Biochemistry

Biochemistry is at the root of all biological systems. But students often find the subject difficult and discouraging. The biochemistry of ocean systems is no less complex. But once students observe the tiny phytoplankton through microscopes, they begin to understand the vast numbers of these cells that would be necessary to provide the amount of oxygen and food that is generated through ocean processes. The continual interaction of photosynthesis and decomposition is a key to living systems and can be illustrated using the ocean environment. In this unit, students can experiment with these processes, using oxygen testing methods and paper chromatography—hands-on activities that bring biochemistry alive.
ACTIVITY 1
Photosynthesis and Decomposition

Purpose
To show the relationship between photosynthesis, decomposition and life in the ocean.

Background
Chemical reactions can be illustrated with numerous examples. The problem with simple reactions is that students see them as unrelated to real life. The processes by which the bodies of plants and animals are formed and disappear are reactions related to everyone’s life and survival. This, coupled with an interest in the ocean, suggests that a discussion of photosynthesis and decomposition in the marine environment may intrigue physical and biological science students.

Photosynthesis and decomposition are useful examples of chemical reactions for beginning students because they can be made more complex and realistic by adding terms to the equation. How and why you would do that can be explained to students. But the drawback is that organic matter occurs in the reactions, and the chemical composition of this material is variable. Students seem not to be concerned about this problem. But the fact should be pointed out to avoid misunderstanding and future problems.

What students’ interest with a discussion of the oceans’ harvest, food chain and energy characteristics. A knowledge of photosynthesis can be combined with a modest exaggeration of the vertical separation between photosynthesis and decomposition to promote understanding of the oceans’ food production for man. Use of oceanic photosynthesis and decomposition as a teaching tool for chemical reactions can accomplish several learning goals at one time.

The advantage of using marine photosynthesis and decomposition as examples of chemical reactions stems from the vertical separation of the processes. Since photosynthesis requires light and seawater absorbs light, photosynthesis can only occur near the ocean surface. Organic matter created by photosynthesis is usually more dense than seawater and sinks. Sinking takes it out of the photosynthesis zone into a zone where decomposition predominates. This vertical transport of organic matter has major implications for global geochemistry.

The downward transport of photosynthesized products also has implications for ocean chemistry and biology. In most oceans, the area below the photosynthesis zone has a reduced oxygen level (the oxygen-minimum zone). Here, decomposition reduces access to atmospheric oxygen, and light is too low to supply oxygen as a product of photosynthesis. These conditions lead to an increased concentration of dissolved plant nutrients. But these nutrients remain at depths where light is too low to support photosynthesis and spur the food chain.

Occasionally, the nutrient-rich water from this zone is pulled to the lighted surface layers and promotes photosynthesis. Areas where such movement occurs are ocean upwellings. They account for 50 percent of ocean foodchain growth even though they occupy only about 1 percent of the ocean area.

Famous fishing grounds are almost all upwelling areas—the Grand Banks off Nova Scotia, the ocean around Antarctica, the waters off Peru and Ecuador, and the equatorial tuna grounds. The physical processes that cause water to upwell in these areas are different in each, but the chemical, biological and commercial implications are the same. (See Part 1, Activity 2, Ocean Upwellings, for further information.)

These ocean characteristics can be used to provide an interesting context for the chemical reactions that describe the reversible reactions of photosynthesis and decomposition.

Introduction
Plants have the unique ability to convert the energy of sunlight into chemical energy. This reaction is called photosynthesis because chemicals (nutrients) are synthesized into plant material in the presence of light. Once plants have carried out this chemical reaction, people and other animals can use the chemical products for food, fiber and energy. During this use, further chemical reactions take place. These reactions are termed decomposition because the original composition of the chemical is destroyed in the process.

Photosynthesis occurs when nutrients containing carbon, nitrogen and phosphorus combine with the hydrogen and oxygen in water to produce solid organic matter and gaseous oxygen. The energy necessary to accomplish this comes from sunlight that is trapped by the green chlorophyll pigments in the plant. This process occurs in every field, forest and body of water—wherever there are
chlorophyll-containing organisms and the sun's energy is strong enough.

Once formed, organic matter can be used as food. This changes the chemical composition of the organic matter and may even result in its being decomposed into the basic chemicals from which it was formed. Think about a wood fire. The energy that warms you is the sun's energy trapped by the process of photosynthesis and set free by the process of burning. Oxygen is needed to keep the fire going. The products of a wood fire are carbon dioxide and ashes that contain nitrogen and phosphorus.

Since these two reactions are each the reverse of the other, a single equation with the symbol — between reactants and products can be used to indicate both equations. This method of writing the chemical reaction should explain the nature of the energy used in photosynthesis and produced in decomposition. The energy differs depending on the direction in which the reversible reaction is proceeding.

Procedure

1. Write a word equation that describes the chemical reaction of photosynthesis and another for organic matter decomposition. Are these two equations related to one another? How?

2. A general chemical formula for organic matter is $C_{100}H_{40}O_{20}N_{10}P$, indicating that for every phosphorus atom there are 16 nitrogen atoms, 46 oxygen atoms, 190 hydrogen atoms, and 106 carbon atoms combined together. When one phosphorus atom is synthesized into organic matter, 154 molecules of oxygen gas ($O_2$) are formed.

Here is an equation showing photosynthesis and decomposition. The right side is correct. You fill in the blanks to create a balanced equation.

\[ \underline{?} CO_2 + \underline{?} NO_3^- + \underline{?} PO_4^{3-} + \underline{?} H_2O + \text{energy} \rightarrow C_{100}H_{40}O_{20}N_{10}P + 154O_2 \]

a. Which direction is photosynthesis?
b. Which direction is decomposition?
c. Which process uses the sun's energy?
d. Which process produces energy?
e. What is the energy produced used for?
f. Is any energy left? Is so, in what form?

The two chemical equations you have balanced symbolize chemical reactions that occur in nature. They can occur in a single plant cell (the decomposition equation is then called respiration) or they can occur in ecological systems. In the ocean, the ability of water to absorb light limits the process of photosynthesis to the upper layers. These layers are where most ocean organisms live. But decomposition occurs at all depths as organic matter from the surface sinks toward the sea floor.

Oxygen from the earth's atmosphere is absorbed into the ocean waters at their interface. Oxygen is also a product of photosynthesis that occurs in the upper ocean layers.

3. Knowing that the $O_2$ content in the ocean has two sources, would you expect dissolved oxygen concentrations to be higher or lower at the ocean floor than the $O_2$ content in the surface layers?

4. What about the concentrations of dissolved nitrate and phosphate in the surface layers compared to layers below?

5. If the concentration of one of the dissolved nutrients ($CO_3$, $NO_3^-$, or $PO_4^{3-}$) is exhausted, what happens to the rate of photosynthesis?

6. What conditions of physical mixing of surface and deeper layers of water would you expect to find in ocean areas with the highest photosynthesis rates?
ACTIVITY 2
The Effect of Decay on $O_2$

Purpose
To investigate how oxygen concentration changes with decay.

Materials
3 quart jars
sliced vegetables
tap water
Lamott or Hach dissolved oxygen testing kits

Procedure
Label the jars 1 and 2.
Fill jar 1 with tap water and test the oxygen content using the dissolved oxygen testing kit. Record the results on the data sheet. Discard water. Refill one-half of jar 1 with sliced vegetables. Fill with tap water and cap. Jar 2 is the control jar. Fill the jar with tap water and cap.
Place the jars out of direct sunlight and extreme temperatures. Do not disturb the jars for three to five days.

After three to five days, measure the dissolved oxygen in jars 1 and 2. Make sure the water is poured very gently from each jar into the test kit. This is necessary to prevent atmospheric oxygen from entering the specimens.

Record the amount of oxygen in jars 1 and 2 on the data sheet. How does the oxygen content compare to the initial oxygen test?

Questions
1. What caused the difference, if any, in oxygen between fresh tap water and the water with vegetables (jar 1)?

2. What caused the difference, if any, in oxygen between fresh tap water and the tap water that sat for several days?

3. What caused the difference, if any, in oxygen between the water with vegetables and the tap water that sat for several days?

Data sheet

<table>
<thead>
<tr>
<th>Jar 1</th>
<th>DO ppm — Day 1</th>
<th>DO ppm — After 3 days</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>tap water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jar 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with vegetables</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ACTIVITY 3

Effects of Plants on O₂

Purpose
To investigate how plants affect the amount of oxygen in water.

Materials
- pond algae or Elodea
- 2 jars of fresh tap water
- Laiton or Hach dissolved oxygen testing kits

Procedure
Label two jars 1 and 2. Fill each jar three-fourths full with fresh tap water. Test each jar for the dissolved oxygen content using the dissolved oxygen testing kit. Record the results on a data sheet.

In jar 1, place a freshwater plant in the water, and place the jar in direct sunlight. Place jar 2 into direct sunlight as well. Make sure the lids are on both jars. After several days test the dissolved oxygen in both jars. Record your results.

Questions
1. What caused the difference, if any, in oxygen between the fresh tap water and the water with the freshwater plant?
2. What caused the difference, if any, in oxygen between the fresh tap water and the water that sat for several days?
3. What caused the difference, if any, in oxygen between the water with the freshwater plant and the water that sat for several days?
4. What is the purpose of jar 2, the control?

Data sheet

<table>
<thead>
<tr>
<th></th>
<th>DO ppm — Day 1</th>
<th>DO ppm — Day 2-4</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jar 1 with plant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jar 2 tap water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Activity 4
Temperature and O₂

Purpose
To investigate the effects of temperature on oxygen.

Materials
2 beakers
tap water
thermometer
heat source
Lamott or Hach dissolved oxygen test kits

Procedure
Fill each beaker three-fourths full of tap water. Measure the dissolved oxygen in both beakers using the dissolved oxygen testing kit. Record your results on a data sheet.

Heat the water in beaker 1 to 10°C above the present room temperature. Test for O₂. Record the results. Simultaneously retest for the dissolved oxygen content in beaker 2.

Increase the heat in beaker 1 by 20°C above room temperature, then 30°C above room temperature. Test the oxygen content after each increase. Also test the oxygen content in beaker 2 each time you test for dissolved O₂ in beaker 1. Record your results.

Graph your results.

Questions
1. What happens to the amount of O₂ as the water is heated? (What is the purpose of beaker 2?)

2. Apply what you have learned about dissolved oxygen to the situation described below:

A news report indicates that a fish kill of 100,000 menhaden occurred in the Neuse River in July. There has been an algae bloom on the river. Some people are blaming toxic chemicals for the kill; others think it could be due to natural causes. Relate your observations to this news story.

Data sheet

<table>
<thead>
<tr>
<th></th>
<th>DO ppm before heating</th>
<th>DO ppm 10°C increase</th>
<th>DO ppm 20°C increase</th>
<th>DO ppm 30°C increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaker 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beaker 2 (control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ACTIVITY 5

Determining Pigments in Marine Algae Using Paper Chromatography

Purpose

To observe different pigments in various marine algae, to compare these pigments to those in spinach and to become acquainted with a very useful biological and chemical technique.

Background

The term chromatography (Greek for "to write in color") was introduced in 1906 by a Russian botanist, Mikhail Tsveit. He used a column of calcium carbonate to separate the components of a petroleum ether chlorophyll extract and suggested that the method might be applicable to other chemical compounds. The process went unnoticed for 30 to 40 years. But in the last 50 years, it has become one of the most valuable tools of biology and chemistry.

In paper chromatography, the separation of the components of a mixture depends on their different affinities for a stationary phase (paper) and their different solubilities in a moving phase (liquid). The molecules that are very soluble in the moving phase and have little affinity for the paper will move faster and a greater distance than those having less affinity for the solvent and a greater affinity for the absorbent (paper).

In paper chromatography, the mixture to be separated is applied as a spot or a line. The chromatogram is developed by placing the bottom edge of the paper in the solvent and letting the solvent flow up the paper. The distance traveled by a particular compound under a specific set of conditions (temperature, solvent system, direction of flow, type of paper, etc.) is characteristic and may be used to identify it. The ratio of the distance traveled by a compound to that of the solvent front is known as the R value:

\[ R = \frac{\text{distance from origin traveled by compound}}{\text{distance of solvent front from origin}} \]

Pigments are molecules that have color. They have color because they selectively absorb some wavelengths of light and reflect others. The wavelengths that they reflect are the ones that we see and identify as the color of the substance. Pigments are in plants and animals. They are used to attract mates and pollinators. Pigments can provide camouflage or warning indicators—both useful in avoiding predators.

In plants, several pigments are necessary for photosynthesis. These pigments absorb light energy from the sun and make it available for the splitting of the water molecule in the light reaction. Without these pigments, plants would be unable to produce the high energy carbohydrates (sugars) from carbon dioxide and water. Without this process, life as we know it could not exist.

The primary pigments are chlorophylls. There are a few different types of chlorophyll in addition to accessory pigments such as carotenoids (carotenes and xanthophylls). Carotenoids are yellowish pigments. They trap other wavelengths of sunlight and pass the energy along to the chlorophyll. Some carotenoids, known as carotenes, are made of carbon and hydrogen. Xanthophylls have oxygen in addition to carbon and hydrogen. Other carotenoids have uses other than as accessory pigments. They may be various colors of yellow, orange, red or brown—explaining the beauty of our autumn.

The three major groups of marine algae are distinguished by a variety of characteristics including the pigments that they have. Here is a table listing some of the differences.

<table>
<thead>
<tr>
<th>Division</th>
<th>Photosynthetic Pigments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyta</td>
<td>Chlorophyll a and b, carotenoids</td>
</tr>
<tr>
<td>(green algae)</td>
<td></td>
</tr>
<tr>
<td>Phaeophyta</td>
<td>Chlorophyll a and c, carotenoids</td>
</tr>
<tr>
<td>(brown algae)</td>
<td>including fucoxanthin</td>
</tr>
<tr>
<td>Rhodophyta</td>
<td>Chlorophyll a (Chlorophyll d in some), carotenoids</td>
</tr>
<tr>
<td>(red algae)</td>
<td>and phycobilins</td>
</tr>
</tbody>
</table>

You can help your students observe these pigments through the technique of paper chromatography. It is advisable to have them also chromatograph spinach pigments for comparison. Spinach may be processed the same way as the algae and is entirely reliable. The algae works best when very fresh and when blotted dry before processing.

Teacher Materials

spinach (fresh)
marine algae
acetone
petroleum ether (white vinegar can be substituted)
Student Information

When you observe a plant, it appears to have one color. In fact, you might say the plant has one pigment in it. But colors among terrestrial and marine plants are due to various concentrations and types of pigments combined in the cells.

These pigments range in color from red to orange to yellow to green. Most plants appear to be predominantly green because green chlorophyll pigments are the most predominant. In autumn, when the chlorophyll pigments break down in the leaves of deciduous trees, you can see the other pigments that are present.

Pigments have color because their molecules reflect the light waves of that color and absorb other light waves. For example, green chlorophylls reflect green light waves and absorb the other light waves (colors).

What is the purpose of these pigments? The chlorophyll molecule traps the energy from the sun for the plant cells to use in the formation of glucose (a molecule with high energy bonds). Several other pigments can absorb other colors of light waves and pass the energy on to the chlorophyll. These are called accessory pigments.

How does paper chromatography work? You will put a concentrated dot of pigment mixture on your chromatography paper. Then touch the bottom of your paper to a liquid solvent that will "crawl" up the paper. This solvent will pick up any pigments that dissolve in it. Some pigments will dissolve easily in the solvent. Other pigments will be held more tightly by the paper and will not easily dissolve in the solvent. Consequently, the pigments will travel at different speeds. When you remove the paper from the solvent and let it dry, the pigments will be fixed at the place on the paper that they had reached when you removed the paper.

You will probably find the following pigments:

- chlorophyll a: blue-green
- chlorophyll b: green (in green algae only)
- chlorophyll c: green (in brown algae only)
- chlorophyll d: green (in red algae only)
- xanthophyll: yellow to brown
- carotene: yellow-orange

Student Materials

- spinach pigment extract (in acetone)
- algae pigment extract (in acetone)
- thumbtack
- test tubes with corks
- holder for test tube
- petroleum ether/acetone mix (92 parts petroleum ether to 8 parts acetone. You can use white vinegar instead of petroleum ether)
capillary tubes  
pencil  
strips of chromatography paper  
hair dryer (cool setting only)  

**Procedure**

Put 6 to 7 millimeters petroleum-acetone or white vinegar-acetone mix in the bottom of your test tube.

Center a pencil dot about 2 cm from the bottom of your chromatography paper strip. Be careful to handle the paper by the edges only. Oily fingerprints will ruin it.

Dip a capillary tube in the pigment-acetone mixture and touch it to the pencil dot briefly. Blow until the acetone evaporates. Repeat often until the pigment dot gets dark green or brown. Be sure you let the paper dry between dots. You can use a hair dryer to speed the process, but only use the cool setting. Heat will destroy the pigments.

Tack the paper with the dot to the bottom of your cork. Drop the paper into the petroleum ether/acetone in your test tube and cover quickly. Be sure that your dot of pigment is not in the petroleum ether/acetone mix, and make sure that your paper strip is not touching the sides of the test tube. Do not move the setup while it is developing (Figure 1). You can place the test tube in a flask to keep it steady.

Five to 10 minutes later or when the petroleum ether/acetone mix has traveled to the top of your paper, remove the strip and observe the pigments.

Repeat for other algae samples and the spinach sample.

**NOTE:** If you run all your samples for the same amount of time, you can compare and decide if the pigments are the same. Or, you can measure the distance each pigment traveled from the original dot and divide that by the distance that the solvent (petroleum ether/acetone) traveled from the dot. If you compare these numbers, they should be the same for identical pigments. This is called the R number.

$$R = \frac{\text{pigment distance from dot}}{\text{solvent distance from dot}}$$

The algae you use will determine the exact results. But in general, carotenes travel fastest and will be at the top of the paper. Below them are the xanthophylls. Next will be chlorophyll a, d or c, depending upon the algae used. Finally, you will find the slow moving chlorophyll b.

**Questions**

1. Algae are classified according to their pigments. The algae are assigned to phyla based on which pigments they have. According to your results, would you place the algae you tested in different phyla? Why or why not?

2. Describe the difference between the spinach pigments and the algae pigments.

3. Why do you think acetone is used to extract chlorophyll pigments instead of water?

4. Does the chromatogram provide information about the relative concentration of the pigments? Explain.

5. Which wavelength of light (color) is the least useful in providing energy for photosynthesis?

6. There are other pigments in algae and spinach that we did not separate. Do you know why we were not able to separate these pigments?

7. Why do you need to be careful not to let your dot of pigment touch the petroleum ether/acetone mixture in your test tube?

8. What is the function of the variety of pigments in the algae and the spinach?
Competency Factors/References

Competency Indicators

Physical Sciences—
3.4 know that chemical reactions occur when two or more elements interact and form one or more new substances.

Biological Science/Applied Technical—
2.4 understand the chemical processes of life.

Biology/Academic—
1.2 know the method of science; and
2.4 know chemical processes of life.

Competency Measures

Physical Sciences—
3.4.1 write chemical equations from word equations;
3.4.2 demonstrate knowledge of the law of conservation of mass by writing balanced equations; and
3.4.3 classify chemical reactions as one of the four basic types: synthesis, decomposition, single and double replacement.

Biological Science/Applied Technical—
2.4.6 explain the significant events in such cell processes as intracellular respiration, digestion, photosynthesis and protein synthesis; and
2.4.7 describe the effects of various environmental variables on respiration, digestion and/or photosynthesis.

Biology/Academic—
1.2.1 perform laboratory exercises that use process skills such as observing, hypothesizing, interpreting data and formulating conclusions; and
2.4.6 explain the significant events in such cell processes as intracellular respiration, digestion, photosynthesis and protein synthesis.

References


Seaweed Chromatography. National Aquarium Education Department, Baltimore.