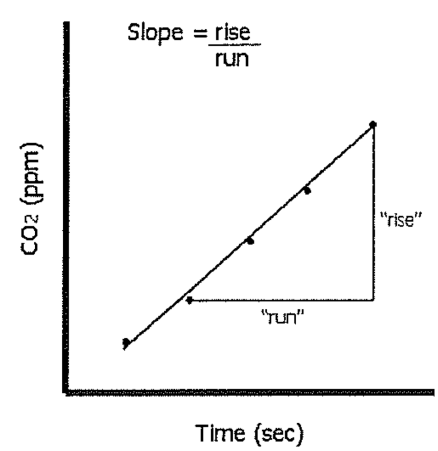
What goes on the Y-axis? (What did you measure? In this case, you measured the distance the water moved in the pipette, which indicates oxygen consumption. And, we have class data, and an average, so you can stress the importance of **replication**!

What are some variables that should be **Controlled** for? (again, there is a difference between controlled variables and the control experiment (here, the ungerminated seeds)

What can be considered our Control experiment? (seeds that were not germinated – these seeds are in a dormant state and all metabolic reactions are veeerrrrrrryyyy slow.

Last, determine the RATE of respiration using slope = rise / run. The slope is the rate of the 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__:

- **What is the function of using a respirometer without any bean seeds in this experiment?** (control)
- **What two gasses are involved in aerobic respiration? How should they have affected the dye in the tube that contained germinated bean seeds? To answer this question, review the equation for cellular respiration and review the discussion of KOH above.** (oxygen and carbon dioxide. We breath in O2 and breathe out CO2. Plants also take in O2 and respire CO2.
- **Explain how temperature fluctuations affect the apparatus and how these were corrected.** Respiration can produce waste heat. We used a water bath to try to keep temperature constant.

__Models and Explanations__: Plants, just like animals, undergo aerobic cellular respiration in their mitochondria. SO, just like animals, plants NEED oxygen too (its true plants produce oxygen in photosynthesis – much of this is released through the stomata in plant leaves. Much of the oxygen that plants need comes from the soil – in through the roots. This is why when you overwater a plant, and the soil becomes waterlogged, a plant may die - if water fills all the air spaces in the soil (instead of air which has 21% oxygen) then oxygen content of the soil decreases as water doesn't hold as much oxygen as air does (maybe 5%). So to prove that plants need oxygen, and use it in a process called aerobic respiration, a respirometer is set up. **A student who demonstrates understanding** of these concepts can explain that both plants and animals undergo aerobic cellular respiration (though only plants do photosynthesis. This explanation should include the effects of temperature on the rates of respiration.

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Extension: During **aerobic** cell respiration, **oxygen** is needed to release the stored energy of glucose, and CO_2 and H_2O are produced. The reaction for aerobic cell respiration is shown here:

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

The increasing concentration of CO_2 in a closed environment can be observed using a pH indicator solution, as when the CO_2 dissolves in a water solution it is converted to carbonic acid, which makes the solution more acidic (lower in pH). To observe the formation of CO2 you can use an indicator solution (bromothymol blue – which turns yellow in the presence of CO_2, or when pH lowers).

Start by discussing indicator solutions (like the yellow-brown iodine, which, when placed on a starchy potato, turns blue-black to indicate the presence if starch). Using a straw and a test tube or cup filled halfway with water and bromothymol blue, blow into the blue solution until it turns, first green, then yellow. Be careful not to blow to hard – BB stains clothing!

Next, take about 50 pea seeds – 25 are dry and 25 have been soaked for 4 days in wet paper towels, and are germinating, Put the 25 dry seeds in a closed container (like a cleaned peanut butter jar). Place a test tube with water and bromothymol blue in the container as well, and then seal with the top.   Take the 25 germinating seeds and repeat, in a new container, with a second test tube of BB/water, and seal that top. Last, make a container that has no seeds, add the test tube of BB/water, and seal it. Talk about controlled variables, like same size / type container in all three set-ups, and talk about control experiments. This is also a good time to talk about qualitative (color, or feel, or sound for example) versus quantitative data (good hard numbers). Observe the test tubes over the next few hours, and days.   In the container with germinating seeds, the color of the liquid in the test tube should turn yellow as CO_2 from the germinating seeds is released.   You can make a data table like the following, recording the color of the test tube each day.

Date	Jar with dry beans	Jar with germinating beans	Jar with no beans
Day zero			

Table 1. Carbon dioxide production by germinating seeds as seen by color change.

Furthermore, there is a lab on enzymes (see table of contents) that tests for enzyme production in germinating versus non-germinating seeds. For a seed to undergo cellular respiration, it will use a variety of enzymes to break down carbohydrates in a series of enzyme-mediated steps. You can test for the presence of enzymes by testing whether the complex starch (a good energy rich storage form found in the bean seeds) is converted to glucose sugar (which is a smaller carbohydrate and which is an easier starting point for aerobic cellular respiration. Seeds that have been germinated show the presence of glucose (as tested for using glucose urine test strips, from the pharmacy, as used by diabetics) but seeds that are dry, and not germinating yet, will not.

Student Worksheet:

ALL living things need energy, and ALL living things process food into ATP by a process called cellular respiration. So – both plants and animals do cellular respiration, and both plants and animals have organelles called mitochondria in which this processing of food takes place. Plants and animals both do respiration; but plants alone do photosynthesis (including the photosynthetic protists and bacteria) Plants and Animals both have mitochondria, but only plants (not animals) have chloroplasts (the organelle of photosynthesis). Seeds germinate, which means they take in water, swell, and begin metabolic reactions including cellular respiration which produces the energy needed to grow the seedling's roots and shoots. In this lab we will measure the rate of aerobic cellular respiration in germinating bean seeds (a plant) by measuring the volume of O_2 consumed.

Question: Do plants undergo aerobic cellular respiration?

Hypothesis: If aerobic cellular respiration is occurring, then as oxygen gas is consumed in the test tube, the marker dye will move towards the test tube.

Predict: which of the following reactions (germinated or ungerminated seeds, or both, or neither?) will produce an aerobic cellular respiration 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** experiment(s)?

Also remember that temperature affects oxygen gas in that gases expand when warmed and contract when cooled.

Hypothesis: If the gasses are warmed they will expand.

Prediction: when the respirometer is in cold water, the dye will move towards the test tube (circle one) ___slowly / quickly___ Which direction does it move? ___________

Prediction: when the respirometer is in warm water, the dye will move (circle one) ___slowly ___ / ___quickly___. Which direction does it move? ___________.

Data:
You are measuring the distance the marker dye moved in the pipette (in mm) at each 5 minute interval, for twenty five minutes total. You have 2 different treatment groups.

TIME (min)	Germinated Bean Seeds							Ungerminated Bean Seeds						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average distance (mm)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average distance (mm)
0														
5														
10														
15														
20														
25														

Table 1. Distance Dye Moved in Respirometer (mm)

Graph your data.

What was the conclusion of your experiment?

Was your hypothesis supported or rejected?