Topic 4: Energetics – 4d. Cellular Respiration Measured by Titration

Resources:

Campbell, N., Reece, J. (2005). *Biology: Concepts and Connections,* 5th edition. Pearson/Benjamin Cummings.

Bowen, R. Free Radicals and Reactive Oxygen [Internet]. Colorado State University. 16 Aug. 2003. Available from: http://www.vivo.colostate.edu/hbooks/pathphys/misc_topics/radicals.html

Antioxidants and Free Radicals [Internet]. Rice University. Sept. 1996. Available from: http://www.rice.edu/~jenky/sports/antiox.html

Building on:

All living things require *energy*. Energy is needed to grow, move, reproduce and to combat *entropy*. The ultimate source of energy on our planet is sunlight. Plants and other *producers* can take the energy of the sun and use *photosynthesis* to store that energy in the form of *organic molecules* like *glucose*. *Cellular respiration* and *fermentation* are needed to release the potential energy in those organic molecules and convert it into a useful form of energy capable of doing *work*, usually *ATP*.

Links to Chemistry:

Bond energy
Endothermic versus exothermic reactions
Organic molecules
Conservation of matter
Conservation of energy
Entropy
Oxidation – Reduction reactions
Gas laws
Stoichiometry
pH
Titration

Links to Physics:

Work
Potential and kinetic energy
Thermodynamics
Efficiency
Phase changes
Gas laws

Stories:

Cellular respiration requires oxygen and yet students read and hear about antioxidants that help to prevent the damage done by oxygen when it forms free radicals. This can be very confusing.

Free radicals are atoms or molecules that contain unpaired electrons in their outer most orbitals. They are often formed when oxygen reacts with other substances; for instance, when oxygen is being reduced during the electron transport system of cellular respiration. When oxygen is involved in the free radical, it is called a *reactive oxygen species*. These are known to damage cell membranes and macromolecules like lipids, proteins, and nucleic acids.

Some free radicals are intentionally made by white blood cells like neutrophils to kill off invading pathogens, others are used in cell-to-cell signaling. But, free radicals have also been implicated in diabetes, heart disease, and aging. Antioxidants are substances that render the free radicals harmless. Vitamins C, E and the element selenium are a few of the known antioxidants that are abundant in fruits and vegetables. The human body has natural pathways that also function to reduce the number of free radicals; catalase found in cells and used in many enzyme experiments is one chemical in a pathway used to decrease the number of free radicals.

Studies have been done to determine how an increase in cellular respiration affects the level of free radicals. Increase in exercise increases cellular respiration, but does it increase the amount of free radicals in the body? Are athletes in greater danger of free radical damage? Should athletes increase the amount of fruits and vegetables in their diets? Preliminary research shows that increase in exercise does increase the number of free radicals initially, but with continued exercise, the body adapts and the number of free radicals is reduced. This takes time to occur, just like it takes time to build up muscle and endurance. A continuous program of exercise is healthy and supports your own free radical fighting systems. Eating more fruits and vegetables is never a bad idea and more research needs to be done with respect to athletes.

Two very good websites with more information are:
http://www.vivo.colostate.edu/hbooks/pathphys/misc_topics/radicals.html
http://www.rice.edu/~jenky/sports/antiox.html

Materials for the Lab:

- Goldfish
- Elodea (aquatic plant, also known as anacris and can be bought at pet stores)
- Burret assembly
- Dropper bottles of phenolphthalein
- Small beakers
- Graduated cylinders (25 ml)
- Fluorescent light (produces very little heat)
- Container or cabinet that can serve as the dark room
- .0025 M NaOH
- Digital balance

Lab Instructions for the Teacher:

This lab requires that the students get the mass of the creatures being used. The easiest way to do this is to have them add the water to their beaker, put the beaker with the water on the digital balance, zero the balance and then add the creature. Having mentioned putting the water into the beaker, it is important to remind students to place the fish in a sufficient amount of water. When setting up the two plant beakers, they will each need a sprig of elodea about three inches long and students should make sure that the elodea is completely submerged in the water.

Students can carry out the baseline titration while the creatures are incubating in their respective beakers. It is important that they save their baseline beaker to serve as a standard for the color of pink they are trying to achieve when they later titrate the water from their other beakers.

Since they only titrate 10 ml from a test beaker, they should keep the remaining water in case they mess up. That way they will still have some of the water so they can try again.

Impress on the students that they should not add phenolphthalein to the beaker while the fish is in it (phenolphthalein used to be the active ingredient in ex-lax).

The NaOH is very weak. Students should be wearing goggles, but if they get some of the NaOH on themselves or their clothes, they just need to rinse it off with water.

Check to make sure your aquarium water is *slightly* acidic right from the beginning. If needed, you can add a very *small amount* of club soda. It should not take very much of the NaOH to complete each titration. If a lab group is using over 10 ml, check to see if they remembered to put the phenolphthalein in the test beaker (this is the most common mistake).

Most student have done a titration before, however, it is still a good idea to demonstrate the titration at the beginning of the class so they can see the importance of only letting a few drops out of the burret at a time and that the beaker needs to be swirled each time.

Cellular Respiration Lab

Introduction: Cellular respiration allows cells to convert the energy stored in food to ATP. This requires oxygen and generates carbon dioxide. If fish or aquatic plants are placed in water, the carbon dioxide they give off forms carbonic acid in the water. The increase in the acidity of the water directly reflects the amount of cellular respiration that has taken place over a unit of time. The amount of cellular respiration for a goldfish, an aquatic plant in the light, and an aquatic plant in the dark will be measured.

Hypothesis: Which creature and condition will have the highest rate of respiration and why?

Procedure:

1. Mass your creatures and then place each in 25 ml of aquarium water. (If the creature is large, place it in a larger amount of water, but be sure to record the amount of water.
2. Allow the creatures to remain in their water under the assigned conditions for 30 minutes.
3. After 30 minutes, carefully return each creature to its original containers and retain the water for testing.
4. Collect data by first creating a *baseline* for your titrations. To do this, measure 10 ml of the aquarium water, add four drops of phenolpthalein, and then titrate with .0025 M NaOH until the water is a consistent pink color.
5. Record the amount of NaOH used in the titration. It represents the starting acidity of the water and will be used to compare your next titrations.
6. Follow the titration procedure for each creature, titrating 10 ml of their water with four drops of phenolpthalein and NaOH until it matches the same pink color of the baseline. Record all results. Note that if your creature has been in 25 ml of water and you titrate only 10 ml of that water, you must multiply your value for NaOH by 2.5 to determine the total amount of carbon dioxide given off by the creature.
7. Graph the results. You determine the best way to represent these data on the graph.

Evidence:

Creature and Condition	Mass (g)	Vol. of Water Incubated (ml)	Vol. of Water Titrated (ml)	NaOH used in Titration (ml)	Total CO_2 Given Off (ml)	Respiration (ml/gram of creature)
Control						
Fish/Light						
Plant/Light						
Plant/Dark						

Analysis Questions:

1. Is it possible to have a negative value for respiration/g in this lab? If a negative value is possible, how could you explain it?

2. According to your data, which appears to have the higher rate of respiration, the fish or the plant in the dark?

3. Explain the difference between the plant in the dark and the plant in the light.

Error Analysis: Do you think your data is accurate? If you feel that some of your data is incorrect, explain which pieces of data you question and why you suspect that data to be wrong.