# Lesson 3 | Case Study: Biodiesel from Microalgae

<table>
<thead>
<tr>
<th>Student Labs and Activities</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content Vocabulary</td>
<td>39</td>
</tr>
<tr>
<td>Lesson Outline</td>
<td>40</td>
</tr>
<tr>
<td>Content Practice A</td>
<td>42</td>
</tr>
<tr>
<td>Content Practice B</td>
<td>43</td>
</tr>
<tr>
<td>Language Arts Support</td>
<td>44</td>
</tr>
<tr>
<td>School to Home</td>
<td>45</td>
</tr>
<tr>
<td>Key Concept Builders</td>
<td>46</td>
</tr>
<tr>
<td>Enrichment</td>
<td>50</td>
</tr>
<tr>
<td>Challenge</td>
<td>51</td>
</tr>
<tr>
<td>Lab A</td>
<td>54</td>
</tr>
<tr>
<td>Lab B</td>
<td>57</td>
</tr>
<tr>
<td>Lab C</td>
<td>60</td>
</tr>
<tr>
<td>Chapter Key Concepts Builder</td>
<td>61</td>
</tr>
</tbody>
</table>
Case Study: Biodiesel from Microalgae

Directions: Study the Venn diagrams below. For each term, write its definition. Where circles overlap, explain the relationship between the terms. Write your answers on the lines provided.

Constants

1.

2.

Variable

3.

Dependent variable

4.

5.

Independent variable

6.
**Case Study**

**A. Biodiesel from Microalgae**

1. Scientists have been exploring the use of protists to produce ________________.
2. Biodiesel is a(n) ________________ made mostly from living organisms.

**B. Designing a Controlled Experiment**

1. A type of scientific investigation that tests how one variable affects another variable is called a(n) ________________ experiment.
2. In a controlled experiment, the ________________ group contains the same factors as the experimental group, but the independent variable does not change.

**C. Biodiesel**

1. Rudolph Diesel invented the ________________ engine.
2. Replacing food crops with ________________ crops is not a good solution because there is a shortage of food in many parts of the world.

**D. Aquatic Species Program**

1. The Aquatic Species Program (ASP) initially studied possible ways that microalgae could capture excess ________________ in the air.
2. ASP project leaders noticed that some microalgae strains produced large amounts of ________________.

**E. Which Microalgae?**

1. Microscopic organisms that live in marine or freshwater environments are called ________________.
2. During ________________, microalgae produce ________________ that can be converted into biodiesel.

**F. Oil Production in Microalgae**

1. Starving microalgae of nutrients, such as nitrogen, increases the amount of ________________ they produced.
2. Starving the microalgae also caused their size to ________________, resulting in no overall increase in oil production.
Lesson Outline continued

G. Outdoor Testing v. Bioreactors
   1. Growing microalgae in open ______________________ can be challenging but might be less expensive than other methods.
   2. Some researchers are now growing algae under controlled conditions in closed glass containers called ______________________.

H. Why So Many Hypotheses?
   1. Dr. Richard Sayre, a biofuel researcher, said that all the ASP research was based on forming ______________________.
   2. According to Dr. Sayre, to get research support, a scientist has to develop a(n) ______________________ and propose some ______________________.

I. Increasing Oil Yield
   1. Microalgae use ______________________ energy, water, and carbon dioxide to make sugar.
   2. Scientists from a biofuel company wondered whether microalgae oil yields could be ________________ by distributing light to all microalgae.

J. Bringing Light to Microalgae
   1. Researchers and engineers used ______________________ to feed artificial light to microalgae in a(n) ______________________.
   2. ______________________ continuously rotate microalgae to the surface so the organisms are exposed to more light.

K. Why Grow Microalgae?
   1. Power plants that burn ______________________ release carbon dioxide into the ______________________, which contributes to ______________________.
   2. Microalgae use carbon dioxide during ______________________ and produce sugar, which can then be converted to oil.

L. Are microalgae the future?
   1. The costs of growing microalgae are currently too high to compete with ______________________-based diesel.
   2. ______________________-based biodiesel might one day become an affordable reality in the United States.
Case Study: Biodiesel from Microalgae

Directions: On the line before each statement, write T if the statement is true or F if the statement is false. If the statement is false, change the underlined word(s) to make it true. Write your changes on the lines provided.

____  1. Biodiesel is fuel made primarily from fossil fuels. ________________________

____  2. During the past few decades, scientists have explored the use of protists to produce biodiesel. ________________________

____  3. Rudolph Diesel, who invented the diesel engine, used soybean oil to demonstrate how the engine worked. ________________________

____  4. Some studies suggest that giving microalgae large amounts of nitrogen could increase the amount of oil they produced. ________________________

____  5. Microalgae can be grown in controlled conditions in bioreactors, but these containers are more expensive than open ponds. ________________________

____  6. Light rods can be used to increase microalgae growth and biodiesel production. ________________________

____  7. Paddlewheels can be used to make sure the microalgae's environment does not change. ________________________

____  8. Microalgae can remove pollutants from the air. ________________________

____  9. Scientists currently face few challenges in their quest to produce biodiesel from microalgae. ________________________
### Case Study: Biodiesel from Microalgae

**Directions:** Answer each question or respond to each statement in the space provided.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. What source of energy is used by most people for industry and transportation?</td>
<td></td>
</tr>
<tr>
<td>2. What problems are associated with using these kinds of fuels?</td>
<td></td>
</tr>
<tr>
<td>3. What is biodiesel?</td>
<td></td>
</tr>
<tr>
<td>4. What are protists?</td>
<td></td>
</tr>
<tr>
<td>5. <strong>Identify</strong> one challenge scientists have faced as they attempt to make biodiesel from microalgae.</td>
<td></td>
</tr>
<tr>
<td>6. <strong>Describe</strong> how scientists have addressed this challenge.</td>
<td></td>
</tr>
<tr>
<td>7. What are some benefits of cultivating microalgae?</td>
<td></td>
</tr>
</tbody>
</table>
Text-Analysis Activity: Identifying Variables and Constants

A controlled experiment tests how one variable affects another variable. The variable that is being changed is called the independent variable. The variable that is being measured or observed is the dependent variable. Constants are factors that remain the same.

Directions: Read each scenario below. Then answer each question on the lines provided.

A group of scientists conducted an experiment to determine the temperature at which a certain species of microalgae grow most rapidly. They set up 25 closed glass containers, or bioreactors. Each bioreactor held 25 L of water, equal amounts of dissolved nutrients, and equal starting populations of algae and were exposed to 12 hours of light each day. Each bioreactor was held at a different temperature. Bioreactor 1 was set at 5°C. Each subsequent bioreactor was set one degree higher, with Bioreactor 25 set at 29°C. After seven days, the scientists measured the size of the algae population in each bioreactor.

1. In this experiment, what is the independent variable? What is the dependent variable?

2. What are two variables that are held constant in the experiment above?

Tila and Jabal want to attract hummingbirds to their school garden by hanging hummingbird feeders that contain sugar water. They did some research to find out how much sugar they should put in the water. One source said to add 1,000 g to 1 L of water. Another source said to add 250 g sugar to 1 L of water. A third source said to add between 500 and 750 g to 1 L of water. Tila and Jabal want to conduct an experiment to determine which sugar solution will attract the most hummingbirds to feeders in their school garden.

3. What will be the independent and dependent variables?

4. What are four variables that should be held constant in Tila and Jabal's experiment?
Case Study: Biodiesel from Microalgae

Directions: Use your textbook to respond to each statement.

1. Terms associated with a controlled experiment are listed in the table below. In your own words, write a definition for each term.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variable</td>
<td>a.</td>
</tr>
<tr>
<td>Dependent variable</td>
<td>b.</td>
</tr>
<tr>
<td>Constant</td>
<td>c.</td>
</tr>
<tr>
<td>Experimental group</td>
<td>d.</td>
</tr>
<tr>
<td>Control group</td>
<td>e.</td>
</tr>
</tbody>
</table>

2. Explain how scientific inquiry has been used in a real scientific investigation by writing a short paragraph that summarizes how scientists have investigated the use of microalgae as a source of fuel.
Case Study: Biodiesel from Microalgae

Key Concept  How do independent and dependent variables differ?

Directions: On the line before each definition, write the letter of the term that matches it correctly. Each term is used only once.

1. a type of scientific investigation that tests how one variable affects another variable
2. any factor in an experiment that can have more than one value
3. the factor measured or observed during an experiment
4. a factor in an experiment that is changed by the investigator
5. factors in an experiment that remain the same
6. a group in a controlled experiment that is used to study relationships among variables
7. a group that contains the same factors as the experimental group, but the independent variable does not change

A. constants  B. control group  C. controlled experiment  D. dependent variable  E. experimental group  F. independent variable  G. variable
### Key Concept Builder

**Case Study: Biodiesel from Microalgae**

**Key Concept** How do independent and dependent variables differ?

**Directions:** Answer each question or respond to each statement in the space provided.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. What is a dependent variable?</td>
<td></td>
</tr>
<tr>
<td>2. What is an independent variable?</td>
<td></td>
</tr>
<tr>
<td>3. What are constants?</td>
<td></td>
</tr>
<tr>
<td>4. What was the dependent variable in the controlled experiment that investigated how nitrogen affected rates of oil production by microalgae?</td>
<td></td>
</tr>
<tr>
<td>5. What was the independent variable in that controlled experiment?</td>
<td></td>
</tr>
<tr>
<td>6. What were the constants in that controlled experiment?</td>
<td></td>
</tr>
<tr>
<td>7. What was the outcome of that controlled experiment?</td>
<td></td>
</tr>
</tbody>
</table>
Case Study: Biodiesel from Microalgae

Key Concept How is scientific inquiry used in a real-life scientific investigation?

Directions: On the line before each statement, write the letter of the correct answer.

1. Scientists used scientific inquiry to explore the idea of using protists to make
   A. nitrogen.
   B. biodiesel.
   C. fossil fuels.

2. When first studying microalgae as part of the Aquatic Species Program, scientists focused on microalgae's ability to capture atmospheric
   A. oxygen.
   B. nitrogen.
   C. carbon dioxide.

3. Microalgae produce oil during photosynthesis, which is a process that requires
   A. soil.
   B. light.
   C. sugar.

4. Scientists have found that the least expensive way to grow microalgae is in
   A. bioreactors.
   B. plastic bags.
   C. open ponds.

5. Recently, scientists hypothesized that they could increase microalgae's production of oil by increasing
   A. pond size.
   B. nitrogen levels.
   C. light distribution.

6. After conducting laboratory tests on how to increase microalgae's oil production, scientists plan to
   A. market the oil.
   B. perform field tests.
   C. change the hypothesis.
Case Study: Biodiesel from Microalgae

Key Concept  How is scientific inquiry used in a real-life scientific investigation?

Directions: Place the events in chronological order by writing a number 1 through 7 on the line before each statement.

1. Scientists develop a test to identify microalgae that have high oil content.
2. Plastic bioreactors are tested to replace more expensive glass bioreactors.
3. The U.S. Department of Energy begins funding its Aquatic Species Program to investigate ways to capture air pollutants.
4. Scientists begin experimenting with growing microalgae in bioreactors to bypass problems associated with growing microalgae in outdoor ponds.
5. Scientists find that starving microalgae of nutrients increases their oil production but also reduces the size of the microalgae.
6. Rudolph Diesel uses peanut oil to demonstrate how his newly invented diesel engine works.
7. Scientists in Washington State experiment on increasing biodiesel yields by distributing light to microalgae.

8. Give an example of how the process of scientific inquiry led to a better understanding of how microalgae produce oils.

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

9. Do you think that scientists eventually will find solutions to the challenges they now face in using microalgae to make biodiesel? Explain.

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
Plant-Based Fuels

Surplus corn and other grains, such as wheat and barley, can be processed into ethanol. A clear, colorless liquid, ethanol is used as fuel in internal-combustion engines such as those found in automobiles. Ethanol already is blended into most gasoline sold in the United States. This use of ethanol saves nearly 1 billion gallons of oil per year.

Biomass Ethanol

Scientists are investigating advanced ways, such as biomass feedstocks, to produce ethanol. The term biomass refers to any organic matter that is renewable, including wood, crops, plants, and animal wastes. Biomass feedstocks include corn fiber, plant residue, and rice straw. The feedstocks contain cellulose, which can be converted into sugars that are fermented into ethanol. These biomass feedstocks are considered low- or no-cost waste material.

Using inexpensive resources reduces the cost of producing ethanol. Also, using these waste materials has environmental benefits; they otherwise would be burned or put into a landfill. As an added bonus, waste materials of the ethanol conversion process are high in protein and other nutrients, making them an excellent feed ingredient for livestock.

Soy Diesel

Biodiesel is being developed as an alternative to petroleum for heavy vehicles. This fuel is made from natural, renewable sources, such as new or used vegetable oils. It is a cleaner-burning fuel and can operate in existing combustion-ignition engines. Soybean oil and methanol produce a product called methyl soyate, or soy diesel. Soy diesel is the main type of biodiesel used in the United States. Alternative oils that are being considered are animal fat wastes and used frying oil, which are cheaper than soybean oil.

Methanol

Methanol is another plant-based fuel. It is made from wood. Like ethanol, methanol can be mixed with gasoline. Pure methanol, or neat methanol, is used as a racing fuel. Methanol is a promising hydrogen source for fuel-cell vehicles. Hydrogen gas also is being tested for use in combustion engines. Because it is a gas instead of liquid, it is easier to store and transport.

Ethanol and methanol are renewable resources, which means they can be replaced by natural processes in less than 100 years. These clean-burning fuels are promising fuel alternatives for the future.

Applying Critical-Thinking Skills

Directions: Answer each question.

1. Infer Why do you think some people would be reluctant to change from using gasoline or diesel fuel in their automobiles to ethanol or methanol?

2. Analyze Do you think the United States should continue working toward using renewable agricultural products for fuel energy? Why or why not?
Observations and Questions

Curiosity about the natural world is a fundamental characteristic that is necessary to scientific inquiry. An observation of some event or condition in the environment can stimulate questions, such as “Why did this happen?” “Does this happen the same way every time?” “Can I make this happen?” “What caused this to happen?”

Often, we get involved in our daily routine, and we fail to pay attention to the world around us just beyond our immediate view. Practice making observations and then developing questions about your observations.

Use the graphic organizers below to make three observations of the natural world around you. For each observation, use the question generator to develop four questions.

Copy the graphic organizers onto your own paper. Leave space on each line for writing.

<table>
<thead>
<tr>
<th>Observation Chart</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Event or Subject Observed</strong></td>
</tr>
<tr>
<td><strong>Visual Details</strong></td>
</tr>
<tr>
<td><strong>Sounds</strong></td>
</tr>
<tr>
<td><strong>Smells</strong></td>
</tr>
<tr>
<td><strong>Feel/Textures</strong></td>
</tr>
<tr>
<td><strong>Other Features:</strong> duration, function, condition, location, importance, value</td>
</tr>
</tbody>
</table>

**Question Generator:**
What are four questions you could ask about this observation that could lead to a hypothesis?

| **Initial Understanding?** |
| **Interpretation?** |
| **Cause and Effect?** |
| **Importance?** |
How can you design a bioreactor?

You are part of a scientific team studying how yeast grows in a bioreactor. In a bioreactor, yeast use sugar as an energy source and release carbon dioxide gas as a waste product. One way you can tell how fast yeast grow is to measure the volume of gas yeast produce.

Ask a Question
How do water temperature and sugar concentration affect yeast growth?

Materials
500-mL Erlenmeyer flask
one-hole stopper with a short piece of glass or plastic tubing in hole
Also needed: rubber tubing (15 cm), water, 100-mL graduated cylinder, plastic wrap (10 cm × 30 cm), scissors, bendable straw, yeast, sugar, triple-beam balance, stopwatch, ice, thermometer, 500-mL beaker

Safety

Make Observations

1. Read and complete a lab safety form.
2. Draw a data table, like the one shown in your textbook, to record your data.
3. Place weighing paper or waxed paper on the triple-beam balance, and then zero the balance. Do not place solids directly on the balance.
   - Measure 3 g of yeast.
   - Use the paper to transport the yeast back to your lab station.
4. Repeat step 3 to measure 4 g of sugar.
5. Measure and pour 350 mL of water into both the Erlenmeyer flask and the beaker.
   - Measure 100 mL of water in the graduated cylinder.
6. Seal the graduated cylinder with plastic wrap.
   - Place your hand over the plastic wrap and turn the graduated cylinder upside down. Carefully place the sealed end of the graduated cylinder into a beaker of water.
   - Pull off the plastic wrap without losing any water from the cylinder. Have a team member hold the graduated cylinder so that it doesn’t tip over.

7. Place one end of a 15-cm piece of rubber tubing over the short plastic or glass tubing in the stopper.
   - Without lifting the cylinder above the water’s surface, insert the free end of the long piece of tubing inside the graduated cylinder. Have a team member continue to hold it.
   - Record the initial reading of the water level in the graduated cylinder.

8. Add the sugar and then the yeast to the Erlenmeyer flask.
   - Place the stopper in the flask and swirl it to mix the contents. This flask is your bioreactor.

9. Record in your data table the volume of gas produced every 10 min for half an hour.
   - To calculate the volume of gas produced for each 10-min time interval, subtract the initial volume from the final volume.

Form a Hypothesis

10. As a class, form a hypothesis that explains how a change in the amount of sugar in your bioreactor affects carbon dioxide production.

11. Form a second hypothesis that explains how a change in temperature of the water affects carbon dioxide production.

Test Your Hypotheses

11. As a class, develop procedures to test your hypotheses. Use a range of temperatures and different amounts of sugar in your tests.

Lab Tips

- Make sure the graduated cylinder is not tilted when you take readings.
- If you use a recycled water bottle as your bioreactor, do not squeeze the bottle once you place the stopper in it or you can force air into the eudiometer.
Lab A continued

12. With your teammates, set up several bioreactors with the conditions you outlined in your procedures.
   - Record the results from each bioreactor in a separate data table.

13. Using the class data, create two line graphs—one graph for each hypothesis.

Analyze and Conclude

14. **Analyze** What conditions resulted in the fastest growth of yeast?

15. **Compare** Which of the two variables had a greater influence on the growth of yeast?
   - How did you draw that conclusion?

16. **The Big Idea** Which scientific processes did you use in your investigation of bioreactors?

Communicate Your Results
Present your team’s results to your class. Include visual aids and at least one graph.

Remember to use scientific methods.
- Make Observations
- Ask a Question
- Form a Hypothesis
- Test your Hypothesis
- Analyze and Conclude
- Communicate Results
How can you design a bioreactor?

You are part of scientific team studying how yeast grows in a bioreactor. In a bioreactor, yeast use sugar as an energy source and release carbon dioxide gas as a waste product. One way you can tell how fast yeast grow is to use a eudiometer to measure the volume of gas yeast produce.

Ask a Question
How do water temperature and sugar concentration affect yeast growth?

Materials
500-mL Erlenmeyer flask
one-hole stopper with a short piece of glass or plastic tubing in hole

Also needed: rubber tubing (15 cm), water, 100-mL graduated cylinder, plastic wrap (10 cm × 30 cm), scissors, bendable straw, yeast, sugar, triple-beam balance, stopwatch, ice, thermometer, 500-mL beaker

Safety

Make Observations
1. Read and complete a lab safety form.
2. Draw a data table, like the one shown in your textbook, to record your data.
3. Place weighing paper or waxed paper on the triple-beam balance, and then zero the balance. Do not place solids directly on the balance. Measure 3 g of yeast. Use the paper to transport your solids back to your lab station.
4. Repeat step 3 to measure 4 g of sugar.
5. Measure and pour 350 mL of water into both the Erlenmeyer flask and the beaker. Measure 100 mL of water in the graduated cylinder.
6. Seal the graduated cylinder with plastic wrap. Place your hand over the plastic wrap and turn the graduated cylinder upside down. Carefully place the sealed end of the graduated cylinder into a beaker of water. Pull off the plastic wrap without losing any water from the cylinder. Have a team member hold the graduated cylinder so that it doesn’t tip over.
7. Place one end of a 15-cm piece of rubber tubing over the short plastic or glass tubing in the stopper. Without lifting the cylinder above the water’s surface, insert the free end of the long piece of rubber tubing inside the graduated cylinder. Have a team member continue to hold it. Record the initial reading of the water level in the graduated cylinder.

8. Add the sugar and yeast to the Erlenmeyer flask. Place the stopper in the flask and swirl it to mix the contents. This flask is your bioreactor.

9. Record the volume of gas produced every 10 min for half an hour. To calculate the volume of gas produced for each 10-min time interval, subtract the initial volume from the final volume.

Form a Hypothesis

10. As a class, form a hypothesis that explains how a change in the amount of sugar in your bioreactor affects carbon dioxide production. Form a second hypothesis that explains how a change in temperature of the water affects carbon dioxide production.

Test Your Hypotheses

11. As a class, develop procedures to test your hypotheses. Use a range of temperatures and different amounts of sugar in your tests.

12. With your teammates, set up several bioreactors with the conditions you outlined in your procedures. Record the results from each bioreactor in a separate data table.

13. Using the class data, create two line graphs—one graph for each hypothesis.
Lab Tips
- Make sure the graduated cylinder is not tilted when you take readings.
- If you use a recycled water bottle as your bioreactor, do not squeeze the bottle once you place the stopper in it or you can force air into the eudiometer.

Analyze and Conclude
14. Analyze What conditions resulted in the fastest growth of yeast?

15. Compare Which of the two variables had a greater influence on the growth of yeast? How did you draw that conclusion?

16. The Big Idea Which scientific processes did you use in your investigation of bioreactors?

Communicate Your Results
Present your team’s results to your class. Include visual aids and at least one graph.

Extension
As part of your presentation, propose future research that your team will conduct on bioreactors. Describe other variables or other organisms your team will investigate. Explain the goal of your future research. Will you develop a product that can be marketed? Will you provide an explanation to solve a scientific problem? Will you develop a new technology?
Lab C

Testing Different Sugar Sources

Directions: Use the information and data from the Lab How can you design a bioreactor? to perform this lab.

You have learned that organisms such as microalgae can be grown under controlled conditions in closed containers called bioreactors. This process can be used to make valuable products such as biodiesel. In Lab B, you designed experiments to test how changes in the amount of sugar and the temperature of water affected the growth of bacteria in a bioreactor. Now design an experiment to test how different sources of sugar affect the growth of yeast. For example, you could test honey and molasses.

Please note that you must complete Lab B before beginning Lab C. Also, have your teacher approve your design and safety procedures before beginning your experiment.
Scientific Explanations

End-of-Chapter Practice

Directions: Work with a group to use scientific inquiry to solve a real-life problem.

As a group, decide on a real-life problem you would like to solve. Then discuss the following questions:

<table>
<thead>
<tr>
<th>Can the problem be answered by science?</th>
<th>How can we address the problem using scientific inquiry?</th>
<th>How can we ensure that bias is not part of our investigation?</th>
</tr>
</thead>
</table>

Design your scientific investigation. Be sure to address the following issues:

<table>
<thead>
<tr>
<th>What steps will we take?</th>
<th>What materials and safety measures will we use?</th>
<th>What are our dependent variable, independent variable, and constants?</th>
</tr>
</thead>
</table>

After your teacher has approved your plan, conduct your investigation. Share your results with the class.

<table>
<thead>
<tr>
<th>What format will we use to present our results?</th>
<th>How can we best communicate the results?</th>
</tr>
</thead>
</table>

Consider posting your results online to share with your peers. Evaluate the feedback you receive and incorporate constructive suggestions into your experimental design.

Experimental design requirements:
- includes steps in logical order
- includes a hypothesis, a dependent variable, an independent variable, and constants
- effectively communicates the results of the investigation
- includes contributions from all group members