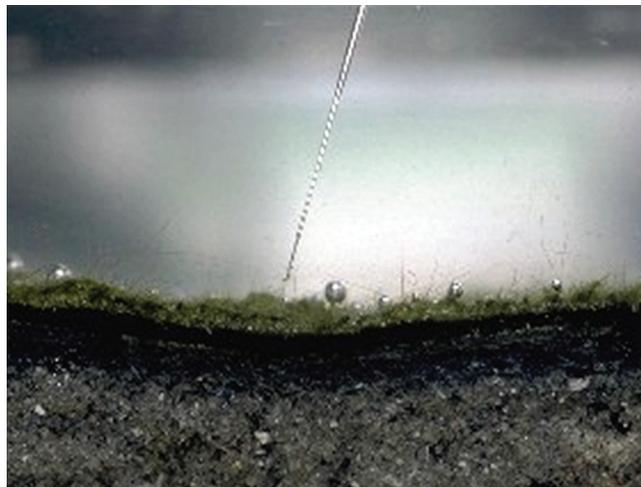


Web Lab: Oxygen Concentration Profiles

Robin Bucaria, Dartmouth Middle School
Dr. Brad Bebout, NASA Ames Research Center

Because they are so directly tied to the energy coming from our sun, the metabolic activities of microorganisms living in microbial mats dramatically change over a 24-hour (diel) cycle. Oxygen is produced by photosynthesis (which only occurs in the light) and is consumed by respiration. The diel changes in activity dramatically alter the concentrations of oxygen present in the mat over the same diel period. By measuring the concentrations of oxygen in a mat, microbial ecologists can determine the net balance of photosynthesis and respiration in the community. A set of measurements of oxygen with depth is called a profile of oxygen concentration.



A close-up view of an oxygen microsensor positioned above a microbial mat. Notice the oxygen bubbles on the surface of the mat. Photo Credit: Henry Page and Brad Bebout

In this experiment, students will expose a microbial mat to light in the Web Lab. A microsensor will record the amount of oxygen present in varying depths of the mat during the exposure to light. Through analyzing profiles of oxygen concentration data, students will determine the relationship between light and the net export or import of oxygen from the mat, and the ability of cyanobacteria to produce and consume oxygen.

The Web Lab is located in the Microbiology Ecology/Biogeochemistry Research Lab at NASA Ames Research Center in Mountain View, California and is classroom accessible through an internet connection to the Microbes @ NASA website: <http://microbes.arc.nasa.gov/>. A microsensor in the lab can be remotely controlled by users to collect data on oxygen concentrations within a microbial mat. In order to participate in Web Lab experiments, remote sites will need to download LabVIEW run time engine version 6.1 <http://ni.com>. Users will need to register with National Instruments in order to download the LabVIEW run time engine. In order to ensure that users can access the Web Lab for classroom experiments at specific times, please email Dr. Brad Bebout (Brad.M.Bebout@nasa.gov) to reserve a run time.

Main Concepts

Explanations of natural systems can be formulated and tested through investigations using observation, experiments, and mathematical models. Daily concentrations of oxygen in a microbial mat vary according to light and depth, as well as consumption of oxygen by respiration.

Scientific Question

How does light change the distribution of oxygen in a microbial mat?

Objectives

1. The student will formulate and test a hypothesis that is an explanation for oxygen concentration at different levels of the mat using observation, experimentation and mathematical models.
2. The student will develop a better understanding of photosynthesis, respiration, and diffusion in a microbial mat.

Abstract of Lesson

Students will test their hypotheses on oxygen concentration in a microbial mat using a demonstration module in which microbial mats maintained in a Web Lab are accessible with a web cam. Microsensor measurements within the community can be automated, or can be remotely controlled by users to collect data on oxygen concentrations within the mat. Students will interpret data recorded in scatter plots (oxygen concentration profiles) in LabVIEW to draw conclusions about the mat community that they are studying.

Prerequisite Concepts

1. Photosynthesis is the process of using light as an energy source to convert carbon dioxide and water to carbohydrates.
2. Oxygen is both produced and consumed by microorganisms. Major Concepts:
3. Aerobic metabolism both requires and produces oxygen. Cyanobacteria produce oxygen in the light. Cyanobacteria consume oxygen in the dark
4. Oxygen moves from places of higher concentration to places with lower concentration. Oxygen not consumed by the microbes in the mat leaves the mat and is added to the atmosphere.
 - An anoxic zone contains zero oxygen.
 - The term oxic describes conditions where oxygen is present.
 - There are places in the mat that are both anoxic and highly oxic at different times in the day, as well as places in a mat that stay anoxic or oxic.
5. Light decreases with depth in a mat.
6. Cyanobacteria consume oxygen at the same time that they produce it. In the light, however, more oxygen is produced than consumed and oxygen escapes. This is called “net oxygen production”
7. Photosynthesis is the fixation of carbon dioxide into biomass. Usually, but not always, photosynthesis results in the production of oxygen.

Misconceptions

Respiration is not breathing. Rather it is the chemical process of converting sugars to usable energy. Aerobic respiration releases energy and produces carbon dioxide.

National Education Standards:

Fully Met	Partially Met	Addressed
<p>NSES A1(5-8): Ability Necessary to do Scientific Inquiry g, h</p> <p>NSES A2(5-8): Understanding about Scientific Inquiry c, d</p>	<p>NSES A1(5-8): Ability Necessary to do Scientific Inquiry b, c, d, e</p> <p>NSES C4(5-8): Structure and Function in Living Systems</p> <p>NSES C6(5-8): Regulation and Behavior a</p> <p>NSES C7(5-8): Population and Ecosystems c</p> <p>NSES G2(5-8): Nature of Science a</p>	<p>NSES A2(5-8): Understanding about Scientific Inquiry g</p>
<p>2061: 1B(6-8) #1</p> <p>2061: 1B(6-8) #6</p> <p>2061: 9B(6-8) #3</p> <p>2061: 12A(6-8) #1</p>	<p>2061: 1C(6-8) #7</p> <p>2061: 2B(6-8) #1</p> <p>2061: 3A(6-8) #2</p> <p>2061: 5D(6-8) #1</p> <p>2061: 12D(6-8) #1,2</p>	

California Science Standards:

Fully Met	Partially Met	Addressed
Grade 6: Investigation and Experimentation #7 a, b, c, d, e Grade 7: Investigation and Experimentation #7 a, b, c, e Grade 8: Investigation and Experimentation #9 b, c, e	Grade 6: Ecology #5 a Grade 8: Investigation and Experimentation #9 a	Grade 7: Cell Biology #1: a Grade 8: Chemistry of Living Systems #6

Readings on Topic Related to Study

Microbes @ NASA website (<http://microbes.arc.nasa.gov/>)

Materials List

1. Internet connection to Microbes @ NASA website
2. Links to Web Lab on the Microbes @ NASA website
3. Copies of scatter plot from the oxygen concentration profiles from LabVIEW in the Web Lab
4. LCD projector

Handouts

1. Copies of scatter plot from the oxygen concentration profile after the profile is finished using LabVIEW
2. Microbes @ NASA website and Web Lab data may be printed and handed out if there are few computers for students to use.

Preparation

1. *Make certain that you have a live Internet connection to the Web Lab. Make certain that you will be able to show data sampling at the time of your class. Contact Dr. Brad Bebout one week before you want to use the Web Lab in your classroom to request a specific run time.*
2. *Download LabVIEW run time engine (version 6.1) from <http://ni.com>. Users will need to register with National Instruments in order to download the free LabVIEW run time engine. Only one computer will be able to control the microsensor. Other computers may view the screen.*
3. *Arrange for a LCD projector to display the web cam and oxygen concentration profiles live from the web site.*
4. *Copies of scatter plot data from an oxygen concentrate profile are available as a Microsoft Excel file (7-14-04 oxygen concentration profile data.xls) on the website in case you are not able to run the experiment live or you have technical difficulties.*

Procedure

Engage: Activities to introduce concepts in the mat (pick one of the activities)

Activity Possibility 1: Dye Diffusion Activity

Objective

Students will understand that gases move from high levels of concentration to lower levels of concentration through simulating the characteristics of a microbial mat.

Materials

Clear rectangular container. (4.1 centimeters wide X 5.7 centimeters high)

Agar

Water

Red food coloring

Blue food coloring

Methods

Please note that this activity took seven hours to complete. A teacher might want prepared several containers of agar with dye added to them at different times. These containers can be labeled and displayed at the front of the class in addition to the demonstration completed during class. Pictures of the diffusion activity are included below.

1. Before class, pour 50 ml of agar solution into a clear rectangular container and allow to set.
2. Measure 5 ml of red food coloring and set aside. Measure 5 ml of blue food coloring and set aside. Measure 5 ml of water and set aside. Do not mix food coloring and water.
3. During class, explain to the class that gases in a microbial mat move from areas of higher concentration to areas of lower concentration. The agar in the container simulates a microbial mat. The food coloring and water represents oxygen concentration in a mat.
4. Add red food coloring, water and blue food coloring separately to the agar block in the clear container. Explain to the class that the colored liquid at the top of the agar represents the oxygen in the mat. Now, oxygen as shown by the dye is concentrated at the top of the mat.

5. **Ask:** What do you think will happen to the food coloring in the agar?

Answer: The food coloring will change the agar from clear to purple.

Ask: If the food coloring represents oxygen in a microbial mat, what is happening to the oxygen in the mat and why?

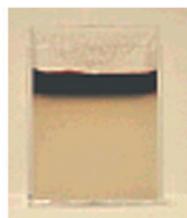
Answer: The oxygen is moving to different areas of the mat where oxygen was not as concentrated, because gases move from areas of higher concentration to lower concentration.

6. Explain that just as the food coloring moves to color the agar, so does oxygen move to fill areas of lower concentration. This is diffusion. Diffusion is a random process. The net result of there being more of something in one place than another, combined with the random process, is that substances are transported from high to low concentration. The line where there is a difference between the higher area of concentration and the lower area of concentration is the diffusion gradient. This gradient shows the change in oxygen concentration over distance.
7. Leave the box filled with agar at the front of the room for students to observe the progress of the diffusion of the food coloring in the agar. Note that as the red and blue dye diffuse, they mix, creating purple. This shows how gases diffuse in an area.
8. During breaks in the Web Lab activity, return to the agar box and have students take measurements of how far the food coloring has diffused in the agar. Students should record the measurements to graph.

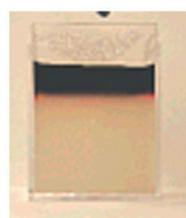
Diffusion Pictures



1:16 p.m.



1:17 p.m. red
and water



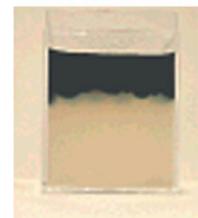
1:18 p.m. blue



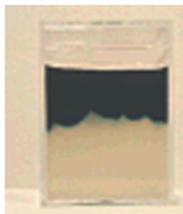
1:30 p.m.



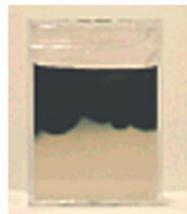
1:38 p.m.



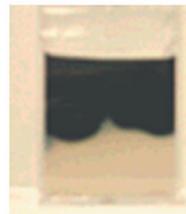
1:50 p.m.



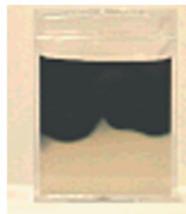
2:03 p.m.



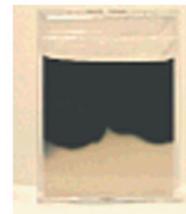
2:34 p.m.



2:54 p.m.



3:16 pm



3:45 p.m.



4:17 p.m.



5:05 p.m.



5:36 p.m.



6:40 p.m.



8:47 p.m.



9:43 p.m.



12:53 a.m.

Activity Possibility 2: Kinesthetic Diffusion Activity¹

Objective

Students will understand that gases move from high levels of concentration to lower levels of concentration through simulating the characteristics of a microbial mat.

Materials

open space in gym or outside

blindfolds(one for each student)

Procedure

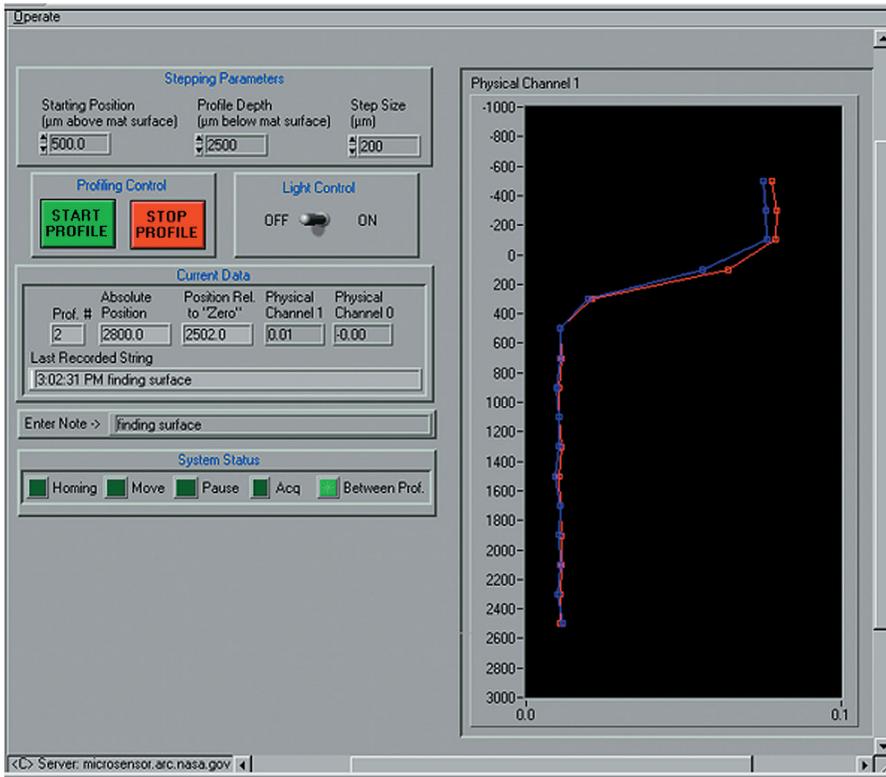
1. Have students line up on one side of the room or space.
2. Explain that gases in a microbial mat move from areas of higher concentration to areas of lower concentration. This principle is known as diffusion. Diffusion is a random process. Today you will act out this process and become oxygen inside a microbial mat.
3. Establish rules of behavior for the activity: slow walking, no pushing or shoving, etc. After you put on your blindfold, you are to walk slowly until you bump into something, then move in a different direction until you do not bump into something. When you hear the word, “freeze,” you will stop.
4. Have students put on their blindfolds.
5. Stop students by saying, “freeze” when they are randomly distributed.
6. **Ask:** What did you learn about diffusion from this activity?
7. **Answer:** Diffusion is random. If you look around the room, oxygen is widely distributed. In the beginning, oxygen was highly concentrated at one side of the room, and then it moved to fill areas of lower concentration. This is diffusion. Now oxygen is randomly dispersed in a more even concentration throughout the area. The net result of there being more of something in one place than another, combined with the random process, is that substances are transported from high to low concentration.

¹activity idea from Dr. Tori Hoehler, NASA Ames Research Center, Mountain View, CA

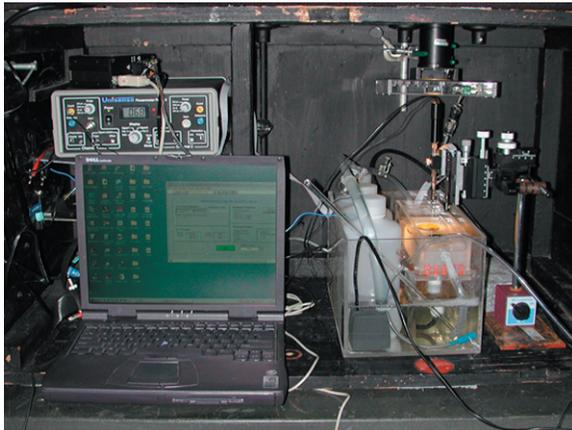
Explore

Classroom Lab Procedure

1. Project live connection to the Web Lab and LabVIEW remote panel. Directions for LabVIEW operation are included at the end of this lesson. While students can observe LabVIEW from individual computers, only one computer can control the experiment in the Web Lab. The teacher computer, which is attached to the LCD projector, should be the computer used to control the Web Lab experiment.



A screen shot of the LabView remote panel available on the Internet to remotely control a light and oxygen microelectrodes, and to obtain oxygen profiles in a microbial mat.



The experimental set-up in our lab that you can control remotely using LabView.



The same experimental setup being used in a hot spring in Yellowstone National Park.

2. Ten minutes before the start of class, turn the light off in the Web Lab by selecting and clicking the light on/off button on the LabVIEW remote panel. Wait ten minutes to make certain that the microbes are at a steady state. In a steady state, things are not changing with time.
3. Make certain students write down the hypothesis that they will be testing. Make certain that the hypothesis is testable. Sometimes an If...then statement is a good format for creating a hypothesis. Example—If the light is on the microbial mat, then _____ will happen.
4. Start profile by selecting and clicking the start profile button.

5. When the first profile is finished, confirm that the profile is indeed a steady state profile by running it again. When profiles are plotted similarly, one knows that the profile is at a steady state because readings have not changed with time.
6. Once a steady state is reached, turn on the light in the Web Lab on the LabVIEW remote panel by selecting and clicking the light on/off button.
7. When a minute has passed, the mat is ready to profile at a non-steady state with the light on. Select and click on start profile in LabVIEW in order to begin the profiling of oxygen.
8. After this profile is completed, wait five minutes to attain a steady state in the mat before making the next profile.
9. The computer will display scatter plot of oxygen profiles in LabVIEW. Make copies of the LabVIEW Oxygen profile scatter plots for students. If profiles are run one day, scatter plots can be copied for data analysis the next day. Scatter plots can be copied from LabVIEW with a right click on the physical channel panel.

Explain

Analysis Questions

Have students study the graph and answer the following questions:

- Are the mats producing oxygen?
- How do we know that they are producing oxygen?
- Are the mats consuming oxygen?
- How do we know that they are consuming oxygen?
- What happens to the oxygen in the mat when the light is on?
- What happens to the oxygen in the mat when the light is off?
- At what depths are production and consumption highest?
- At what depths are production and consumption the lowest?
- Are these mats a source or sink of oxygen?

Teacher note: These questions are explained with a sample scatter plot of oxygen concentration profiles that is attached to this unit (See the handout titled, Oxygen Concentration Profile Model Explained for an explanation.)the area. The net result of there being more of something in one place than another, combined with the random process, is that substances are transported from high to low concentration.

Questions About the Graph of Oxygen Concentration Versus Depth

Question: Is there more oxygen in the mat or in the water in the light?

Answer: There is a higher concentration of oxygen in the mat than in the water since photosynthetic processes produce oxygen. Deeper penetration of oxygen occurs in the mat when the light is on. Remember when the stromatolite explorer ran out of power, it latched onto filamentous bacteria to catch a free ride back to the surface where the solar radiation present would recharge the fuel cell? Those bacteria make that trip every day because they need to follow the oxygen.

Question: In the dark, is the highest oxygen concentration in the mat or in the water? Then, in the dark, which way would oxygen move, from the water to the mat, or from the mat to the water?

Answer: The organisms consume oxygen in the dark through the process of respiration. When respiration occurs, less oxygen is available in the mat than in the water column. Therefore, oxygen is moving, by diffusion from the water to the mat. Oxygen does not penetrate as deeply in a mat when it is dark.

Question: What is the difference in the depth where zero oxygen is in the mat during dark and light periods?

Answer: This depends on the graph studied. To determine this distance, subtract the depth where zero oxygen occurs in the dark from the light reading.

Question: What factors account for this difference in depth?

Answer: Production of oxygen through photosynthesis accounts for the difference in depth. Oxygen producing microorganisms in the mat, such as cyanobacteria use light as an energy source to convert carbon dioxide and water to carbohydrates, releasing oxygen. Also, oxygen concentration decreases in the dark due to the consumption of oxygen through the process of respiration.

Question: When and where does oxygen leave the mat?

Answer: Along the diffusion gradient. Diffusion is a random process, but the net result of diffusion is the movement of substances from areas of high concentration to areas of lower concentration.

Question: Which places in the mat are oxic?

Answer: The oxic places in the mat are the locations that contain oxygen. Any location above zero on the x-axis indicates the presence of oxygen.

Question: Which places in the mat are anoxic?

Answer: Anoxic places are locations in the mat that do not contain oxygen. Any location at zero or below on the x-axis indicates the absence of oxygen or an anoxic zone.

Question: Are there places in the mat that are oxic and anoxic?

Answer: Depending on the time of day and light conditions, certain places in the mat are oxic during conditions where light is present and oxygen can be produced through photosynthesis. These same places are anoxic when it is dark due to consumption of oxygen through respiration and the fact that oxygen is not being produced through photosynthesis. Look for depths shown on the graph where oxygen is at zero in the dark and above zero in the light.

Question: What might microorganisms that live in the zones of the mat that are oxic and anoxic do during oxic periods?

Answer: Anoxic organisms might move to a place in the mat where oxygen is not present. Organisms that use oxygen will be more active in their chemical processes. Organisms that produce oxygen will be conducting photosynthesis.

Question: What might microorganisms that deal with both oxic and anoxic conditions do during anoxic periods?

Answer: Organisms that are living in oxic conditions use aerobic metabolism, whereas organisms in anoxic conditions use anaerobic metabolism. Organisms that are primarily oxic, but can deal with anaerobic conditions are called “facultative anaerobes”. Some organisms with aerobic metabolism may move to a place in the mat where oxygen is still available (this may only be at the surface of the mat). Some organisms may also conduct anaerobic processes.

Scientific Inquiry Questions

Question: Does the data confirm your initial hypothesis? Why or Why not?

Answer: Answers will vary depending on the initial hypothesis and the data collected. Make certain that students base explanations on the data displayed by the graph and science content.

Question: Why would you repeat the profiling process with the same conditions?

Answer: Until the experiment is replicated several times under the same conditions, one cannot be certain of the outcome of the results.

Return to the dye or kinesthetic diffusion activities at the beginning of the lesson to make a connection between diffusion and the oxygen concentration profile. Have students look at the profile of oxygen in the light and have them predict where the diffusion of oxygen will occur.

Question: Where will the diffusion of oxygen occur?

Answer: Oxygen will move from areas of higher concentration to lower concentration. Notice that the water contains less oxygen than the mat. Some oxygen will leave the mat and enter the water. Some oxygen will move to areas in the mat that contain less oxygen, either by moving up or down. Remember that some of the oxygen is consumed through the energy transfer processes of microorganisms living within the mat.

Question: How does the process of diffusion that occurs in the mat relate to the diffusion of food coloring in the agar?

Answer: Just like the red and blue food coloring mixed throughout the agar and colored it purple, oxygen also randomly moves from areas of higher concentration to areas of lower concentration. If a mat is kept at the same state, oxygen will diffuse throughout the mat and into the water column above the mat.

Question: How does the process of diffusion that occurs in the mat relate to the kinesthetic diffusion activity?

Answer: Just like each student moved randomly away from the line of students to a location without other students to completely fill the area with students, oxygen also randomly moves from areas of higher concentration to areas of lower concentration. If a mat is kept at the same state, oxygen will diffuse throughout the mat and into the water column above the mat.

Have students study the profile of oxygen concentration that was made when the light was off and compare this profile with the one taken in the light.

Question: Compare this profile taken in the dark, with the profile taken in the light. What happened to the oxygen that was available during light conditions?

Answer: Although bacteria and other organisms consumed some oxygen when they used oxygen for energy transfers (creating a sink in oxygen), some oxygen moved out of the mat into the water. Oxygen also moved within the mat because oxygen moves from areas of higher concentration to areas of lower concentration.

Discussion

Discuss the results of the experiment and analysis questions as a class.
What other experiments can you design for oxygen profiling? What conditions can you change and still profile oxygen?

Evaluation

Lab Notebook: Using the Lab Notebook form, have students complete a written lab report. Use the rubric to evaluate student work.

Follow-up:

Additional activities are available in Microbial Mat Investigations at the Microbes @ NASA website: <http://microbes.arc.nasa.gov>. For example, to see the difference between consumption of oxygen by organisms and diffusion, arrange with Dr. Bebout at NASA Ames Research Center to conduct the following experiment using the Web Lab. Take oxygen profile readings of the mat at a steady state in both light and dark conditions. Then the organisms in the mat would be killed with mercury, then all of the O₂ would be removed by letting it diffuse out into nitrogen bubbled water over time (nitrogen bubbled water has no O₂). Next the N₂ bubbled water would be replaced with high oxygen water and profiled with an oxygen microsensor to see how long it takes the O₂ to go into the mat. These results would be compared with the oxygen profile taken before the organisms were killed.

Dr. Brad Bebout can be contacted at Brad.M.Bebout@nasa.gov

Lab Report

Complete the following lab report in your Lab Notebook giving specific details and writing in complete sentences.

I. Purpose: What are you trying to learn in this experiment?

II. Hypothesis: What are you testing in this experiment?

III. Materials: What materials and equipment did you use to conduct this experiment?
(Give specific details.)

IV. Procedures: What methods did you use to conduct the experiment? List in step-by-step detail what you did during the experiment.

V. Observations: What did you observe during the experiment? What data did you collect?
Make certain graphs, data collection tables, etc. are included.

VI. Conclusions: Does your data support or reject your hypothesis? Explain why. Relate your conclusions to the information that you are learning in science.

VII. Further Study: What ideas for further research do you have? Explain your idea(s) in a complete paragraph.

Lab Report Checklist

Your lab being evaluated on the following criteria:

- The purpose for the experiment is clearly explained.
- A testable hypothesis is written.
- The materials and equipment used to conduct this experiment are given using specific details.
- The procedures taken to conduct the experiment are listed in detail in sequential order. Transition words such as First, Next, Last, etc. are used to indicate the order of the methods used in the experiment.
- Observations describe changes in the dependent variable and include data in the form of graphs, data collection tables, etc.
- Conclusions explain whether the data supports or rejects the hypothesis and why. This section relates conclusions to the information learned in science.
- Ideas for further research are explained in a complete paragraph.
- The notebook is neat, complete, detailed, and written in complete sentences.