Grass Roots GK-12 Laboratory Activity

Title: **ALGAE: A Little Green Alternative Energizer**

Initial Version: 8/2012

Second Revision: 7/2013 & 7/2015

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Course & Appropriate Level: Environmental Science (can be used in a Living Environment classroom); Appropriate for High School students; could be modified for use in a 7th Grade Life Science Course

Abstract:

Due to increasing population and standard of life, global energy use is constantly growing and is a major issue currently facing human civilization. The heavy reliance on fossil fuels as sources of energy needs to change due to climate change and their limited supply. Currently, the only renewable replacements for these fuels are those derived from biomass. Those from algae offer an advantage from other biomass-based sources due to easy conversion using existing infrastructure, high yields, and growth on non-arable land.

In this module, students will begin to explore the future of energy by questioning their own energy conceptions as well as critically analyzing algae as a potential renewable energy source. They will be presented with current energy demands and look at where the energy comes from to power their daily lives. Due to its advantages, algae will be used as a case study for biomass-based renewable energy. The students will learn what it takes to grow algae, design an experiment to create an optimal bioreactor and culture algae in the classroom. Students will be able to see first hand how biomass can be grown as well as some of the problems associated with culturing algae, including scale up. They will then learn about how biodiesel fuel is produced from algae. Finally, they will be presented with the cradle-to-grave assessment of algae to allow them to see the entire picture of this emerging technology and how they might fit into energy related work (science, political, economical) in the future.

Time Required: Depending on the amount of time spent explaining current energy usage and how algae can help solve the energy crisis, the unit could take between 6-7 class periods with at least one lab period. Time will need to be set aside to measure algae growth over the course of 3 days and to analyze results.

NY Standards Met: *From NYS standards Living Environment: Standard 1; Standard 4;Key Idea 2: 2.1a: 2.1g: 2.1h: 2.1i: Key Idea 5: 5.1a. 5.1b. 5.1c. 5.1d. Key Idea 7: 7.1a 7.2c.*

Special Notes:	This laboratory activity was created via Cornell University's NSF GK12 Grass Roots Program: Award 1045513

Student Learning Outcomes:

Upon completion of this lab activity, students will:

- Explain the difference between renewable and nonrenewable energy sources.
- Investigate current energy consumption in the United States and how it applies to their energy use.
- Understand the process of photosynthesis and what is required for algae to grow.
- Predict how light absorption will affect algae growth.
- Design and carry out a controlled experiment.
- Defend their reasons for how they design a class bioreactor using data collected from their experiments.
- Analyze and interpret data to combine the "best" variables needed for algae growth.
- Summarize their findings in a one-page paper, which describes their experiment and data collected.
- Design a bioreactor as a class using data collected from individual experiments.
- Discriminate between the human impacts on the environment using current energy sources versus alternative fuels such as algae-based biofuels.
- Critically analyze the potential of algae as a renewable energy source through life cycle assessment and comparing it to other biomass-based energy sources

Class Time Required:

- 6-7 class periods (40 minutes)
    - Class 1: What is energy and where does it come from?
    - Class 2: How do algae grow?
    - Class 3-4: Building an algal bioreactor
    - Class 5: Analysis of the algal bioreactor
    - Class 6: Producing biodiesel from algae
- 10 minutes at the beginning of 3 class periods to make observations of bioreactors
- One class period to analyze results and pool class data

Teacher Preparation Time:

- Teachers should familiarize themselves with the lecture slides and handouts as well as the reading assignment prior to this unit.
- Please allow at least two weeks for algae and media to arrive to the classroom. Finding a local University to provide these materials and advice for culturing specific strains is ideal.
- Making TAP algae growth media takes approximately 1 month. It is recommended that you purchase the either pre-made media or the Hunter's trace element solution to eliminate this step.
- Allow a week to two weeks prior to the first day of the experiment to begin culturing the algae. This step consists of diluting the algae that is received into the algae growth media (approximately 15 to 25 mL) and culturing it in a similar way that students will later culture their own algae. This will become the culture that the students will use to start their reactors. Depending on the amount of algae cells added to the media, it will take between 5 days to two weeks for the algae to reach a maximum population. The cultures will become dark green at that time (see picture). If the cultures remain dark green for over a week, the algae will begin to die due to lack of nutrients. It is recommended that after the cultures are green for a few days (3 to 5), they are diluted into fresh algae growth media (1:20 to 1:50

dilution) to keep the algae alive. The dilution process can be repeated indefinitely provided the cultures are not contaminated.

Tips for the Teacher:

- Read through the lectures presentation, laboratory handouts, and background information prior to teaching the unit. Additionally, new algae developments are happening daily, a simple search through science news sites could provide current events to provide further motivation for the unit.
- Growing the algae prior to the students growing it provides excellent practice for the laboratory. During this time, you can watch how the algae grows to ensure the laboratory days are spaced appropriately as well as begin figuring out how the “bioreactors” will be set up in your classroom.
- Avoid contamination in your cultures by thoroughly cleaning and drying glassware. Try to keep all containers closed as much as possible throughout the experiment to avoid organisms other than algae growing on the nutrients. Contamination is often visible as white clumps in the media and can also be seen under the microscope. If the cultures are contaminated, often no algae will grow. You can also avoid contamination by adding the antibiotic ampicillin. By practicing the lab ahead of time you can ensure your method will not result in contaminated cultures.

- Do not use tap water to grow algae.
- You can allow students to observe algae before or after the experiment. We chose after the experiment so the students could be excited to see what they grew for the first time. Allowing them to see the cells prior would provide a fun activity to open the unit and possibly give them the opportunity to deduce what variables affect algae growth rather than teaching them from photosynthesis.

Assumed Prior Knowledge of Students:

Students must have an understanding of:

- Photosynthesis and Cell Respiration

- Cell organelles
- Scientific method

  This unit is currently designed to reinforce the concepts and steps of the scientific method that have been previously covered. The material could be modified to be used as an introduction to the scientific method.

Placement in the Curriculum:

In the Regents Living Environment curriculum, this Algae module would fit after a unit on Photosynthesis and should be taught in conjunction with a unit on Human Impact on the Environment. Ideally, this unit would be part of a larger unit that discusses the effects of nonrenewable energy technologies and Algae would be presented as a model organism which could be a possible solution to the Energy crisis. This unit also serves as a review of the Scientific Method and designing controlled experiments.

Background Information for Teacher:

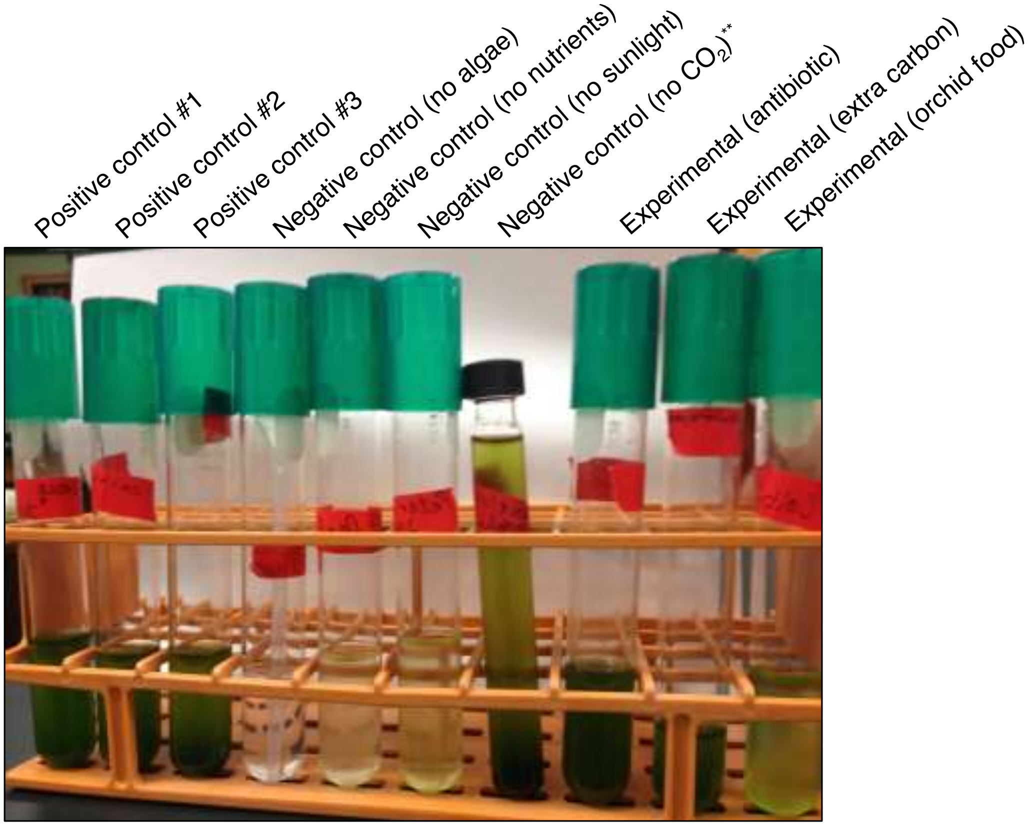
See folder with readings

Best reading is the Scum Solution and Can Biofuels Finally take Center Stage

Discrepant Event:

- Current renewable energy usage in the United States is around 10% and overall energy usage is increasing; what are some ways we can improve the amount of energy that comes from renewable sources in the near future?
- Liquid fuels are valuable due to their high energy density per volume as well as the existing infrastructure that uses them, mainly transportation. The only renewable source of liquid (and gaseous) fuels currently is biomass. Additionally, biomass-based fuels that have high-lipid content can be directly converted into gasoline or diesel (high-energy content fuels). Algae can produce more lipids than many other organisms being considered as renewable energy sources.
- Additional advantages of algae over other biomass sources of energy:
  - High yield per acre (largely lipids)
  - Can use land not available for agriculture
  - Less water needed than in agriculture
  - Can use saline/wastewater
  - Include process in waste streams
- Growing algae on non-arable land is an excellent discrepant event. One of the current problems with cellulosic energy crops is that they compete with farmland for space, the food versus fuel argument. Algae can be grown in areas that plants cannot, provided a source of water (fresh or sea water) is available.
- One of the current limitations in utilizing algae as a source of renewable energy is designing bioreactors and scaling them up to meet energy demands. The interactive learning demonstration will allow the students to see first hand how light is absorbed and how this could be a problem when making large cultures of algae. Students will then explore many variables that will affect the growth of algae. They should be challenged to think of how this might scale to make even more algae.

Experimental Data:



** The negative control of leaving out CO_2 for this experiment did not work due to the stopper not sealing the tube shut

Teacher notes:

What follows are the handouts for the two lectures prior to the experiment as well as helpful hints for the experiment. Following the handouts with the notes are the student handouts themselves, without anything filled in. Presentations are included in a folder with the readings. No handout or lecture notes accompany the final lecture.

Assessment:

- The assessment for this unit consists of a student questionnaire to allow for reflection on what was learned throughout the unit, how conceptions of renewable energy and science changed as a result of the unit, and what students may be interested in learning next. The assessment document used is found in this packet.
- Students also present their hypotheses and data to the class, offering additional opportunities to assess.
- The handouts for this module are meant to be interactive and accompany the lectures. Several questions will allow the students to think about energy concepts in unique ways while others serve as a review of material previously covered. These handouts can be used throughout the module to assess student progress.

Lesson 1: What is energy and where does it come from?

In this activity your class will discuss the definition of **energy** and find out how it is used in our everyday lives. We will investigate the **sources of energy** classifying them as **renewable** or **nonrenewable** and finding out how they are delivered to us. We will look at energy consumption in the United States over history and talk about where it is planning to go in the future. At the end of this handout you will determine which renewable energy sources can replace nonrenewable ones.

Question: List four things that you think of when you hear the word **energy**?

(This is an exercise to get the students thinking about where they have heard the word "energy" before. It is a good idea to write these on the chalkboard.)

1)
2)
3)
4)

Class definition:

Energy is: Energy is defined as the ability to do work

Energy source is: An energy source is something that can be tapped to provide energy (mechanical, heat, chemical, etc…) to do work.

List of energy sources:

1) Solar
2) Oil
3) Natural gas
4) Coal
5) Wind
6) Hydroelectric
7) Geothermal
8) Nuclear
9) Biomass
10) Tidal

Others:

(Answers such as batteries or electricity are not direct sources of energy)

Question: How did you use energy yesterday? List 5 activities.

Energy activity use:	Source of energy:	Electricity? (Y or N)
Watch television	unknown? *(It is okay for students to not know what the source of energy is at this time (especially for electricity), they will be able to trace it to the source in the next question.)*	Yes

For two of these activities list as many steps as you can about how the energy was delivered from the source to your activity. Separate the steps by arrows and list any steps you do not know as question marks.

Activity #1:Watch TV________________________________

Steps:

Watch TV ➔ electrical outlet ➔ power lines ➔ power plant

Sources of energy at a power plant: Coal, natural gas, nuclear, wind, hydroelectric, tidal

(Students may get stuck on a step in the process, this is a good exercise for homework for them to trace all of the steps it takes to have the energy to do everyday activities as well as where the source of it comes from. Additionally you may have to clarify some in between steps for them that they might not think of)

Activity #2:_________________________________

Steps:

Definition:

Renewable means: Renewable is defined as the ability to be replenished.

Question: What does it mean to be a **renewable energy source**?

A **renewable energy source** is:

A renewable energy source is one that can be replenished over the timescale of human life

A **nonrenewable energy source** is:

A nonrenewable energy source is one that once it is used it cannot be replenished quickly enough to be useful.

Go back to your list of **energy sources**, mark the sources as either **renewable (R)** or **nonrenewable (N).** Did any of your activities yesterday use **renewable energy?**

From the original energy source list: renewable sources: Solar, wind, biomass, geothermal, hydro, and tidal. Nonrenewable sources: oil, coal, natural gas, nuclear

(These next few questions are made to have students think about their energy consumption and challenge their current assumptions on renewable energy)

Question: In 2001 what percentage of the energy used in the United States was renewable energy?

Initial guess:______________ Actual answer: 6.4% (from the chart in the presentation)

What is the main type of energy used in the United States? Where did it come from?

The majority comes from fossil fuels, gas, coal, and oil

In 2012, what percentage or electricity in the United States was generated from renewable sources?

Initial guess:______________ Actual answer: ~11% (from Wikipedia, this should be verified prior to lecture)

In 1850, what percentage of the energy used in the United States was renewable energy?

Initial guess:______________ Actual answer: 100%_____________

Where did it come from?

Wood was used to heat homes, cook food and run factories. Coal had yet to be discovered and there was no internal combustion engine.

(This is an excellent discrepant event for students to think about)

The United States consumes **20%** of the energy consumed worldwide, yet only has **4%** of the population.

If all the energy used in the United States was converted to gallons of gasoline, the United States consumes a total of **1 trillion gallons** of gasoline equivalents in a year!

Question: What "states" can energy be stored in? Give an example of each.

1) Electrical (technically this would be a liquid/solid if in a battery)
2) Gaseous (natural gas)
3) Liquid (gasoline)
4) Solid (coal)

(*Other answers such as potential, mechanical or chemical energy could also be appropriate here)*

Question: Why is biomass an important replacement for fossil fuels? Give three examples of biomass-based energy sources?

(Biomass is the only renewable energy source that can replace gaseous-, liquid- and solid-based fossil fuels)

1) Trees
2) Grasses
3) Agricultural waste

(*Long list of answers that can be written on the chalkboard)*

Question: What are the four types carbon-based found in living organisms? Circle the one that looks most like gasoline.

1) Lipids/fats (most similar to gasoline)
2) Sugars/carbohydrates
3) Amino acids/proteins
4) DNA

Question: Why are algae an attractive biomass energy source?
(High lipid content per acre)

Homework: (Go over homework as a class; serves as review of the first lecture.)

1) Read the "Scum Solution" article. Give two more reasons why algae are an attractive renewable energy source.

    1)

    2)

2) List 2 more activities that required energy list as many steps as you can about how the energy was delivered from the source to your activity

Energy activity use:	Source of energy:	Electricity? (Y or N)

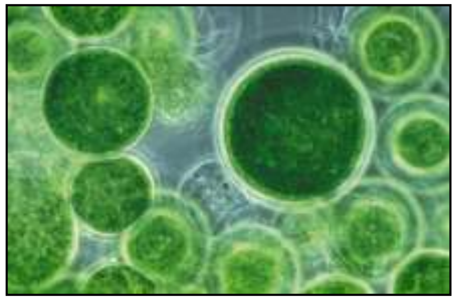
Activity #1:____________________________________

Steps:

Activity #2:_________________________________

Steps:

Lesson 2: How do algae grow?



In this activity we will discuss many of the **variables** that can affect the growth of algae. We will begin by reviewing the advantages of renewable energy and why algae in particular are advantageous as a potential biofuel. Next we will discuss the growth of algae focusing on solar energy. We will learn about the **solar spectrum** and **light absorption** through an interactive learning demonstration. Finally we will talk about other variables that could affect algae growth before designing bioreactors of our own!

Question: What is the only **renewable energy** that can be converted to a liquid fuel?
(Biomass)

Question: List three reasons why we need **renewable liquid fuels**?
(Answers will vary but may include: can make more of it, can be transported, current infrastructure requires liquid fuels)

Question: List four advantages to using **algae** as a source of **renewable liquid fuels**?
(Several possible answers including: Cheap to grow, Can be grown in areas not useful for other purposes, Does not use land needed for food crops, High lipid content, Fast growing)

Algae grow using a process known as:______(Photosynthesis)__________
Describe this process in your own words:
(Autotrophs convert solar energy into chemical energy of glucose)

For the rest of this activity we will be using a **spectrometer.** A spectrometer measures the properties of light over a spectrum of wavelengths. We will begin to understand these properties through a demonstration.

(If a spectrometer is not available, answers can be drawn on the chalkboard with the most important ones being the solar spectrum and absorbance of solutions)

Question: What defines a color? *(No correct answer; allows students to think about what a color is and how they would describe it.)*

Initial guess:

Draw the spectrum for green light *(Show students using the spectrometer. A flashlight app for the iPhone enables the class to look at pure colors with the spectrometer)*

Question: What does the spectrum of red light look like?

Initial guess: Final answer:

Question: What does the spectrum of blue light look like?

Initial guess: Final answer:

Question: What does the spectrum of white light look like?

(combination of the previous three colors)

Initial guess: Final answer:

Label the visible light spectrum

____

400nm 500nm 600nm 700nm

Draw the spectrum of fluorescent lights and an LED light

Fluorescent: LED:

Question: What does the **solar spectrum** look like?

Initial guess: Final answer:

Question: Is the solar spectrum the same every day? What could affect the solar spectrum? (No, the intensity of the solar spectrum is affected by the amount of cloud cover.)

Question: What happens to light when it passes through a leaf?

Initial guess:

Final answer: (Green light is absorbed by the chlorophyll in the leaf; the amount of light that passes through depends on the thickness of the leaf. You can ask students why they think leaves are so thin. You can show students this step using a leaf and the spectrometer)

Question: What happens to light when it passes through algae?

Initial guess:

Final answer: (The denser the algae, the less light will pass through. This is why algae are best grown in shallow pools. If you have already started your algae culture, you can show students this using the spectrometer as well)

How do algae grow when there is not enough light? (If there is not enough light, the algae at the bottom will not be able to survive unless the water is circulated allowing all the algae to be in contact with and absorb light or the algae have to swim around.)

Question: What is required to grow algae? (Water, nutrients, light, carbon dioxide)

Homework question: Name three variables you could like to test in your bioreactor? Circle your favorite. (Answers will vary)

Lesson 3: Building an Algal Bioreactor

In the previous activity, we looked in depth at several **variables** that can affect the growth of algae. We looked at the **solar spectrum** and how the amount of light passing through a reactor can be influenced by algae growth. Now we will analyze many of the variables that affect algae growth by testing them in your own bioreactors!
(Teacher notes: Prior to beginning the lab, split students into small groups or pairs depending on class size and materials available. As the positive and negative controls are discussed, assign students to a group to ensure all negative and positive controls are tested. This leads to greater variability in results which will allow students to observe the growth of algae under different conditions. Depending on class size, other experimental variables may be tested. This gives more data students can use when building the best reactor based on initial results.)

Note: The combined cost of algae and growth medium should be around $50

Materials:

10 mL of growth medium
0.5 mL of algae culture
1 test tube for each group
Test tube shaker (optional)
Plant growth light

Name the five requirements for algae to grow:

1) Sunlight
2) Water
3) Nutrients
4) Carbon dioxide
5) Algae

What is a **positive control**? What do you expect to happen with this culture?

(A control group is used to compare to the experimental group. The positive control group contains all the requirements above and should grow well (turn green). We will compare our experimental groups to the positive control to see if they grow better or worse)

What is the reason why we have a **negative control**? What are the negative controls we are setting up for this experiment:

(The negative control group shows what happens if we leave out a required element. These should not grow and should be used to compare to the experimental group to make sure our hypothesis about the requirements is correct and that no contamination occurred. We have four negative controls (no sunlight, no nutrients, no carbon dioxide, no algae; each left out individually)

In addition to groups testing the positive and negative controls, groups can also test: (these are possible experimental groups. As an extension to the lab, have students brainstorm how they could test each of these variables and ask for additional variables that could be tested.)

Aeration (more carbon dioxide)
Reproducibility
Distance from light
Additional carbon source (add 0.1 mL of additional carbon source)

Other growth media
Antibiotics (avoid contamination) (add 0.1 mL of ampicillin)
Other:

List the variable you are testing:

To set up your reactor: (This is best done one small group at a time. As students work on answering the questions above, call one group over at a time to set up the reactor.)

For all steps, keep containers closed to avoid contamination

1) Determine the media that should be used to test the variable you selected
2) Add 10 mL of media to a test tube. Add additional solutions as described by the variable you selected
3) Add 0.5 mL of algae to your media. Mix gently
4) Reserve a small amount to look at under the microscope
5) Place reactors in desired environments (aluminum foil works well to eliminate light, stoppered tubes can be used to reduce the likelihood of CO_2 entering the reactor.)

(Set the algae reactors about 4 to 8 inches from the plant growth light. If a shaker is used, set it to a low speed (~100 rpm is fast enough))

Building an Algal Bioreactor Worksheet

List the reason(s) why you chose to test the variable you picked:

Describe the environment for your reactor:

What is your hypothesis for your experiment? Why? (Have students share their hypotheses with class.)

Describe the appearance of your reactor:
(Quantitative measurements can also be carried out. Absorbance measurements will accurately track algae concentration when the concentration is below saturation. A wide range of wavelengths should be able to be used, but the wavelengths may differ for different strains. Conductivity and pH can also be measured to understand how algae perturbs it environment as it grows.)
Draw a picture of what the algae looks like under the microscope:

(See pictures on pages 5 and 6.)

Building an Algal Bioreactor Data Log: **(For all observations, it may be conducive to compile a list of class observations to use when designing the class reactor. Students record their individual observations on their lab sheets and on the class data sheet.)**

Day 2:

Describe the appearance of your reactor:

How does this compare to the positive and negative controls:

Day 3:

Describe the appearance of your reactor:

How does this compare to the positive and negative controls:

Day 4:

Describe the appearance of your reactor:

(For reactors in which significant algae growth occurs, students will observe a green color evenly distributed throughout the reactor. Over time, if the culture becomes very dense, they may observe a more intense green color at the bottom of the reactor because the algae may settle to the bottom. Contamination is often seen in the form of white mold spots sticking to the container near the top of the water level of the reactor. For absorbance measurements, students should observe exponential growth for at least a few days, followed by a slower increase in absorbance.)

How does this compare to the positive and negative controls:

Lesson 4: Analysis of an Algae Bioreactor Worksheet

What was the variable you chose to study? Why did you pick this variable? Describe why this variable could affect algae growth?

What was your original hypothesis for your experiment?

What was the result for your reactor?
(Students should be able to use quantitative data to support their conclusions.)

Did the **positive control** work as expected? How does the positive control compare to your reactor (better growth, same growth, no growth…)?

Did the **negative controls** work as expected? How does the negative control compare to your reactor?

Provide a reason to explain your results:

Now let's look at the algae under a microscope.

Draw a picture of your algae, label with as much detail as you can observe: (If your algae did not grow, show a picture of what you observe under the microscope as well as a second picture from the positive control group). (Ideally, students should observe several algae cells swimming. If students do not see swimming algae, make sure they observe a sample that is active. Contaminants, such as bacteria, may also be seen: those cells may have a different shape or color.)

Did you learn anything by looking at the algae under the microscope?

(Students may be able to use hemacytometers or other cell-counting devices to correlate their absorbances with numbers of cells.)

Summarize your results (in no more than two sentences):

Was your hypothesis confirmed? Why or why not:

What variables affected algae growth for the classroom bioreactors?

What went right/what needs to be improved? What would you like to test next?

Now let's design a classroom algae reactor out of recyclable materials! What do you need for your reactor to optimally grow algae? Sketch a picture (Depending on time available, students can look at data from each group reactor and design a new class reactor that will provide optimal growth conditions for algae. This reactor can remain hypothetical or can be set up and observed over the course of a few days. Observations from the class reactor should be recorded and compared to observations from the individual reactors. These observations can then be used to determine if the reactor designed by the class provides a better growing environment for the algae. If this lab is done with multiple classes, each class can compare the growth of algae in their class reactor to the reactors designed in other classes. This extends the scientific method and allows students to work within a community of scientists conducting similar research.

Lesson 5: Producing Biodiesel from Algae

In the previous activity, we grew algae under different conditions and designed our own bioreactors to maximize growth. In this activity, we will explore the process of converting algal lipids into biodiesel fuel.

(Teacher notes: Conversion of algae to biofuel requires three steps: separation of algae from the water it grows in, separation of lipids from other cellular components, and chemical conversion of the lipids to biodiesel molecules. We have found that the first and third processes are relatively easy to carry out, but the second one is difficult. In this activity we will have students use vegetable oil to carry out the third step, but it may be instructive for the students to have them try to produce biofuel from the algae they have grown. This could give them some idea of the difficulties involved in producing biodiesel.)

Materials:

Previously grown algae culture
Vegetable oil
Weigh scales
Separatory funnels
Sodium hydroxide
Methanol
Glass wool
Metal tongs
Lighter or Bunsen burner

Producing biodiesel from algae requires three processes

1) Separation of the algae from the water it grows in
2) Isolation of the lipids in the algae from the other components of an algae cell
3) Conversion of the lipids into biodiesel

What are some ways you could separate the algae you have grown from the growth medium?

(There are many possibilities: among them are:
Centrifugation
Electrophoresis (algal cell surfaces are negatively charged)
Flocculation (use of a molecule that causes algal cells to aggregate)
Some strains of algae will settle on their own
Filtration
Flocculation, centrifugation, and filtration are most common industrially)

Use one of these methods to separate your algae from your growth medium and determine the mass of algae you have grown.

(There are many ways that this could be done, depending on the resources available. One possibility is to use vacuum filtration: Line a funnel with filter paper and put the funnel into a side-arm flask that is hooked up to a vacuum pump. Another possibility is to simply dry the algae over time. A third possibility is to use a centrifuge to collect the algae at the bottom of a container and decant the supernatant.)

Mass of Algae Grown ____________________

After separating the algae from water, the parts of algae cells that are used to make biodiesel fuel need to be isolated from the rest of the cellular components. Lipids, which make up parts of the membranes in cells, and fats can be chemically converted to biodiesel fuel. They can be isolated from the other cellular components by adding a solvent that only solvates lipids and fats. Hexane and supercritical carbon dioxide are common. Supercritical methanol can be even more convenient to use. (Why?)

(The last two steps in the biodiesel production process can be combined if supercritical methanol is used.)

Assuming that your algae culture is 30 % by mass of lipids and fats, and assuming that the mass of biodiesel produced is approximately equal to the mass of lipids and fats, what mass of biodiesel could you produce with your algae culture?

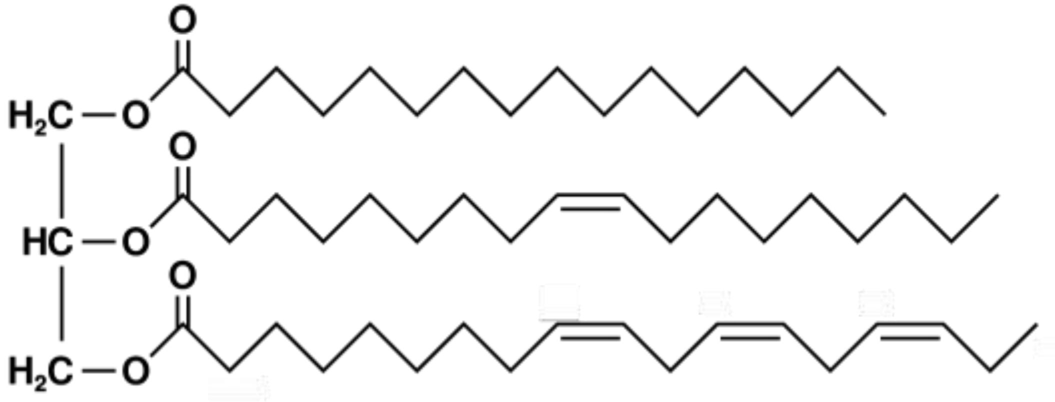
Mass of Biodiesel ____________________

The density of biodiesel fuel is about 0.88 g/cm^3. How many gallons of biodiesel fuel could you produce from your culture?

______________ Gallons of Biodiesel Fuel

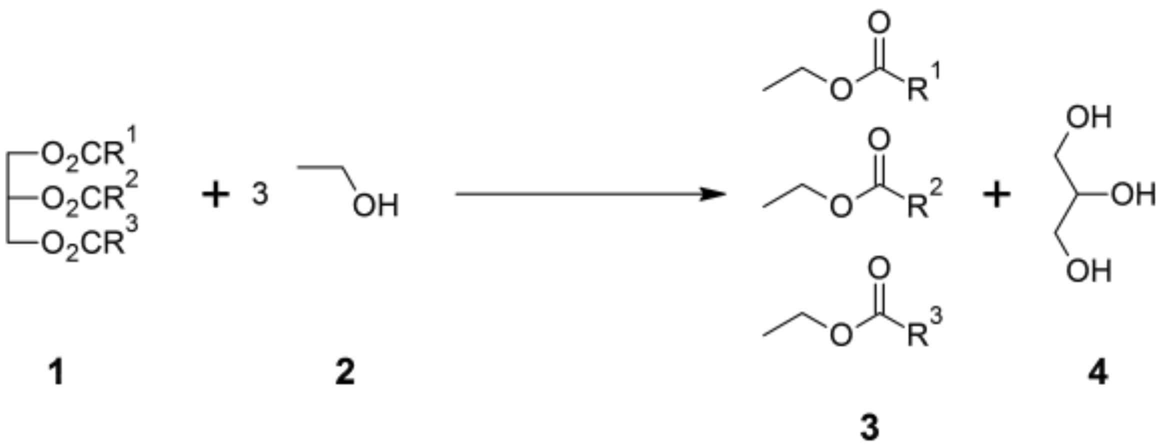
Because the separation of lipids from other cellular components is difficult and because it is difficult to produce significant amounts of biodiesel from our own cultures, we will skip this second step. Instead, we will start with vegetable oil and chemically convert it to biodiesel fuel.

Vegetable oil is made up of triglycerides.



A triglyceride is a molecule with three chains of hydrocarbons linked together. The three long chains are linked to a small three-carbon chain (derived from glycerol) through ester bonds. To form biodiesel fuel, the three chains need to be separated from each other by breaking the ester bonds and reforming them. This process is called transesterification.

Transesterification of triglycerides in vegetable oil can be carried out by reacting the tryglycerides with methanol. This reaction is sketched out below.



Each triglyceride (1) reacts with three methanol molecules (2) to form methyl esters (biodiesel, 3) and glycerol.

Let's carry out this reaction and see what happens.

(This reaction is catalyzed by a base. Before this activity takes place, a solution of sodium hydroxide in methanol must be prepared. Measure out an amount of methanol equal to 1/5th the amount of vegetable oil you intend to mix (methanol:oil ratio of 1:5). For every 50 mL of methanol, add 0.875 g NaOH. You may need to stir and heat the solution slightly to dissolve the NaOH. The amounts of oil and methanol used in the lab can be changed appropriately to meet your needs.)

Add 10 mL of the solution of methanol to 50 mL of vegetable oil. Mix vigorously for a few minutes and place the solution into a separatory funnel. After some time, you should see two different liquid phases. One of them is the glycerol, and the other is the biodiesel. Which one is which?

(It may take a long time to see two different layers. Therefore, it may be convenient to wait one day. The glycerol is denser than the biodiesel, so the biodiesel should be on top and the glycerol should be on the bottom.)

Now, we will make observations of what happens when these products are burned. Prepare three different samples:

1) The lower fraction after reaction
2) The upper fraction after reaction
3) Vegetable oil

(Only a few mL of each are needed.)

Dip a small piece of glass wool into the first sample (the lower fraction) so that it is coated. Hold the wool with tongs and light it on fire in a hood. What color is the flame? What sounds are made as the wool is burning? Do you see any smoke? If so, what color? Record your observations.

Now repeat this process for the other two samples.

(The biodiesel (the upper fraction) should burn easily and should give off thick, black smoke. The glycerol and vegetable oil do not burn as readily and do not give off black smoke. If there is unreacted methanol in any of the fractions, this may produce a crackling sound.)

Observations:

Sample 1:

Sample 2:

Sample 3:

Based on your observations, can you tell which fraction is biodiesel and which fraction is glycerol?

Grass Roots GK-12 Laboratory Activity

Lesson 1: What is energy and where does it come from?

In this activity your class will discuss the definition of **energy** and find out how it is used in our everyday lives. We will investigate the **sources of energy** classifying them as **renewable** or **nonrenewable** and finding out how they are delivered to us. We will look at energy consumption in the United States over history and talk about where it is planning to go in the future. At the end of this handout you will determine which renewable energy sources can replace nonrenewable ones.

Question: List four things that you think of when you hear the word **energy**?

1)

2)

3)

4)

Class definition:

Energy is:

Energy source is:

List of energy sources:

1)	5)	9)
2)	6)	10)
3)	7)	Others:
4)	8)	

Question: How did you use energy yesterday? List 5 activities.

Energy activity use:	Source of energy:	Electricity? (Y or N)

For two of these activities list as many steps as you can about how the energy was delivered from the source to your activity. Separate the steps by arrows and list any steps you do not know as question marks.

Energy Activity #1:___________________________________

Steps:

Energy Activity #2:_________________________________

Steps:

Definition:

Renewable means:

Question: What does it mean to be a **renewable energy source**?

A **renewable energy source** is:

A **nonrenewable energy source** is:

Go back to your list of **energy sources**, mark the sources as either **renewable (R)** or **nonrenewable (N).** Did any of your activities yesterday use **renewable energy?**

Question: In 2001 what percentage of the energy used in the United States was renewable energy?

Initial guess:_______________ Actual answer:_____________

What is the main type of energy used in the United States? Where did it come from?

In 2012, what percentage or electricity in the United States was generated from renewable sources?

Initial guess:_______________ Actual answer:______________

In 1850, what percentage of the energy used in the United States was renewable energy?

Initial guess:_______________ Actual answer:______________

Where did it come from?

The United States consumes **20%** of the energy consumed worldwide, yet only has **4%** of the population.

If all the energy used in the United States was converted to gallons of gasoline, the United States consumes a total of **1 trillion gallons** of gasoline equivalents in a year!

Question: What "states" can energy be stored in? Give an example of each.

1)

2)

3)

4)

Question: Why is biomass an important replacement for fossil fuels? Give three examples of biomass-based energy sources?

1)

2)

3)

Question: What are the four types carbon-based found in living organisms? Circle the one that looks most like gasoline.

1)

2)

3)

4)

Question: Why are algae an attractive biomass energy source?

Homework:

1) Read the "Scum Solution" article. Give two more reasons why algae are an attractive renewable energy source.

    1)

    2)

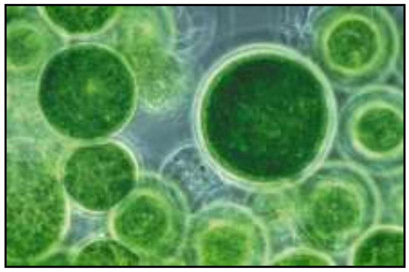
2) List 2 more activities that required energy list as many steps as you can about how the energy was delivered from the source to your activity

Energy activity use:	**Source of energy:**	**Electricity? (Y or N)**

Activity #1:______________________________________
Steps:

Activity #2:______________________________________
Steps:

Lesson 2: How do algae grow?



In this activity we will discuss many of the **variables** that can affect the growth of algae. We will begin by reviewing the advantages of renewable energy and why algae in particular are advantageous as a potential biofuel. Next we will discuss the growth of algae focusing on solar energy. We will learn about the **solar spectrum** and **light absorption** through an interactive learning demonstration. Finally we will talk about other variables that could affect algae growth before designing bioreactors of our own!

Question: What is the only **renewable energy** that can be converted to a liquid fuel?

Question: List three reasons why we need **renewable liquid fuels**?

1)

2)

3)

Question: List four advantages to using **algae** as a source of **renewable liquid fuels**?

1)

2)

3)

4)

Algae grow using a process known as:______________________________

Describe this process in your own words:

For the rest of this activity we will be using a **spectrometer.** A spectrometer measures the properties of light over a spectrum of wavelengths. We will begin to understand these properties through a demonstration.

Question: What defines a color?

Initial guess:

Draw the spectrum for green light

Question: What does the spectrum of red light look like?

Initial guess: Final answer:

Question: What does the spectrum of blue light look like?

Initial guess: Final answer:

Question: What does the spectrum of white light look like?
Initial guess: Final answer:

Label the visible light spectrum

400nm 500nm 600nm
700nm

Draw the spectrum of fluorescent lights and an LED light
Fluorescent: LED:

Question: What does the **solar spectrum** look like?
Initial guess: Final answer:

Question: Is the solar spectrum the same every day? What could affect the solar spectrum?

Question: What happens to light when it passes through a leaf?

Initial guess:

Final answer:

Question: What happens to light when it passes through algae?

Initial guess:

Final answer:

How do algae grow when there is not enough light?

Question: What is required to grow algae?

1)

2)

3)

4)

Homework question: Name three variables you could like to test in your bioreactor? Circle your favorite.

1)

2)

3)

Lesson 3: Building an Algal Bioreactor

Material List

1) 0.5 mL of algae culture per group, approximately 15 mL of algae. *Chlamydomonas reinhardtii* works well and can be purchased from: http://chlamycollection.org . Algae cultures should be fully grown prior to giving them to students.
2) 10 mL of TAP media per group, approximately 500 mL of media. TAP media can be purchased from: http://chlamycollection.org/media/ or made according to the recipe found at: http://www.chlamy.org/TAP.html
3) Distilled water
4) Cleaned and air dried glassware for algae cultures. 20 mL test tubes work well as do Erlenmeyer flasks
5) Test tube rack
6) Stoppers for test tubes or flasks
7) Tin foil
8) Indoor plant grow lamp
9) Pipettes (glass or plastic) and graduated cylinders
10) Microscope
11) Blank microscope slides

Optional:

12) Shaker for test tubes or flasks
13) Spectrophotometer
14) Other algae growth medium (plant food, orchid food caused the algae to grow slower or not at all compared to TAP media)
15) Additional carbon sources (ie glucose, acetate). 0.1 mL per group of a 20% w/v solution.
16) Penicillin-based antibiotic. 0.1 mL per group of a 10 mg/mL ampicillin solution.
17) Spectrometer (for interactive learning demonstration)

Note: The combined cost of algae and growth medium should be around $50

Lesson 3: Building an Algal Bioreactor

In the previous activity, we looked in depth at several **variables** that can affect the growth of algae. We looked at the **solar spectrum** and how the amount of light passing through a reactor can be influenced by algae growth. Now we will analyze many of the variables that affect algae growth by testing them in your own bioreactors!

Materials:

- 10 mL of growth medium
- 0.5 mL of algae culture
- 1 test tube per group
- Test tube shaker
- Plant growth light

Name the five requirements for algae to grow:

1)

2)

3)

4)

5)

What is a **positive control**? What do you expect to happen with this culture?

What is the reason why we have a **negative control**? What are the negative controls we are setting up for this experiment?

In addition to groups testing the positive and negative controls, groups can also test:

Aeration (more carbon dioxide)
Reproducibility
Distance from light
Additional carbon source (add 0.1 mL of additional carbon source)
Other growth media
Antibiotics (avoid contamination) (add 0.1 mL of ampicillin)
Other:

List the variable you are testing:

To set up your reactor

For all steps, keep containers closed to avoid contamination

6) Determine the media that should be used to test the variable you selected
7) Add 10 mL of media to a test tube. Add additional solutions as described by the variable you selected
8) Add 0.5 mL of algae to your media. Mix gently
9) Reserve a small amount to look at under the microscope
10) Place reactors in desired environments

Building an Algal Bioreactor Worksheet

List the reason(s) why you chose to test the variable you picked:

Describe the environment for your reactor:

What is your hypothesis for your experiment? Why?

Describe the appearance of your reactor:

Draw a picture of what the algae looks like under the microscope:

Building an Algal Bioreactor Data Log:

Day 2

Describe the appearance of your reactor:

How does this compare to the positive and negative controls:

Day 3

Describe the appearance of your reactor:

How does this compare to the positive and negative controls:

Day 4

Describe the appearance of your reactor:

How does this compare to the positive and negative controls:

Lesson 4: Analysis of an Algae Bioreactor Worksheet

What was the variable you chose to study? Why did you pick this variable? Describe why this variable could affect algae growth?

What was your original hypothesis for your experiment?

What was the result for your reactor?

Did the **positive control** work as expected? How does the positive control compare to your reactor (better growth, same growth, no growth…)?

Did the **negative controls** work as expected? How does the negative control compare to your reactor?

Provide a reason to explain your results:

Now let's look at the algae under a microscope.

Draw a picture of your algae, label with as much detail as you can observe: (If your algae did not grow, show a picture of what you observe under the microscope as well as a second picture from the positive control group).

Did you learn anything by looking at the algae under the microscope?

Summarize your results (in no more than two sentences):

Was your hypothesis confirmed? Why or why not:

What variables affected algae growth for the classroom bioreactors?

What went right/what needs to be improved? What would you like to test next?

Now let's design a classroom algae reactor out of recyclable materials! What do you need for your reactor to optimally grow algae? Sketch a picture

Lesson 5: Producing Biodiesel from Algae

In the previous activity, we grew algae under different conditions and designed our own bioreactors to maximize growth. In this activity, we will explore the process of converting algal lipids into biodiesel fuel.

Materials:

- Previously grown algae culture
- Vegetable oil
- Weigh scales
- Separatory funnels
- Sodium hydroxide
- Methanol
- Glass wool
- Metal tongs
- Lighter or Bunsen burner

Producing biodiesel from algae requires three processes

1) Separation of the algae from the water it grows in
2) Isolation of the lipids in the algae from the other components of an algae cell
3) Conversion of the lipids into biodiesel

What are some ways you could separate the algae you have grown from the growth medium?

Use one of these methods to separate your algae from your growth medium and determine the mass of algae you have grown.

Mass of Algae Grown ___________________

After separating the algae from water, the parts of algae cells that are used to make biodiesel fuel need to be isolated from the rest of the cellular components. Lipids, which make up parts of the membranes in cells, and fats can be chemically converted to biodiesel fuel. They can be isolated from the other cellular components by adding a solvent that only solvates lipids and fats. Hexane and supercritical carbon dioxide are common. Supercritical methanol can be even more convenient to use. (Why?)

Assuming that your algae culture is 30 % by mass of lipids and fats, and assuming that the mass of biodiesel produced is approximately equal to the mass of lipids and fats, what mass of biodiesel could you produce with your algae culture?

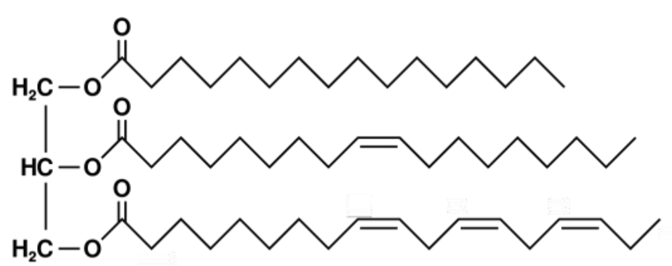
Mass of Biodiesel ____________________

The density of biodiesel fuel is about 0.88 g/cm^3. How many gallons of biodiesel fuel could you produce from your culture?

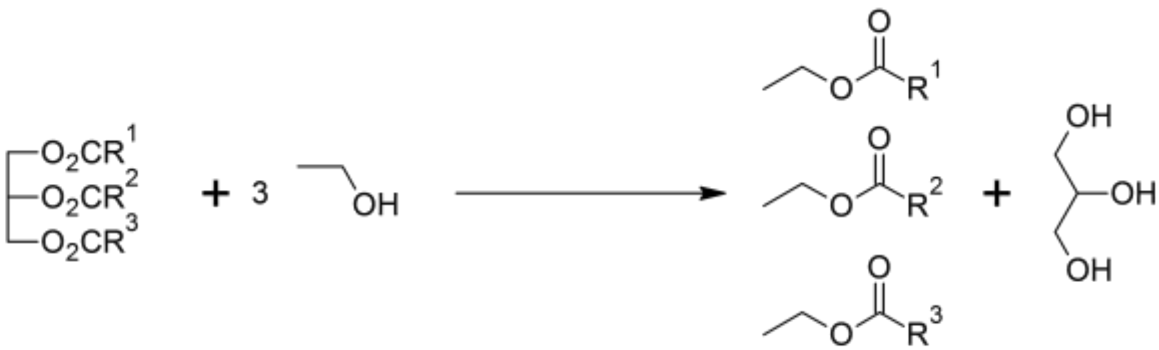
______________ Gallons of Biodiesel Fuel

Because the separation of lipids from other cellular components is difficult and because it is difficult to produce significant amounts of biodiesel from our own cultures, we will skip this second step. Instead, we will start with vegetable oil and chemically convert it to biodiesel fuel.

Vegetable oil is made up of triglycerides. A triglyceride is a molecule with three chains of hydrocarbons linked together. The three long chains are linked to a small three-carbon chain (derived from glycerol) through ester bonds. To form biodiesel fuel, the three chains need to be separated from each other by breaking the ester bonds and reforming them. This process is called transesterification.



Transesterification of triglycerides in vegetable oil can be carried out by reacting the tryglycerides with methanol. This reaction is sketched out below.



1 2 3 4

Each triglyceride (1) reacts with three methanol molecules (2) to form methyl esters (biodiesel, 3) and glycerol.

Let’s carry out this reaction and see what happens.

Add 10 mL of the solution of methanol to 50 mL of vegetable oil. Mix vigorously for a few minutes and place the solution into a separatory funnel. After some time, you should see two different liquid phases. One of them is the glycerol, and the other is the biodiesel. Which one is which?

Now, we will make observations of what happens when these products are burned. Prepare three different samples:

1) The lower fraction after reaction
2) The upper fraction after reaction
3) Vegetable oil

Dip a small piece of glass wool into the first sample (the lower fraction) so that it is coated. Hold the wool with tongs and light it on fire in a hood. What color is the flame? What sounds are made as the wool is burning? Do you see any smoke? If so, what color? Record your observations.

Now repeat this process for the other two samples.

Observations:

Sample 1:

Sample 2:

Sample 3:

Based on your observations, can you tell which fraction is biodiesel and which fraction is glycerol?

Renewable Energy Algae Unit Wrap-up

1) What did you find most interesting about the renewable energy and algae unit?

2) Write down three facts you did not know previously about renewable energy and/or algae:

3) Was anything surprising to you when learning about our current energy use and its future?

4) Did you enjoy growing algae in the classroom? What was the best part of the experiment? Did anything surprising happen? What did you learn about the scientific method?

5) Was there anything confusing mentioned in the unit on algae and renewable energy?

6) What questions do you still have about renewable energy and algae?

7) Do you feel like algae can solve the impending energy crisis? Why? What other information might you need to decide this?

8) After learning about our current energy use, do you plan to change your energy consumption at all? Why or why not? How might you be able to cut back your energy use?

9) Summarize what you learned about renewable energy and how algae is a possible solution in one sentence:

10) Did growing algae in the classroom teach you anything about a possible career in science and research? What questions do you still have about what it is like to be a scientist?