The Tools of Science—Methods for Collecting Data in High School Biology

by Connie Scercy Wood, 2016 CTI Fellow
East Mecklenburg High School

This curriculum unit is recommended for:
All levels of high school Biology

Keywords: Data collection, Research, Biochemistry, Respiration, Photosynthesis

Teaching Standards: See Appendix 1 for teaching standards addressed in this unit.

Synopsis: Students in science should be designing and carrying out their own investigations. However, their inexperience with the tools and methods of scientific research hinders their ability to envision how they can collect the data they need to answer the question they want to investigate. This curriculum unit is designed to equip high school biology students with ideas for the kinds of tools and methods they could use to reach their goal. It contains a "Toolkit" of equipment and techniques for collecting data on pH, organic compounds, enzymatic reactions, cellular respiration, and photosynthesis that students can turn to when designing experiments. The unit includes background information for the teacher on the methods used for collecting data and links to resources for instructions on their use and where they can be purchased. The teacher can present the "tools" one at a time over a period of time, or in its entirety as a resource for students to explore. I intend to continue adding to this "Toolkit" with additional tools and methods to explore other areas of biology, so look for updates in the Comments section.

I plan to teach this unit during the coming year to 65 students in IB Biology, and 60 students in MYP Biology I.

The Tools of Science—Methods for Collecting Data in High School Biology

Connie Scercy Wood

Introduction

Science students should be able to design and conduct experiments to search for answers to a scientific problem. They often struggle with this because of a lack of experience, not only with the design of the experiment, but how to go about collecting the kind of data that will lead them to the answer to the problem. They don't know what they don't know. They need the right tools to do the job, but they have never seen the tools nor do they know how to use them. A student might hypothesize that different sugars would affect the rate of fermentation by yeast and produce different amounts of carbon dioxide, but how do they quantify that? Typical biology labs will often introduce students to a few techniques for collecting data, but what are the limitations and margins of error for these techniques? What if a student wants to go deeper and use more quantifiable and reliable methods? What if the next experiment requires them to design their own procedure and they want to measure something different? Where is the toolbox of scientific instruments and techniques they can pull from to do the job?

This challenge has faced scientists since the beginning of science. Scientists doing research often cannot just open a catalog and find the exact equipment that will do what they need to do in the lab. Each problem being studied and each lab space has its own unique requirements for the collection of data, so the scientist may have to duct tape, splice together, and jury-rig equipment and methods together in order to do what they want to do.

Many new discoveries are made when new processes and technologies allow for the collection of new kinds of data. The invention of microscopes led to the field of cytology. The process of polymerase chain reaction (PCR) has revolutionized the study of DNA and how it works. The need to be able to collect certain kinds of data drives the invention of new ways to do so. Part of science is coming up with new ways to collect data, but in the classroom, we don't often have the time for students to do this. So I wanted to put together a basic "tool kit" that would give my students at least a place to start when they are designing experiments and need to know how to collect data. From these basics, I hope students will see how the tools and techniques might be adapted for the specific kinds of data they are trying to collect.

Science teachers are often far removed from the world of scientific research. We teach science concepts; we even teach students how to use the scientific method and we discuss famous experiments that have been done in the past. But very few science teachers are

able to keep up with the latest tools and techniques being used in scientific research. This unit will also discuss some techniques being done in laboratories today and how they might be applied in the classroom. The biology students I teach are in grades 9 through 12, regular Biology I to upper level International Baccalaureate and Advanced Placement Biology. The tools and techniques in this paper are selected with them in mind as well as the standards for science which require students at all levels to design scientific experiments. However, many of these methods should be applicable to middle school and to earth and environmental students as well.

Background Information

When deciding how to organize my "Tool Kit" of biology, I decided to divide it up by topics. So this information will be presented in the order in which I teach the basic topics of biology.

Biochemistry

Measuring pH

One of the first topics in biochemistry is pH. pH is a measure of the concentration of hydrogen ions in a solution and is expressed by the formula:

$$pH = -\log[H^+]$$

The pH scale ranges from 0 to 14. Every pH value represents a ten-fold change in the concentration of hydrogen ions. (As a side note, I had always wondered why pH values only went to 14. Why not a pH of 15 or 16? The reason has to do with water. Pure water will have a concentration of 1×10^{-7} moles of H^+ and 1×10^{-7} moles of OH^-. This is how many of the water molecules will dissociate per mole of water. If either hydrogen ions or hydroxide ions are added, the concentration of the other goes down. So together the product of the two concentrations always equals 1×10^{-14}.)[1]

Common substances used in biology for changing the pH of something include lemon juice (pH 2), vinegar (pH 2.2), hydrochloric acid (pH 0), ammonia (pH 11), baking soda (pH 8.3), and sodium hydroxide (pH 14).[2] Students will often measure the pH of one of these, but then want to get something in between. One way to do this is to dilute the above substances. Changing the concentration by a power of ten—from 1.0 molar to 0.1 molar, for example—changes the pH by 1 pH unit.[3]

The most common method in classrooms for measuring pH is to use pH paper. This paper is impregnated with chemicals which change color in proportion to the concentration of hydrogen ions and is then matched to a color chart which indicates the pH. Universal pH paper provides a quick measure of pH, but to avoid contamination of the solution being tested, a sample should be removed and the test paper applied to the sample. The results from using pH paper are not very precise, providing only an

approximation of the actual pH value. The color change can be affected by several factors, including the concentration of salts, proteins, temperature and the presence of alcohol as a solvent in the solution being tested.[4]

Indicator solutions work in a similar way and change colors when mixed with a sample of the substance being measured. These are often used in soil and water test kits for stream sampling or in pool test kits. Bromothymol blue is an indicator that changes from blue to yellow as pH approaches 6.0. It can also be used to observe pH changes due to changes in carbon dioxide levels. Phenolphthalein is another indicator that changes color--going from clear to bright pink at pH levels of 10 to 13. It is often used in acid-base titrations to determine the concentration of an acid or base.

For more accurate measurement and for monitoring pH over time, a pH meter or pH sensor connected to a data logger should be used if available. These measure pH much more accurately, taking into account the effects of other ions in the substance on pH. Care should be taken to calibrate the sensor before using and to properly store it when finished. If the glass membrane of the probe dries out it will not function properly. Calibration instructions will come with the pH sensor. Water samples can be measured directly, and soil sample pH can be determined using samples mixed in water. These sensors are becoming more affordable for schools and can often be used with classroom computers or Chromebooks. My school uses PASCO sensors, but there are other companies, such as Vernier, which are also very good. Information on suppliers can be found in the Teacher Resources section of this unit. Having at least one or two pH sensors will allow you to use them in a lab rotation, as a pH measuring station, or for individual students to use for their own research.

For research labs and industry there are a variety of ways to measure pH, including portable pH meters that can test plant tissue samples, powders, and even textile or paper samples in the field.[5] One version—the Horiba Laqua Twin pH Meter—is about $230 on Amazon. While that might be a little expensive for some schools, it would be a great example for students of a tool for measuring pH that is being used in industry, and is very simple to use.

Microsensors allow pH to be measured in very small samples and in living plant and animal tissue with minimal invasion.[6] These are not within the price range for a school. They have been used to test *in vivo* the pH of the urine in the kidneys of pigs and to study *in vivo* changes in blood pH in insects and other organisms.[7]

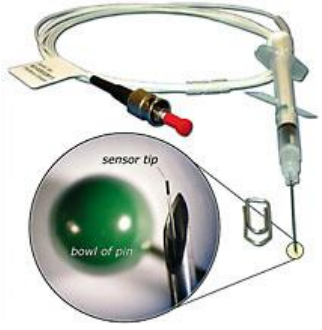


Figure 1.--Needle-type pH Microsensor
http://www.presens.de/products/brochures/category/sensor-probes/brochure/ph-microsensors.html#tab-probes

Organic Compound Testing

The presence of organic compounds can be detected using various chemical indicators. However, the tests typically done in science classrooms are qualitative. Reducing sugars such as glucose and fructose can be detected using Benedicts solution, which changes from blue to green to orange to red as sugar concentrations increase. One can get a general idea about the amount of sugar present from this. To make this quantitative, you can test a series of known sugar concentrations, filter out the precipitate, and run the remaining unreduced benedicts through a colorimeter or spectrophotometer to measure the absorbance. The less Benedicts reduced, the more red light is absorbed. A calibration curve is then made from this data and is used to determine the concentration of the unknown sugar solution. A good video on doing this is at https://www.youtube.com/watch?v=ssgq1QbgJrM .[8]

There is a quantitative Benedicts solution, which can be used if a student wants to get a better idea of the quantity of sugar in a sample. This version of Benedicts solution can be ordered wherever qualitative Benedicts solution is sold, and there is a link in Teacher Resources for a recipe to make your own. The test involves a titration of the Benedicts with the sample containing sugar until the Benedicts turns from blue to clear. The amount of Benedicts reduced correlates to the amount of glucose present in the sample.[9] Instructions for this test are at http://www.biologydiscussion.com/carbohydrates/test/qualitative-and-quantitative-tests-for-carbohydrates/13042 under the heading "Estimation of Glucose by Benedict's Method".

Glucose test strips can also be used to detect the presence of glucose. The strip contains toluidine and an enzyme—glucose oxidase. The enzyme converts glucose into gluconic acid and hydrogen peroxide. The peroxide reacts with toluidine, causing a color change. Aspirin, penicillin and some other antibiotics, as well as vitamin C, may produce false positives.[10] The strips come in bottles of 100 strips for about $20. Diastix and Clinistix are two major brands. Diastix strips measure glucose in increments that range from 0 to >110mmol/L.[11] It's somewhat like using pH paper—not extremely precise. The strips may be ordered online, or purchased from behind the counter at most pharmacies.

A quantitative protein test using biuret can also be similarly done by creating a standard curve using know protein solutions, and measuring the absorbance as the color changes from blue to violet, using a spectrophotometer or colorimeter. A detailed procedure is can be found at https://biologyalive.files.wordpress.com/2015/09/5-quantitative_biuret_test-amended.doc.

Spectrophotometers and Colorimeters

The use of spectrophotometers and colorimeters in the tests above, and in some to follow, allow students to use a tool that is often used in scientific research. Most schools have these if they teach chemistry or AP Biology. The difference between a colorimeter and a spectrophotometer is that the colorimeter measures how much of a certain color of light is absorbed by a substance, while a spectrophotometer can measure the transmittance as well as absorbance for a wider range of wavelengths of light. The cost of a colorimeter may be lower if purchased new, but used spectrophotometers may be found online for less than $200. Be sure to purchase cuvettes that will fit the spectrophotometer.

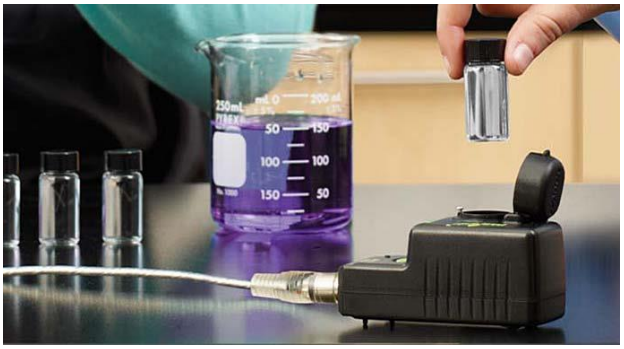


Figure 2--Colorimeter
https://www.pasco.com/prodCatalog/PS/PS-2121_pasport-colorimeter-sensor/

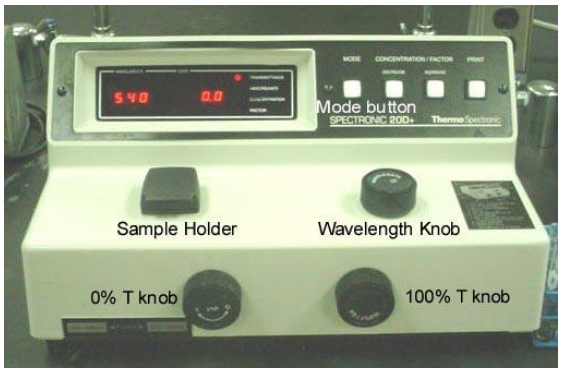


Figure 3--Spectrophotometer
http://iws.collin.edu/jbeck/Spectrophotometryweb/Spectrophotometer.jpg

Spectrophotometers measure the absorbance or transmittance of particular wavelengths of light through a sample. A wavelength of light is selected that is absorbed best by the substance being tested. A "blank" solution is prepared that contains everything except the molecule that is being tested. This is used to set the spectrophotometer to ignore everything except the substance of interest. Then the sample is placed into the spectrometer and the amount of light absorbed by the substance of interest is measured. There is a linear relationship between absorbance and the concentration of the substance as described by the Beer-Lambert Law.[12]

Spectrophotometers can be used to measure color changes, which can often be related to concentrations of a particular substance, such as the amount of biuret that is being oxidized which relates to the amount of protein. By using known sample concentrations, a standard curve can be created for the substance being

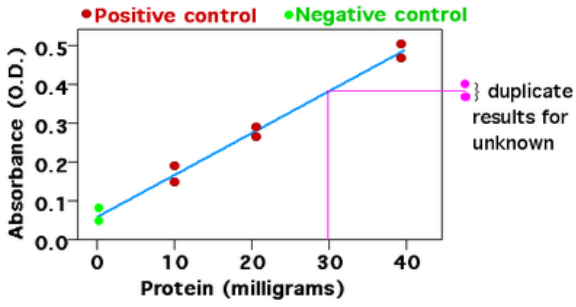


Figure 4.Standard Curve for Absorption versus Concentration of a Protein
https://upload.wikimedia.org/wikipedia/commons/thumb/9/9c/Standard_curve.png/400px-Standard_curve.png

tested. Absorbance would be measured for each of the known samples, and then graphed. The curve can then be used to determine the concentrations of unknown samples.

Students need to be aware that spectrophotometers and colorimeters were not designed for just one lab, but have applications for many things. Frequently they are used to measure the concentration of a substance, but can also be used to identify a substance based on the variety of wavelengths of light that the substance absorbs. They can also be used to get an estimate of bacterial populations. In this case, the instrument is not measuring light absorbance, but the light that is scattered by the bacteria, a reading called Optical Density. However there is a direct relationship between the absorbance and the number of bacteria.[13] An interesting experiment might be for students to test the absorbance of known concentrations of bacteria with light absorbance to determine the relationship for themselves.

Spectrophotometers can also be used to determine the results of enzymatic reactions. Spectrophotometry has been used with Dinitrosalicylic acid (DNS), which changes color when it reacts with reducing sugars, to determine the presence of reducing sugars leaking from bacteria being treated with silver nanoparticles.[14] Students can use the spectrophotometers, combined with color-changing indicators, to measure the presence of reactants or products from various enzymatic reactions. For example, they could measure the reaction when amylase breaks down starch, using iodine as an indicator and measure the color change of the iodine using the spectrophotometer.

Other uses for spectrophotometry include the determination of DNA or protein concentrations in a sample using ultra-violet spectrophotometry.[15] DNA has a maximum absorption at 260nm[16] and proteins absorb best at 280nm, depending on the proportion of the amino acids tryptophan and tyrosine in the particular protein.[17] Most spectrophotometers used in the classroom only use wavelengths in the visible light spectrum—340nm to 950nm. It is still good to discuss with students that the UV and infrared wavelengths can also be used with spectrophotometers that have that capability.

Cell Energy

Respiration

Cellular respiration is the breakdown of organic compounds in cells to produce the energy molecule adenosine triphosphate, or ATP. In the process, certain waste materials are produced. Aerobic respiration follows the summary equation:

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

Anaerobic respiration uses the following formulas, depending on the type of organism:

$$C_6H_{12}O_6 \longrightarrow CO_2 + \text{Alcohol}$$

$$\text{or} \quad C_6H_{12}O_6 \longrightarrow \text{Lactic acid}$$

Using either formula, sugar consumption could possibly be measured taking periodic samples, stopping the reaction by inactivating the enzymes involved, and using a quantitative Benedicts test as previously described to measure the sugar. Most often, however, the reaction is measured by observing the amount of oxygen consumed or the amount of carbon dioxide being produced.

Oxygen consumption can be measured using a respirometer. There are several different kinds of respirometers. Most teachers will be familiar with the type used in the AP Biology Respiration Lab which uses glass vials, a rubber stopper and a graduated pipet. Living organisms such as germinating peas or very small animals are places into the vial. Potassium hydroxide is placed on cotton in the tube to absorb any carbon dioxide produced, so that the only changes in gas volume in the tube are due to consumption of oxygen. A control vial is set up to account for changes due to air pressure and temperature fluctuations which can also affect the volume of gas in the chamber. The respirometer may be placed into a water bath which maintains temperature and allows the water to enter the pipet as the oxygen is consumed. Alternatively, colored water or soap bubbles can be introduced into the tip of the pipet, which will move into the pipet.[18] The change in the volume of oxygen is measured directly on the pipet. There are issues that students will run into with these respirometers. If they are not sealed properly around the pipet, they can leak. The water, dye or bubbles entering the tip may take a while to reach the point where the graduations start, delaying the collection of data, and even then it is often difficult for students to read the volumes. Bumping the respirometers can result in errors in the volume due to fluid coming into the pipet. Students should be made aware of these limitations and take care to avoid the possible problems.

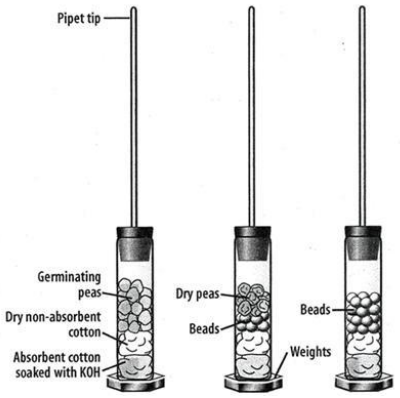


Figure 5–Respirometers.
https://www.biologycorner.com/resources/cell_respiration_pipets

Respirometers that are a little more high tech than our simple vials are used in many ways. Waste water treatment plants utilize aerobic microbes in the breakdown of organic wastes. Too much CO_2, not enough oxygen and these organisms can't do their job. So the conditions are closely monitored for carbon dioxide and oxygen levels as well as for other factors that can affect the respiration ability of these microbes. The respirometer, in Figure 6, can continuously monitor up to 24 reactors at a time.[19] Respirometers are also used to monitor bacteria used to biodegrade oil spills or plastics, and factors that affect their rates of degradation.[20]

Figure 6—EBS Challenge respirometer system for wastewater treatment monitoring
http://www.ebsbiowizard.com/2012/05/respirometry-as-a-tool-for-troubleshooting-of-biological-wastewater/

Another way to measure respiration is to use a carbon dioxide sensor and data-logging software. There are several companies that make these for schools—Vernier and Pasco are two of them. These sensors work using an infrared source and an infrared detector. The more CO_2 that is present, the less infrared is detected as the CO_2 absorbs the IR radiation.[21] The cost for one sensor is about $259. Students use the sensor by connecting it to a collection bottle. The organisms to be tested are place inside the bottle and the sensor fits snugly into the mouth of the bottle. The collection bottles are sold along with the sensors.

Figure 7--Pasco PASPORT CO_2 Gas Sensor
https://www.pasco.com/prodCatalog/PS/PS-2110_pasport-carbon-dioxide-gas-sensor/index.cfm

Another company makes a soil respirometer which can be used with the CO_2 gas sensor to measure respiration by soil organisms.

Figure 8 --Soil Respirometer used with CO_2 Gas Sensor and Temperature Sensor
http://probes.ciderhousetech.com.au/index.php/2014-07-07-06-16-13/ecology/soil-respirometer-detail

Photosynthesis

The formula for photosynthesis is

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

Photosynthesis can be measured by the consumption of carbon dioxide, or the production of oxygen. Simple photosynthesis labs can be done with aquatic plants such as *Elodea* which will release small bubbles of oxygen as they do photosynthesis. Students then count the bubbles of oxygen as the plant is exposed to different temperatures, different amounts of carbon dioxide, or different levels of light. This gives a relative value for the amount of oxygen produced by photosynthesis, but is subject to a great deal of error if bubbles are of different volumes or if students don't see all of the bubbles.

Another simple method qualitative method to observe the effects of photosynthesis or respiration is to use bromothymol blue. As mentioned before, bromothymol blue will change to yellow as pH becomes more acidic. The presence of carbon dioxide in water will make the water acidic. Students can either blow into the water or add baking soda as a source of carbon dioxide. Using baking soda would provide a better measure of control over how much CO_2 the plants were exposed to. When bromothymol blue is added along with the CO_2, it will turn yellow. If plants are allowed to do photosynthesis, after a while, the bromothymol blue will return to blue as pH levels rise due to CO_2 being consumed from the water by the plants. Respiration by plants can be observed by covering the beaker or flask being used with foil, so that the plants will only be doing respiration and releasing CO_2. I have written two activities for a Biology I class in a previous unit. They are activities 3 and 4 in the unit "Wonderful World of LIVING Color" at http://charlotteteachers.org/wp-content/uploads/2012/06/09.01.11.pdf. [22] I would have students do something similar, but create a standard curve for the bromothymol blue using a spectrophotometer.

A better way is to use either an oxygen gas sensor to measure the oxygen, or a carbon dioxide gas sensor (the same one described to measure respiration above) to measure the carbon dioxide consumed by the plant. The oxygen gas sensor contains a gas permeable membrane through which oxygen diffuses and is reduced, setting up a flow of electrons from anode to cathode which is detected and is proportional to the amount of oxygen gas present in the sample.[23]

Figure 9.—PASCO Oxygen Gas Sensor and Sample bottle
http://probes.ciderhousetech.com.au/index.php/2014-07-07-06-16-12/gases/pasport-probeware-2/pasport-oxygen-gas-sensor-detail

Most Biology teachers are familiar with the AP Biology lab that uses the spectrophotometer to measure the rate of photosynthesis. This lab uses

a color changing indicator— 2,6-dichlorophenol-indophenal (DPIP)—which changes from a dark blue to clear as it is reduced. Isolated chloroplasts from fresh spinach or some other plant material are flooded with DPIP. As the light reactions of photosynthesis progress, the DPIP is reduced instead of NADP+. The more photosynthesis that is being done, the more DPIP is reduced and becomes clear. This is measured with the spectrophotometer as an increase in transmittance. College Board has changed the example lab for photosynthesis to one that uses plant disks and measures their rise and fall due to CO_2 production. The old lab using DPIP and the spectrophotometer is far superior, I think and has the advantage of introducing students to an important tool in science. A link to the old version of the lab is in the Teacher Resources.

Some scientists use a form of spectrophotometry that utilizes an infra-red gas analyzer. A reference chamber is used to measure the CO_2 levels in the air. Infra-red wavelengths specific to CO_2 are beamed through the chamber. The more gas that is present, the more light will be absorbed and the less will strike the detector. A plant is placed in a second chamber and allowed to do photosynthesis, then the CO_2 levels are measured. The difference is the amount of CO_2 consumed by the plant for photosynthesis. This instrument can be used in the lab or in the field for plant studies, agricultural research, and to measure uptake of CO_2 over large areas. Monitoring stations are set up across the United States by the National Ecological Observatory Network (NEON) to measure how CO_2 levels are changing. They have data sets on this and many other ecological parameters across almost a dozen states that students could use for data-mining investigations.[24]

Strategies

I plan to use the information on the tools of science in a couple of ways. First, as we use some of the tools in our regular labs, I will discuss the tool and other uses it could have. It's important that students understand that a spectrophotometer is not just something to use to measure photosynthesis. Like a hammer, it is a tool that can be used in many ways if they understand how it works and what it can do. On a regular basis, I will have ToolTime, where students will be introduced to a new tool, technique, or a new use for one they have already seen. These will be brief warmups or demos, done once a week. The complete list of tools—the ToolBox—will be posted on my Edmodo website, where students will have access to them when preparing for "plan-your-own" labs. Another idea I may try is a ToolTime Rotation, where I set up stations with several different tools and have students use them to collect sample data in order to get a "feel" for how they are used to collect data.

This unit has only scratched the surface of the possible types of tools and techniques available for use in biology classes. I expect to keep adding to it as I have the time, learn

about other new methods for collecting data, or have access to new tools. The following is the beginning of the ToolBox that I will give my students:

Student ToolBox

Use this Toolbox of methods and equipment as a resource for determining how you might be able to collect data for your experiment. Feel free to modify them to fit the needs of your data. This ToolBox is a work in progress. There are MANY other ways to collect data and some that you may come up with on your own. Let me know if you come up with a unique way to use an instrument or technique so I can add it to this ToolBox.

To use the Pasco sensors describe in the ToolBox, you must connect them to the Chromebook using the AirLink. View the following video to see how to set up the AirLink and probes: https://www.youtube.com/watch?v=9Z_jU5huOl4

pH Measurement

Changing the pH of a substance—

- Possible solutions to use to change the pH: lemon juice (pH 2), vinegar (pH 2.2), hydrochloric acid (pH 0), ammonia (pH 11), baking soda (pH 8.3), and sodium hydroxide (pH 14).[25]
- Changing the concentration by a power of ten—from 1.0 molar to 0.1 molar, for example—changes the pH by 1 pH unit.[26] To vary your pH values, try diluting the solution you are using to change your pH rather than using a different substance.

pH paper—never leave the paper in the solution being tested, nor lay it down on the lab table. Take readings immediately after dipping the pH paper in the solution being tested or in a sample taken from it. Remember, pH paper has a broad margin of error.

Color changing indicator solutions— Bromothymol blue is an indicator that changes from blue (at a basic pH) to yellow as pH approaches 6.0. It can be used to observe pH changes due to changes in carbon dioxide levels.

Phenolphthalein is another indicator that turns from clear to bright pink at pH levels of 10 to 13.

pH Sensors—To use the pH sensors with our Chromebooks, first read over the instructions that come with the probe. Before beginning measurements, you should calibrate the sensor:

1. Select two buffer solutions that are above and below the pH range you believe you will be measuring. For example, if you will be measuring in the pH 6 area, pick pH 4 and pH 7 buffers. Pour a little of each into two beakers.

2. Remove the sensor from the storage solution, and rinse with distilled water.

3. Follow the manufacturer's instructions for calibration, using first one buffer solution, rinsing, and then the second buffer solution, followed by rinsing with distilled water again.

4. Now place the sensor into the solution to be tested and measure the pH.

5. When finished, rinse the sensor before placing it back into the storage solution.

Watch the video at https://www.youtube.com/watch?v=sP1bSEF654Y on calibrating the PASCO pH sensor using SPARKvue on the Chromebooks.

Detecting Organic Compounds

Quantitative Benedicts Test for Reducing Sugars—

- *Method 1—Use known glucose concentrations and a colorimeter to create a calibration curve.*
  Test samples of known glucose concentrations with Benedicts solution. Place ten drops of each sugar solution in a test tube and add ten drops of Benedicts. Place the test tubes in a warm water bath for the reaction. Filter out the precipitate, leaving the unreduced Benedicts still present. Using red light, which will be absorbed by the blue Benedicts solution, measure the absorbance using a colorimeter. Plot the data from each known sample to create a calibration curve. Add Benedicts to your unknown solution in the same manner, heat, and then filter the precipitate and measure the absorbance with the colorimeter. Use the calibration curve to determine the concentration of sugar in the sample.[6] See the video-- https://www.youtube.com/watch?v=ssgq1QbgJrM
  Caution—Wear goggles when doing this test. Benedicts is caustic. Wash skin immediately if it gets on your skin.
- *Method 2—Using Quantitative Benedicts solution and titration to determine the concentration of glucose.*
  This test uses a special formulation of Benedicts solution. You will boil 25 ml of quantitative Benedicts in a flask with 10g of Na_2CO_3 with a few porcelain chips. Use a titration burette to add your sample to be tested. As sugar is added a white precipitate will start to form, and the blue color will fade. Boil for two to three minutes, and continue to slowly add the sample drop by drop until the point when the blue color has completely disappeared. Record the volume of the sample added. It takes 50 mg of glucose to reduce 25ml of Benedicts. Calculate the amount of sugar in the sample using the following formula:

50mg glucose/the ml of sample used=Unknown mg glucose/100ml

Or…

Unknown mg glucose = 50mg x 100ml/ the ml of sample used[7]

Caution—Wear goggles when doing this test. Care should be taken to set up the hot plate underneath the burette so that it does not tip over while adding the sample. Benedicts is caustic. Wash skin immediately if it gets on your skin.

Glucose Test Strips—Can be purchased from a pharmacy for about $20 for a package of 100 test strips. They work by dipping the strip into the sample and then comparing the color change to the chart on the bottle. These only give results within a range of glucose levels, so are not the most accurate but are easy to use. Note—aspirin, antibiotics, and vitamin C may produce false positives.

Quantitative Biuret Test for Proteins—Biuret can be used to measure the amount of protein by testing standard protein solutions with Biuret, incubating them at 37ºC for 15 to 20 minutes and then measuring the absorbance in a spectrophotometer set at a wavelength of 562nm. Create a standard curve based on the absorbance of each of the standard protein samples, from which you can determine the concentration of any unknown samples. Detailed instructions can be found at https://biologyalive.files.wordpress.com/2015/09/5-quantitative_biuret_test-amended.doc .

Spectrophotometer—A spectrophotometer is used to measure the concentration of a substance based on the amount of light at certain wavelengths that it absorbs. The following videos will introduce you to the spectrophotometer and how to use it:

https://www.youtube.com/watch?v=pxC6F7bK8CU Video-- How does a spectrophotometer work?

https://www.youtube.com/watch?v=EvDwmgSbnnk Video—How to Use a Spectrophotometer

http://abacus.bates.edu/~ganderso/biology/resources/spec20.html Step by step guidelines for using the Spec 20.

The cuvettes for the Spec-20 spectrophotometers look like test tubes. However, treat them with care—they are expensive and sensitive to damage. They are specially made so that light can pass through easily without much distortion. Never clean them with a test tube brush, but rinse several times immediately after use. Scratches can affect the transmission of light through the cuvettes. When handling them, never touch them around the middle with your fingers. Oils from your hand will affect the results. Wipe them off with lens paper or Kim wipes, as regular paper towels will also scratch the glass.

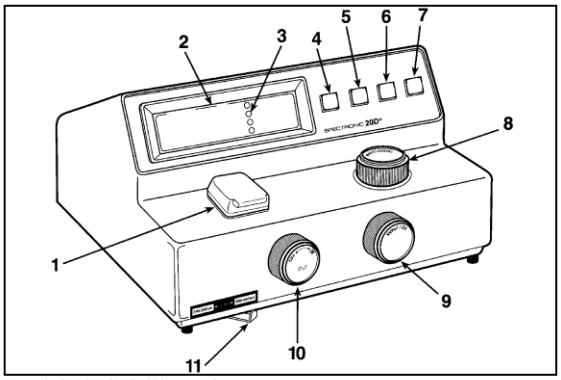


Figure 9 *SPECTRONIC 20D⁺ spectrophotometer*

KEY

1. Sample compartment
2. Digital readout
3. Mode indicators
4. Mode selection
5. Decrease
6. Increase
7. Print
8. Wavelength control
9. Transmittance/Absorbance control (100%T/0A)
10. Power switch/Zero Control
11. Filter lever

Spectrophotometer 20+
http://openwetware.org/wiki/Lidstrom:Tube_Spec

As shown in the instructional videos, when using the spectrophotometer, you will set up one cuvette that is called the blank. This will have everything that your experimental cuvettes have except the molecule you are measuring. Place the blank in the spec first, and follow the instrument instructions to set the spec to 100% transmission with the blank cuvette in place. You are telling the spec to ignore light absorbed by the cuvette, and everything else in it, so that it will only measure the absorbance of the substance you want to measure.

You can use the spectrophotometer to create absorption curves for reactions involving Benedicts and Biuret solutions in order to determine the amount of sugar and protein respectively. An absorption or standard curve is made when you measure the absorption for a molecule at known concentrations. After graphing this data, you should see a linear relationship which can be used to determine the concentration of an unknown sample of the same substance.

Spectrophotometry can be used for most color changing reactions and can be used to determine the concentrations of chemicals. You can also use it to measure the rate of enzymatic reactions by creating a standard curve for the product of the reaction and then measuring the absorbance of that product over the time of the reaction.

You might also be able to use the spectrophotometer to measure bacterial populations. See the article at
http://www.clt.astate.edu/dgilmore/Microbiology/Lab%20instructions/Spectrophotometry%20handout.doc and at

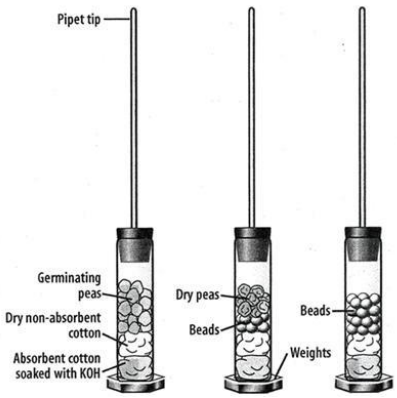
http://classes.midlandstech.edu/carterp/courses/bio225/chap06/Microbial%20Growth%20ss5.htm to see how to develop a standard curve to use to measure unknown bacterial populations with a spectrophotometer.

Cell Energy—measuring respiration or photosynthesis

Respirometer—A respirometer is used to measure the consumption of oxygen as organisms are doing aerobic respiration. It works by measuring the change in the volume

of the oxygen gas in a closed chamber as the organism uses oxygen for aerobic respiration. The following videos show you how to set up the respirometers in two different ways:
https://www.youtube.com/watch?v=oL7C9_3biZQ
https://www.youtube.com/watch?v=W11UDJnervw .

Put a 1ml graduated pipet into a rubber stopper and secure and seal it with aquarium sealant. Let dry. If placing the respirometer in a water bath, take a glass vial and attach a large washer on the end with strong waterproof glue. Add absorbent cotton loosely to the bottom and add two pellets of potassium hydroxide. The potassium hydroxide will absorb any carbon dioxide being produced by the organism, so that the only gas that is changing is the oxygen being used by the organism. Cover with non-absorbent cotton. This will keep the potassium hydroxide away from your organisms. Add the organisms to be tested. Close the vial with the rubber stopper/pipet. Be sure that there are no leaks around the stopper. Before placing the respirometer in the water bath, place a small drop of food coloring in the tip, just before lowering it into the water. The dye will move with the water as it is pulled in, and will make reading the volumes easier on the pipet.

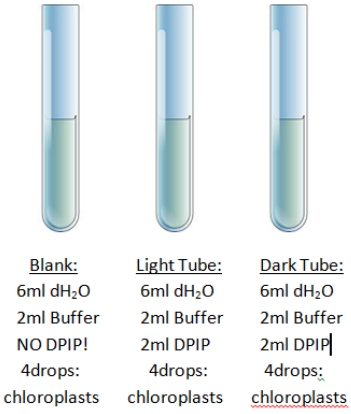


Respirometers
https://www.biologycorner.com/resources/cell_respiration_pipets

CO_2 gas Sensor—This sensor can be connected to our Chromebooks using the AirLink. It will then automatically open the SPARKvue app on the Chromebook. If not, open the app manually. This sensor can be used to measure both respiration and photosynthesis. Respiration produces carbon dioxide and photosynthesis consumes carbon dioxide to make sugar, so you will either be measuring the decrease or the increase in CO_2. An important point is to never let the sensor get wet. Keep it out of any liquids in your sample bottle. Watch the following video on using the CO_2 sensor to measure photosynthesis. It also demonstrates how to use the SPARKvue software to graph and analyze the data: https://www.youtube.com/watch?v=ZnrLbd6eunQ

Pasco PASPORT CO_2 Gas Sensor
https://www.pasco.com/prodCatalog/PS/PS-2110_pasport-carbon-dioxide-gas-sensor/index.cfm

Measuring Photosynthesis using a spectrophotometer—You will need to research the entire protocol for this experiment. You can find the original at http://hinton.harvardmedk12.wikispaces.net/file/view/DPIP%20Plant%20Photosynthesis%20Protocol.pdf/464452360/DPIP%20Plant%20Photosynthesis%20Protocol.pdf. Put your own twist on it. Set up the test tube cuvettes similar to the picture below. The "dark" tube would be covered in foil while the tubes are sitting in the light. You may have additional test tubes depending on your experiment. You must have a Blank test tube that will be used to tell the spectrophotometer what to ignore. In this example, the Blank is being used to tell the spectrophotometer to ignore the light absorbed by the glass, the water, the buffer, and the chloroplasts. What do the other tubes have that the blank does not?—DPIP! The DPIP will change from dark blue to clear as it is reduced by the light reactions. The more photosynthesis, the less light is absorbed by the DPIP, and the higher the transmittance.



Bromothymol Blue—This indicator changes from blue (at a basic pH) to yellow as the pH approaches 6.0. Carbon dioxide dissolved in water forms carbonic acid, making the water more acidic. If Bromothymol blue is added to water it can be used to detect the release of carbon dioxide due to respiration. If you want to observe the consumption of carbon dioxide during photosynthesis, then add bromothymol blue to the water and then add baking soda as a carbon dioxide source. The bromo blue will turn yellow as the pH decreases from the carbon dioxide. After adding a plant, the yellow color will change back to blue as the plant uses the carbon dioxide for photosynthesis and the water becomes less acidic. To quantify this, you should create a standard curve of the amount of baking soda added and the change in color of the bromothymol blue as measured by a spectrophotometer. Using this method, you can select variables to test to see how they might affect the rate of photosynthesis.

Oxygen Gas Sensor-- This sensor can be connected to our Chromebooks using the AirLink. It will then automatically open the SPARKvue app on the Chromebook. If not, open the app manually. This sensor can be used to measure both respiration and photosynthesis. Aerobic respiration consumes oxygen and photosynthesis releases oxygen as a waste product in the process of making sugar, so you will either be measuring the decrease or the increase in O_2.

PASCO Oxygen Gas Sensor and Sample bottle
http://probes.ciderhousetech.com.au/index.php/2014-07-07-06-16-12/gases/pasport-probeware-2/pasport-oxygen-gas-sensor-detail

Assessment

To assess the effectiveness of the ToolBox, I will assess the number of students who utilized the information from it based on their individual plan-your-own experiments, or Internal Assessments, as they are called in IB Biology. I will score students on the appropriateness of their choice of tool or technique for their experiment, how well they used it, and their data collection.

Appendix 1—Implementing Teaching Standards

International Baccalaureate Nature of Science Standards
1.8.--The importance of evidence is a fundamental common understanding. Evidence can be obtained by observation or experiment. It can be gathered by human senses, primarily sight, but much modern science is carried out using instrumentation and sensors that can gather information remotely and automatically in areas that are too small, or too far away, or otherwise beyond human sense perception. Improved instrumentation and new technology have often been the drivers for new discoveries. (This unit addresses various methods and tools, including sensors that students can use to gather data.)

3.1. --Data is the lifeblood of scientists and may be qualitative or quantitative. It can be obtained purely from observations or from specifically designed experiments, remotely using electronic sensors or by direct measurement. The best data for making accurate and precise descriptions and predictions is often quantitative and amenable to mathematical analysis. Scientists analyse data and look for patterns, trends and discrepancies, attempting to discover relationships and establish causal links. This is not always possible, so identifying and classifying observations and artefacts (eg.-types of galaxies or fossils) is still an important aspect of scientific work. (This unit addresses ways that students can turn qualitative measurements, such as the Benedicts and iodine tests for organic compounds into more quantitative measurements. Sensors allow for the collection of a greater amount of accurate data.)

3.2.--Taking repeated measurements and large numbers of readings can improve reliability in data collection. Data can be presented in a variety of formats such as linear and logarithmic graphs that can be analyzed for, say, direct or inverse proportion or for power relationships. (This unit shows students how they can use sensors for the collection of a large amount of data.)

3.3.--Scientists need to be aware of random errors and systematic errors, and use techniques such as error bars and lines of best fit on graphs to portray the data as realistically and honestly as possible. There is a need to consider whether outlying data points should be discarded or not. (This unit points out the sources of error and limitations of using various tools and techniques for collecting data.)

Notes—

[1] pH Technical Handbook:What Is pH and How Is It Measured?--
A Technical Handbook for Industry ." Hach Company. November 2014. Accessed September 25, 2016. www.hach.com/asset-get.download.jsa?id=7639984488.

[2] Helmenstine, Anne Marie. "PH of Common Chemicals." About.com Education. July 22, 2015. Accessed September 25, 2016. http://chemistry.about.com/od/acidsbases/a/phtable.htm.

[3]pH Technical Handbook:What Is pH and How Is It Measured?

[4]"Oops! Errors in PH Measurement - Precision Laboratories." Precision Laboratories. March 9, 2015. Accessed November 23, 2016. https://preclaboratories.com/oops-errors-in-ph-measurement/.

[5] "Measurement of PH in Plant Tissue." Horiba Laquatwin. January 8, 2015. Accessed November 23, 2016. http://www.horiba.com/fileadmin/uploads/Scientific/water_quality/Documents/Application_Notes/HIS/11__-_Measurement_of_pH_in_Plant_Tissue__Hi-Res_.pdf.

[6] "PH Measurement in Plants and Animals." PreSens Precision Sensing. Accessed November 23, 2016. http://www.presens.de/products/brochures/category/sensor-probes/brochure/ph-microsensors.html#tab-applications.

[7] "Selected Publications Showing PH Microsensors." PreSens Precision Sensing. Accessed November 23, 2016. http://www.presens.de/references/publications/product-group/ph-microsensors.html.

[8] *Quantitative Test for Reducing Sugars*. By Dr. Bhavsar. Performed by Dr. Bhavsar. October 10, 2014. Accessed November 23, 2016. https://www.youtube.com/watch?v=ssgq1QbgJrM.

[9] Kumar, Parul. "Qualitative and Quantitative Tests for Carbohydrates." BiologyDiscussion.com. 2016. Accessed November 23, 2016. http://www.biologydiscussion.com/carbohydrates/test/qualitative-and-quantitative-tests-for-carbohydrates/13042.

[10] Ferry, Robert, Jr. "Tips for Managing Type 1 and 2 Diabetes at Home." MedicineNet.com. March 22, 2016. Accessed November 23, 2016. http://www.medicinenet.com/diabetic_home_care_and_monitoring/page6.htm.

[11] "Glucose Test Strips." Flinn Scientific. 2016. Accessed November 23, 2016. http://www.flinnsci.com/store/Scripts/prodView.asp?idproduct=21528.

[12] Vo, Kevin. "Spectrophotometry." LibreTexts. July 22, 2015. Accessed November 23, 2016. http://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry.

[13]Gilmore, David F. "Spectrophotometry and Its Applications in Microbiology." Arkansas State University. Accessed November 23, 2016. www.clt.astate.edu/dgilmore/Microbiology/Lab instructions/Spectrophotometry handout.doc.

[14] Li, J., K. Rong, F. Li, Z. Lu, and R. Chen. "Highly Selective Antibacterial Activities of Silver Nanoparticles against Bacillus Subtilis." NCBI Resources/PubMed. October 2013. Accessed November 23, 2016. https://www.ncbi.nlm.nih.gov/pubmed/24245147.

[15]"UV Spectrophotometric Analysis of DNA and RNA." Advanced Molecular Biology Techniques Laboratory. January 1, 2002. Accessed November 23, 2016. https://people.hofstra.edu/Beverly_Clendening/Adv_Molecular_Biology/Protocols/UV_Spec_Analysis_RNA&DNA.htm.

[16] *Ibid.*

[17] "Spectrophotometry and Its Application in Protein Estimation." G-Biosciences. September 15, 2014. Accessed November 23, 2016. http://info.gbiosciences.com/blog/bid/201326/Spectrophotometry-and-Its-Application-in-Protein-Estimation.

[18] "Respiration Rate in a Germinating Seed." Bio 183 Laboratory Distance Education Section. Accessed November 23, 2016. http://webprojects.oit.ncsu.edu/project/bio183de/Lab/respiration_lab/respiration2.html#2.

[19]@ebsbiowizard. "Respirometry as a Tool for Troubleshooting of Biological Wastewater - EBS - Wastewater Training and Consulting." EBS Wastewater Training and Consulting RSS. 2016. Accessed November 23, 2016. http://www.ebsbiowizard.com/2012/05/respirometry-as-a-tool-for-troubleshooting-of-biological-wastewater/.

[20] "Respirometers Information." Respirometers Information | Engineering360. 2016. Accessed November 23, 2016.

http://www.globalspec.com/learnmore/labware_scientific_instruments/clinical_research_labware/respirometers.

[21] Verniervideo. YouTube. 2012. Accessed November 23, 2016. https://www.youtube.com/watch?v=pmsxa0-cKYU.

[22]Wood, Connie S. *The Wonderful World of LIVING Color*. November 1, 2009. Curriculum Unit, The Charlotte Teachers Institute, Charlotte, NC.

[23] NEONBetaEDU. YouTube. October 22, 2014. Accessed November 23, 2016. https://www.youtube.com/watch?v=PlEzyZadA90.

[24] "NEON." NEON. 2016. Accessed November 23, 2016. http://www.neonscience.org/.

[25] Helmenstine, Anne Marie. "PH of Common Chemicals." About.com Education. July 22, 2015. Accessed September 25, 2016. http://chemistry.about.com/od/acidsbases/a/phtable.htm.

[26]pH Technical Handbook:What Is pH and How Is It Measured?

[27]*IB Diploma Programme--Biology Guide 1st Assessment 2016*. Cardiff, Wales: International Baccalaureate Organization, February 2014. Accessed November 23, 2016. http://sdgj.cdhuaying.com/ckfinder/userfiles/files/9a6ff27578e18e3f1023a4dc046beef1.pdf.

Bibliography--Teacher Resources

Suppliers

https://www.pasco.com/ --Pasco. Sells probeware such as colorimeters, wireless pH sensors, and CO_2 and O_2 gas sensors. The probeware can be used with Chromebooks and a free downloadable SPARKvue app that allows students to collect and analyze the data. The software and equipment are very easy to use for students.

https://www.vernier.com/ --Vernier Software and Technology. This company has been around for a long time and has a good reputation for its probeware.

Biochemistry

file:///C:/Users/CWood/AppData/Local/Temp/LG004%20rev%201.pdf –resource on pH by the HACH company which produces water testing products. Best explanation of pH I have ever seen, and easy to read.

https://preclaboratories.com/category/ph-test-strips-2/ --Precision Laboratories website with short articles addressing pH and how their brand of pH test strips work, as well as examples of experiments to do at home or in the classroom.

https://www.youtube.com/watch?v=vwY-xWMam7o --Video--How to use a pH meter by BioRad

https://www.youtube.com/watch?v=sP1bSEF654Y --Video—Calibrating a PASCO pH sensor using SPARKvue

https://www.youtube.com/watch?v=ssgq1QbgJrM --Video—how to make a calibration curve for the Benedicts test for reducing sugars.

https://www.youtube.com/watch?v=HApJEXLl_ws --Video for making calibration curve for Benedicts test with how to make the glucose solutions.

http://www.biologydiscussion.com/carbohydrates/test/qualitative-and-quantitative-tests-for-carbohydrates/13042 --Contains instructions on preparing a quantitative Benedict's solution and how to do the test.

https://biologyalive.files.wordpress.com/2015/09/5-quantitative_biuret_test-amended.doc. -- *Quantitative Biuret Test for Proteins instructions*

https://www.youtube.com/watch?v=pxC6F7bK8CU --Video-- How does a spectrophotometer work?

https://www.youtube.com/watch?v=EvDwmgSbnnk --Video—How to Use a Spectrophotometer

http://onlinelibrary.wiley.com/doi/10.1002/bmb.20694/full -- "Applied spectrophotometry: Analysis of a biochemical mixture" A college lab that has student explore the use of spectrophotometry with a mixture of DNA, RNA and proteins. Must have UV spec for the lab as written, but could be modified for use with other mixtures.

http://www.ableweb.org/volumes/vol-29/v29reprint.php?ch=1 --Really thorough set of lessons in which students conduct a series of experiments on enzymes that also uses spectrophotometers to measure the reaction.

https://www.colby.edu/academics_cs/courses/BI214/upload/lab4-am-assay.pdf -- Measuring Amylase Activity in Cereal Grains. This lab has students centrifuge to

separate the proteins, and use a spectrophotometer to measure amylase activity. On starch with iodine as a color changing indicator

file:///F:/Assay-of-amylase-enzyme-activity.pdf --Assay of Salivary Amylase Activity using DNS and spectrophotometry.

http://www.sigmaaldrich.com/catalog/product/aldrich/128848?lang=en&region=US -- source for 3,5 Dinitrosalicylic acid for detection of reducing sugars. Catalog item 128848-5G. 5g for $14.60.

Cell Energy

https://www.youtube.com/watch?v=Q7i4BgRdRnc --video on setting up a respirometer for small animals

https://www.youtube.com/watch?v=oL7C9_3biZQ --Bozeman's video on setting up the AP Respiration lab with peas and respirometers.

https://www.youtube.com/watch?v=W11UDJnervw --video that shows alternative way to use AP Bio respirometers, without water bath.

http://biology4alevel.blogspot.com/2015/08/95-using-respirometers.html --IB site that shows several ways to measure oxygen uptake as well as CO_2 production using a respirometer with a manometer.

http://webprojects.oit.ncsu.edu/project/bio183de/Lab/respiration_lab/respiration2.html#2 --NC State University Distance Learning Respiration Lab showing a large respirometer with a mouse. Bubbles used in tip of pipet instead of being submerged in water.

http://probes.ciderhousetech.com.au/index.php/2014-07-07-06-16-13/ecology/soil-respirometer-detail --soil respirometer for use with CO_2 gas sensor to measure CO_2 from soil organisms by Ciderhouse tech

https://www.youtube.com/watch?v=pmsxa0-cKYU --Video on the use of the Vernier CO_2 gas sensor.

https://www.youtube.com/watch?v=LBFKKQZo4kg --Video showing 5 activities with PASCO oxygen and CO_2 gas sensors

https://www.youtube.com/watch?v=ctOO7zjWBvs --Video showing how to calibrate the Pasco Dissolved Oxygen Sensor

https://www.youtube.com/watch?v=PlEzyZadA90 --GREAT video on how an infra-red gas analyzer is used to measure photosynthesis.

http://www.neonscience.org/ --The National Ecological Observation Network. Has lots of data sets in over a dozen states on a variety of variables. Students could use this website with a little training to do a data-mining research paper.

http://hinton.harvardmedk12.wikispaces.net/file/view/DPIP%20Plant%20Photosynthesis%20Protocol.pdf/464452360/DPIP%20Plant%20Photosynthesis%20Protocol.pdf --The old version of the AP Biology Photosynthesis lab using DPIP and a spectrophotometer.