Topic 7. Respiration

Respiration is the cellular process by which some of the chemical energy of a reduced carbon substrate is converted into the chemical energy of **ATP**. While it seems counter intuitive, like animals, plants respire. In the dark, plants have no other source of ATP. In these activities you will explore the various consequences of this fact.

Web Lesson @http://botit.botany.wisc.edu/botany_130/respiration

I. How respiration is partitioned in the plant cell.

For introductory courses such as this one, we consider the sugar, **glucose**, as the starting substrate. However, glucose is only one of a huge number of molecules that can be consumed by respiration to produce ATP. In the case of glucose, respiration starts with **glycolysis**. The process results in the net production of **pyruvate** molecules and **NADH** that go into the two other components of respiration. These pyruvate molecules are eventually consumed in aerobic respiration to produce **CO_2**, **H_2O**, and **ATP**.

Glycolysis occurs in the **cytosol**.

In the absence of oxygen, glycolysis is the only chemical source of ATP for cells not undergoing photosynthesis. For glycolysis to continue, NAD+ must be regenerated from NADH. In yeast cells, NAD+ is regenerated in a reaction that converts pyruvate into CO_2 and alcohol. This process is called **alcoholic fermentation** and we will observe the process in today's lab.

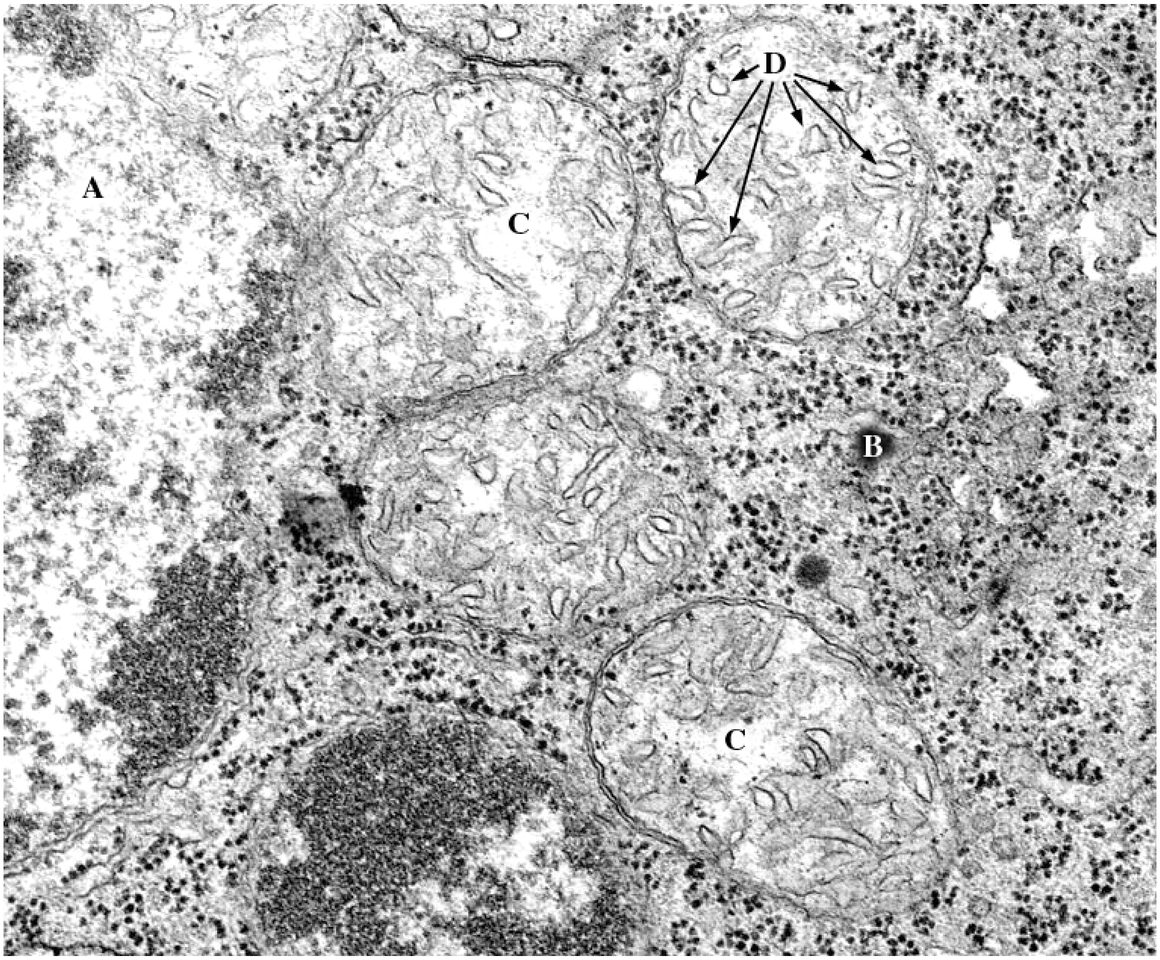
In **aerobic respiration,** the pyruvate first reacts with **CoEnzyme A** in the **matrix of the mitochondrion.**

In the process, pyruvate loses one carbon molecule to form CO_2, and a NADH is produced from NAD+. The two carbon molecule left, is bound to CoEnzyme A to form **Acetyl CoA**. Acetyl CoA enters into the **Krebs Cycle**, also in the matrix, where the two carbon component is broken down into **CO_2**. Some of its energy is captured in the form of **$FADH_2$**, **NADH**, and **ATP**. In today's lab we will detect CO_2 generated from germinating grains respiring aerobically.

In the mitochondrion, electrons from NADH and $FADH_2$ enter into the **electron transport chain** which, in an intact mitochondrion, is coupled to **oxidative phosphorylation** resulting in the production of ATP from ADP.

The electron transport chain occurs in the **cristae of the mitochondria**. High energy electrons taken from NADH and $FADH_2$ are passed from molecule to molecule down an energy gradient, and are eventually passed to oxygen to produce water. Some of the initial energy from these electrons are captured in the form of ATP. In today's lab, we will observe a substrate reduced to a red precipitate by electrons passed to it from the electron transport chain.

Observe the figure on the next page:



1. Where does glycolysis occur? ___________

2. Where does the Krebs Cycle occur? ___________

3. Where does the electron transport chain occur? ___________

4. Where does oxidative phosphorylation occur? ___________

II. Aerobic Respiration of Grains.

<u>Work in groups of two: four groups choose wheat, five groups choose corn.</u>

IIa. Release of carbon dioxide by respiring grains.

Germinating grains are an excellent subject for studying plant respiration as they have no photosynthetic tissues, and, hence, do not consume CO_2. Their entire energy needs are met by the starch stored in their endosperm. In this activity, we will determine the rate of CO_2 generation in germination grains in ml of gas per minute. This is directly related to the rate of oxygen consumption in ml of oxygen per minute, and can be converted into moles. A mole of gas at standard conditions occupies 22.4 liters. Hence, 1 ml of a gas makes up roughly .001 / 22.4 moles of that gas. Considering the balanced equation for respiration,

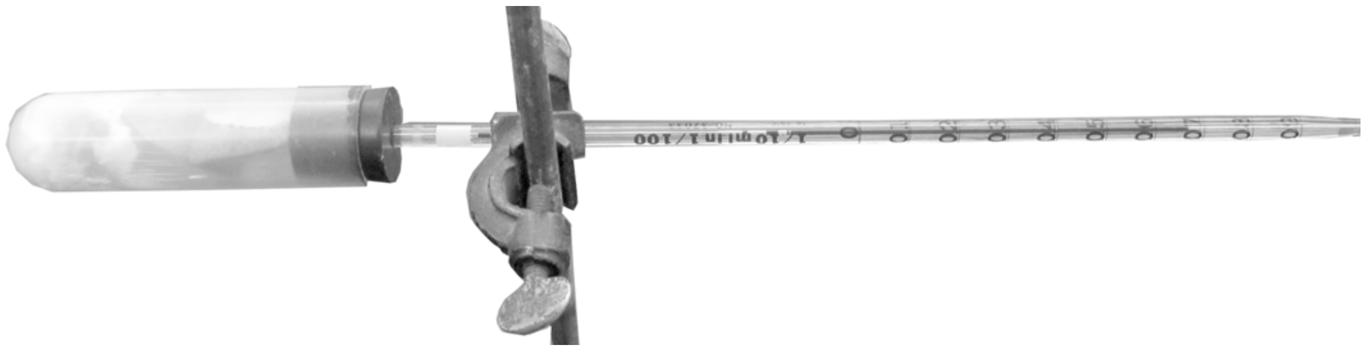
$$C_6H_{12}O_6 + 6\ O_2 \text{ ---------->} 6\ H_2O + 6\ C0_2$$

the moles of glucose consumed = one sixth of the moles $C0_2$ released.

To measure CO_2 release we will use paper saturated in KOH to absorb CO_2 gas generating KCO_3 which is a solid. By observing a change in volume in a closed system with germinating grains and KOH, we will can deduce the rate of evolution of CO_2 and calculate the rate of respiration.

Procedure: Take a 50 ml centrifuge tube and, using your forceps, carefully place a piece of filter paper saturated with KOH in the bottom. Note that KOH is caustic! Try to drop the paper directly to the bottom without touching the sides of the tube. Then take a loose wad of cotton and create a gas permeable barrier between the paper and the rest of the tube. Add 10 grams of germination grains and seal the tube with a stopper attached to a .1 ml pipette. See the figure below.

Take this complex and attach it to a ring stand by the pipette using the ring stand clamp. See the figure on the next page. Wait 5 minutes before initiating your observations. To begin, add a colored solution to the tip using a Pasteur pipette. Carefully monitor the level of the solution and the time lapsed until it the level moves into an uncalibrated area of the pipette. Record both the change in the level and the time lapsed in the table below. If possible repeat this process nine more times by introducing more colored solution at the tip of the pipette to initiate each recording session



Change in level (ml)	Time Lapsed	Rate of CO_2 Evolution*

* ml / minute

Calculate an overall rate: divide the sum of column one (ml) by the sum of column two (seconds):

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Collect data from the entire room and record them below.

Corn

Average rate for corn ______________________

Wheat

Average rate for wheat ______________________

Speculate about the variation found between groups using the same grain.

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Speculate about the variation found between the rates for corn and wheat.

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1. Does this experiment by itself, demonstrate that the grains are respiring aerobically?

2. What process/e of respiration release CO_2?

IIb. Observing Oxygen Consumption in Respiring Grains.

At the beginning of the period your TA placed 100 ml of corn grains into a 4 ounce screw cap jar and sealed it with an oxygen probe. There is a data sheet next to this oxygen meter. Your TA will have recorded the per cent oxygen at the beginning of the period. Please note this number and observe the oxygen concentration at the end of the period.

Oxygen concentration - start	Oxygen concentration - end

3. Does this result demonstrate that the grains are respiring aerobically?

4. What process/es of respiration consume O_2 ?

IId. Heat of Respiration of Respiring Grain.

Observe the thermos of respiring and dead grain on the side bench and fill in the table below:

Temperature of Live Grain	Temperature of Dead Grain

5. From your text or lecture, what is the efficiency of aerobic respiration in plant cells?

6. The chemical energy in glucose not captured as chemical ATP energy is lost in what form/forms of energy?

7. Is the generation of heat ever helpful in the survival of plants (is it ever adaptive?)? If so provide an example.

III. The Electron Transport Chain.

Work in groups of four.

Take a germinating grain of corn and cut both sets of grains in half as illustrated:

To the cut surfaces of these grains add triphenyl tetrazollium chloride (tetrazollium) drop by drop until the cut surface is thoroughly wetted. Set aside and observe after 15 minutes. Record your observations on the next page.

Dead Grains	Living Grains

Tetrazollium "steals" electrons away from the electron transport chain. As tetrazollium becomes reduced (as it accepts electrons) it forms a red precipitate.

8. Without tetrazollium present where do these electrons end up?

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IV. Alcoholic Fermentation in Yeast.

Work in groups of four.

IVa. At the sink, in a cooler of warm water, is a premixed suspension of yeast in 10% glucose. Mix this thoroughly and load a fermentation tube with the mixture (your TA will demonstrate how), and place the tube in the cooler in the warm water. Check on your tube in 30 minutes.

Smell the contents of the beaker and tube. What are your observations of the aroma?

IVb. Wine Making: Demonstration.

Your TA will add brewer's yeast to a flask of grape juice. Please observe what happens during the next seven labs. Be sure to check the water level in the fermentation lock when you make these observations.

9. One of two factors will cause fermentation to stop. What are they?

10. How is alcoholic fermentation different from glycolysis?

10. If an organism is respiring anaerobically, why is glycolysis insufficient?

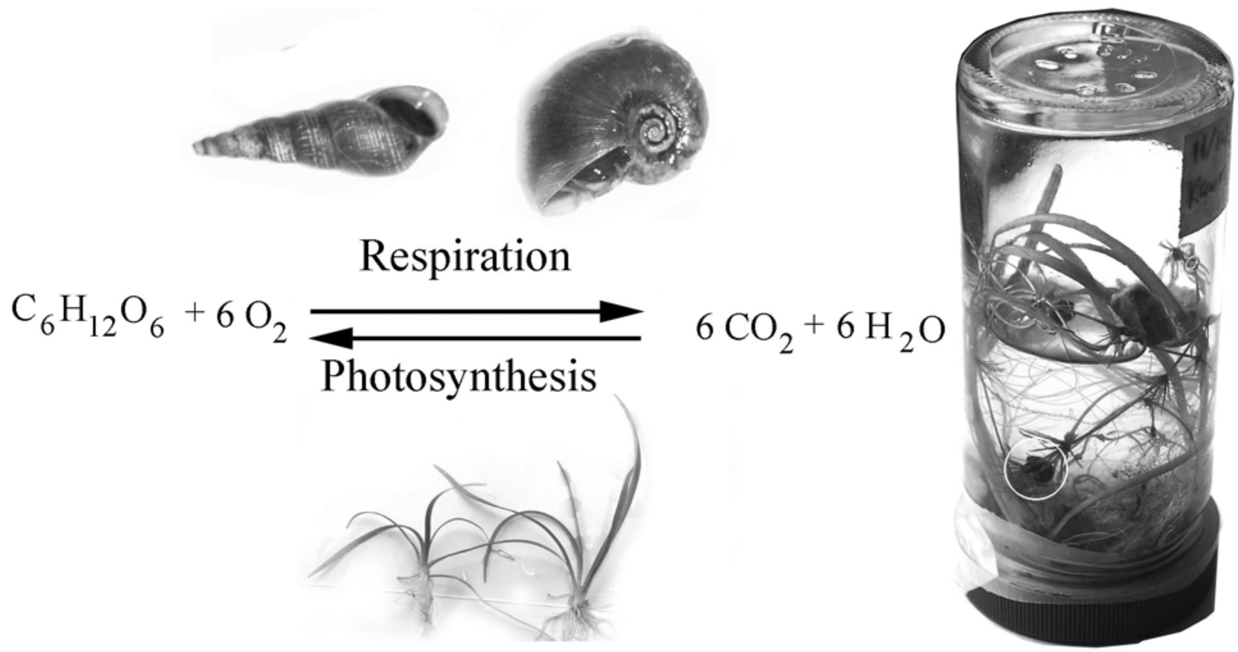
V. Respiration Work Sheet.

Take a work sheet, and complete this sheet in lab. Hand it in when completed, and we will review the questions/answers in discussion.

VI. Life in a Jar: An Exploration of Ecological Stability.

<u>Introduction</u>: Life is possible only within a temperature and pressure range where liquid water occurs. Conditions for animal life are even more restrictive in that animals require food derived from autotrophs and are sensitive to even a short deprivation of oxygen. We know that life has existed on Earth for at least 3.5 billion years. During this interval, astrophysicists tell us, the sun has been growing considerably brighter and, yet, the Earth's climate has remained stable enough for liquid water, and hence, life, to persist. Further, animal life has existed for at least 600 million years. During that time an atmosphere rich in oxygen must have been maintained without interruption. This activity is intended to impart an appreciation of the long-term stability of the biosphere.

One method of studying how ecosystems function, and maintain stability, is through the use of microcosms. A microcosm is a small version of an ecosystem set up to approximate the dynamics of a naturally occurring one. The advantages of working with microcosms are obvious as one can manipulate an artificial system in ways that would be ethically or physically impossible with a natural system. In lab today, you will create your own little self-contained ecosystem in an 8 ounce screw cap jar. You will use either plants or green algae or both as primary producers. Each jar will include snails that will serve as primary consumers. In each jar you will place a gravel substrate, a quantity of aquarium water, and a quantity of nutrient solution (fertilizer). Energy will enter the system from a bank of fluorescent lights that will be on continually.



Procedure: Stability, in these mini ecosystems, can be unambiguously defined as the continuation of physical conditions that allow for the survival of the animals (the most sensitive component of the community). Working with your TA, each bench of students will consider a different starting factors that affect stability. This may be wet weight of the biomass of the rooted aquatic plants, wet weight of the algae, the quantity of added nutrients, size of the snails, or any other condition that you believe will influence the stability of the system. Each group (bench) will then devise a plan to create a stable microcosm. If your system has living animals in March then you are a winner and will receive a reward during the final lab of the semester.

Setting up Biocylinders

1. Each system will be made using 150 ml screw-cap jars on the side bench. Each bench will put together one jar.

2. You may choose the quantity of gravel to be added to your jar.

3. You may choose the quantity of aquarium water you wish to add to your system.
4. You may choose the quantity of nutrient solution you wish to use, but it must be less than 5 ml.
5. Select a combination of rooted aquatic plants (*Sagittaria*), floating aquatic plants (Ceratophyllum), or filamentous algae as your primary producers. If you choose *Sagittaria* you must plant the roots in the substrate (Have things put together so that when you turn the jar upside down the roots will be covered).
6. Choose two snails. You may choose their size. Snails are hermaphroditic, hence, the possibility exists that they may produce a new generation.
7. Put your system together:
    a. Add gravel, aquarium water, and nutrient solution.
    b. Add your primary producers and snails to the jar (rooted plants should be inserted upside down).
    c. Screw the cap on tightly, and, seal it with silicone caulk.
    d. Carefully invert the jar. Avoid burying snails, but bury the roots.
    e. Take your jars to the light bench. Place it a the sheet with your section number. Mark the spot on that sheet where you will keep your cylinder. Label your jars with your table and section number.
8. Observe your jar each lab to check on the status of your animals. If they die please record the date when you first noticed their death. If the snails die continue observing to see if zooplankton appear. The survival of these smaller animals should be considered as evidence that the system remains stable even if the snails have died.

VII. The Carbon Cycle

Typically plants produce oxygen used by animals for respiration which in turn produce carbon dioxide used by plants in photosynthesis. The consequences of plant respiration can be critical for some environments and situations. Here are some situations to be considered with your group in discussion.

1. Oxygen levels in a weed choked stream
2. Effect of global warming on the primary production of tropical rain forests
3. The rate of growth of a tree measured as per cent increase in its mass as the tree ages

   After due consideration of “3” should we remove (log) the unproductive old-growth forests? What other factors should be considered?

4. The growth of plants in waterlogged soils
5. Life on a self sustaining lunar base where the nights are 14 days long

Review Lesson @ http://botit.botany.wisc.edu/botany_130/respiration