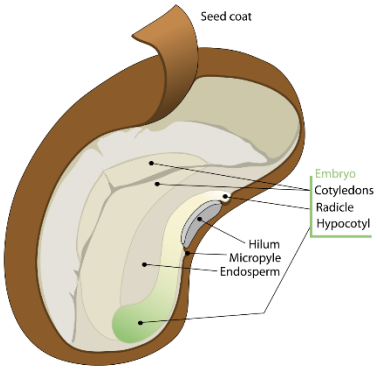
Cellular Respiration: Peas

This activity uses germinating pea seeds. Seeds are the 'offspring' of plants. While to most of us they look relatively inert or dead, seeds are actually viable organisms. Because seeds are generally NOT photosynthetic they must acquire the energy needed for growth and development from stored compounds within the seed. Under less than favorable conditions, they metabolize at a very low rate, but they do metabolize including generating ATP via aerobic cellular respiration. Once the seed is hydrated (imbibition), taken in water, germination begins. Germination includes the production and activity of many enzymes. This is a period of astounding growth for the newly developing plant.



The image to the left is a typical dicot seed. Recall the dicotyledons are a group within the flowering plants. Their seeds include 2 seed leaves or cotyledons. Peas and beans are both dicots. The seed is surrounded by a tough protective covering called the seed coat. The hilum marks the location where the seed was attached to the parent plant. The micropyle is a small pore-opening through which the pollen (plant male sex cells) passed during the fertilization of the ova, which led to the formation of the seed. The endosperm surrounds the embryo. It is produced after fertilization; starch, oils and proteins are stored in the endosperm to nourish the embryo. The embryo consists of the cotyledons, radicle and hypocotyl. The cotyledons are the seed leaves, the first leaves to appear above ground. The radicle is the fastest growing part of the embryo and will become the embryonic root. The hypocotyl becomes the stem below the seed leaves.

Just as a reminder, the summary reaction for aerobic cellular respiration is given below.

Summary Reaction for Aerobic Cellular Respiration

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + ATP$$

Glucose **Oxygen** **Carbon dioxide** **Water** **Adenosine triphosphate**

Aerobic cellular respiration is a multi-step, energy-yielding process that occurs in the cytoplasm and mitochondrion of all eukaryotic organisms, including plants. During the first step (glycolysis) of the process glucose is 'broken' in half. Enzymes in the cytoplasm perform this initial split of glucose (food/carbohydrate). Enzymes located in the mitochondrion complete the breakdown. The complete breakdown of glucose yields carbon dioxide, water, and ATP, the universal energy currency.

In this activity you will be using pea seeds that have been hydrated over night to begin germination. Pea and bean seeds possess the same internal and external structures. You can refer to the picture above during the activity. You will be using pea seeds that were boiled and some which have been germinating for 24 hours and 48 hours.

Write a hypothesis regarding oxygen consumption and pea germination: _____

_____

Materials

Vials or large test tubes (18 mm x 150 mm) – 4
Cotton balls
Potassium hydroxide (KOH) (15 %)
Pipette – Pasteur - 2
Volumeter – cork with bent pipette inserted
Marker
Aluminum foil
Beaker – 250 mL, 4
Beaker – 50 mL, 1
Test tube rack
Glass rod
Paper towel
Dye or tinted glycerol or tinted water
Pea seeds* – un-germinated
Pea seeds* – 24 hour germination
Pea seeds* – 48 hour germination
Pea seeds* – boiled

*other seeds can be substituted for peas. Alternatively, half the class can be asked to do the activity with peas, the other half could use another bean.

Procedure

1. Pick up 4 vials or test tubes, test tube rack and 4 volumeters from the supply table.

2. Use the marker to label the 4 test tube: boiled, un-germinated, 24-hour, and 48 hour.

3. Place a cotton ball in each vial. Use the glass rod to push the cotton ball to the bottom of each vial/test tube. Place the test tubes in the test tube rack.

5. Return to the supply table. Add 30 boiled peas to a beaker. Gently add about 100 mL of tap water to the beaker. Gently swirl the beaker, then pour out the water. Repeat this rinsing procedure one more time. Take this beaker back to your bench and use the marker to label it 'boiled peas'.

*Bacteria often grow in the pea water. Rinsing the peas immediately before use helps lower bacterial numbers.

6. Return to the supply table. Add 30, 24-hour germinated peas to a beaker. Gently add about 100 mL of tap water to the beaker. Gently swirl the beaker, then pour out the water. Repeat this rinsing procedure one more time. Take this beaker back to your bench and use the marker to label it 'germinated'.

7. Return to the supply table. Add 30, 48-hour germinated peas to a beaker. Gently add about 100 mL of tap water to the beaker. Gently swirl the beaker, then pour out the water. Repeat this rinsing procedure one more time. Take this beaker back to your bench and use the marker to label it 'germinated'.

8. Return to the supply table and place 30 un-germinated peas in the beaker. Take this beaker back to your bench and use the marker to label it 'un-germinated'. **Do not rinse these peas.**

9. Return to the supply table and dispense ~15 mL of 15 % potassium hydroxide into the 50 mL beaker.

10. At your bench use a pipette to add 15 % potassium hydroxide to each vial. Saturate the cotton ball, 3 (60 drops) to 4 (80 drops) mL of solution should be adequate.

During aerobic cellular respiration oxygen is consumed and carbon dioxide released. In order to observe and measure the consumption of oxygen, the evolution of carbon dioxide has to be negated. Potassium hydroxide (KOH) absorbs carbon dioxide, therefore any change in the volumeter should be reflect oxygen consumption only. The impact of carbon dioxide release should be negligible on the readings of the volumeter.

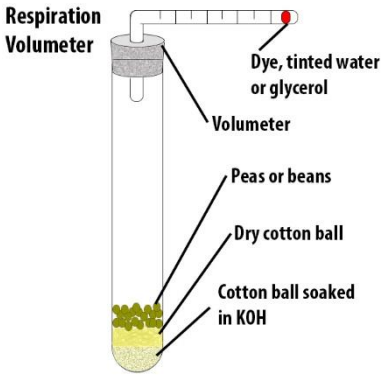
11. Add a second cotton ball to each tube. Use the glass rod to push the cotton down the tube.

12. Transfer 30 un-germinated peas from the un-germinated beaker to the test tube or vial labeled un-germinated.

13. Transfer 30 boiled peas from the 'boiled' beaker to a paper towel, then add the peas to the test tube labeled boiled. The paper towel should remove any extra moisture from the outside of the peas. Repeat this step with the 24 hour-germinated peas and the 48 hour-germinated peas.

14. Insert a volumeter into each vial or test tube. The volumeter must be firmly in place. Your instructor may have you use Vaseline around the stopper to better seal the tubes.

15. Wrap each test tube in aluminum foil and return it to the test tube rack.



16. Use the Pasteur pipette to add ~1 mL of dye or tinted glycerol to the end of the bent volumeter tube. Mark the location of the dye with the marker. Note the time. Repeat this procedure for each vial.

17. At 30 minute intervals, mark the location of the dye. Allow peas to incubate for 2 hours, marking the location of the dye spot on the volumeter every 30 minutes.

18. If the volumeter is graduated in mL, record the mL changes for each test tube and each time period below. If the volumeter is not graduated in mL, measure the distance of each mark for each time period from the initial mark. Record your results below.

	Time intervals (record the change in volume or measure the movement of the dye indicator)					Total volume change
	0	30	60	90	120	
Boiled peas						
Un-germinated peas						
24- hour germinated						
48- hour geminated						

Was your hypothesis supported by your experimental data? If not, why not?

Which sample metabolized the most oxygen? What explanation can you provide for this result?

Which sample used the least amount of oxygen? How do you explain this result?

What was the purpose of boiling the peas for the boiled pea sample?

Why is KOH used in this experiment?

Why were the test tubes or vials covered with aluminum foil?

Graph your results below.