Title: Energy Storage in Plants: Should Biofuels Be Made from Seeds or Leaves and Stems?

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Abstract:

The investigation involves students determining where plants store potential energy (a.k.a., chemical energy). In the investigation, students will grow rapid cycling *Brassica rapa* plants and observe that the dry weight (mass) increases because of photosynthesis. They will measure the amount of energy stored in seeds and vegetative tissues by using a simple calorimeter. The information collected by the students is evaluated as it relates to potential sources of biofuels.

Learning objectives:

This lesson came from a high school curriculum developed for the Ford Next Generation Learning (formally Ford Partnership for Advanced Studies) curriculum on biofuels. The goal of the program is to create an upper level high school curriculum that will prepare students to enter careers in science and engineering (Ford Motor Company Fund, 2015). A description of the overall biofuels curriculum has been published (Schumack *et al*., 2012). The complete curriculum can be downloaded from the University of Detroit Mercy's (2010) website. The instructional materials fulfill many of the Next Generation Science Standards (National Research Council, 2013) for grades 9 -12. Because the instructional material is highly quantitative, the activities support many of the mathematics outcomes of the Common Core Curriculum as well (National Governors Association Center for Best Practices - Council of Chief State School Officers, 2010).

At the end of this activity the student should be able to:

1. Describe the nature of energy
2. Describe how plants capture and use energy
3. Describe aspects of a plant's life cycle
4. Calculate where plants store potential energy
5. Determine the best source of materials for making biofuels to help reduce global warming.

Linkage to the Next Generation Science Standards:

- HS-LS1-5. Use a model to illustrate how photosynthesis transforms light energy into stored chemical energy.
  - Students will measure the mass of seeds and the mass of plants after photosynthesis. Additionally, the students will compare the dry mass of plants grown in light versus grown in the dark. Students will observe that etiolated (dark grown) seedlings gain height faster than seedlings grown in light. However, there will be no overall increases in mass in the etiolated seedlings because they used stored resources to increase height. Once the resources are diminished, the etiolated seedlings stop growing. In contrast, the light grown seedlings gain mass through photosynthesis.
  - The outcome is addressed by having students estimate, using a calorimeter, the amount of energy in a single seed and the amount of energy in a mature plant.
- Science and Engineering Practices: Use a model based on evidence to illustrate the relationships between systems or between components of a system.
  - The students need to look at the summary equation of photosynthesis to determine that germinant seedlings growing in the dark are not photosynthesizing while light grown plants gain dry mass due to photosynthesis.
- Science and Engineering Practices: Constructing explanations and designing solutions in 9–12 builds on K–8 experiences and progresses to explanations and designs that are supported by multiple and independent student-generated sources of evidence consistent with scientific ideas, principles, and theories.
  - The students are not informed of their results before they conduct their experiments; thus, they need to evaluate their own data based on the scientific theories described the law of conservation of mass, the laws of thermodynamics, and the content of the summary equation for photosynthesis.
- Disciplinary Core Ideas: LS1.C: Organization for matter and energy flow in organisms. The process of photosynthesis converts light energy to stored chemical energy by converting carbon dioxide plus water into sugars and released oxygen.

    - From their experimental results, the students should observe that plants only gain dry mass when light is present. Experiments with calorimeters will show the students that plants gain chemical potential energy through photosynthesis. This instructional material was not designed to describe all of the major aspects of photosynthesis. Thus, key topics such as carbon dioxide absorption and oxygen evolution which are components of LS1.C are not covered by this set of exercises.

- Cross Cutting Concepts: Changes of energy and matter in a system can be described in terms of energy and matter flows into, out of and within that system.
    - The students' experiment will show that the plants only gain dry mass when the plants are exposed to light. The calorimeter measurements will show that the plants have gained chemical potential energy.

- HS-LS2-5. Develop a model to illustrate the role of photosynthesis and cellular respiration in the cycling of carbon among the biosphere, atmosphere, hydrosphere, and geosphere.
    - This concept is presented in Figure 1 of the students' handout. The figure shows CO_2 is a key input for photosynthesis and CO_2 is a major product of cellular respiration. The relationship is developed more fully in the investigation involving the synthesis of cellulosic ethanol (University of Detroit Mercy, 2010).

- Science and Engineering Practices: Using Mathematics and Computational Thinking: Mathematical and computational thinking in 9–12 builds on K–8 experiences and progresses to using algebraic thinking and analysis, . . . Simple computational simulations are created and used based on mathematical models of basic assumptions.
    - Since the students do not use the entire plant in determining the amount of energy stored in a plant, they need to use basic algebra to determine the total energy content of both seed and leaves plus stems. The calculations are described in Tables I and III of the students' handout.

Timeframe:

The procedure requires little preparation by the instructor. The first time the exercise is taught, the instructor will need to assemble a simple apparatus which is described in the "List of materials" (below). The investigations work well on a once a week basis. Rapid-cycling *Brassica rapa* plants are used because they complete their life cycle in 35 to 40 day's (University of Wisconsin - Madison, 2015). To do the calorimetry experiments, plant materials need to be dried under modest heat for a few days. All of the laboratory activities can be completed in five to seven weeks.

The author has classroom-tested this lesson in a pre-college program that met once a week. The students were in 8th and 9th grade. Below is the timeframe used to complete the experiments. The exercise can be completed in a shorter period of time if your class meets more than once a week.

Week 1: (Procedure A: Steps 1 - 3) Seeds were weighed and planted. Including lecturing, the activity required 2 hours.
Week 2: (Procedure A: Step 4) Heights of plants were measured. Classroom time needed: 15 minutes.
Week 3: (Procedure A: Steps 4 & 5) Heights of plants were measured and pollinated. Classroom time needed: 30 minutes.
Week 4: (Procedure A: Step 4 & 6) Heights of plants were measured and pollinated. Classroom time needed: 30 minutes.
Week 5: (Procedure A: Step 4 & 7) Heights of plants were measured. Classroom time needed: 15 minutes.
Week 6: (Procedure A: Steps 8 - 10). Harvest seeds and stems with leaves. Place plant material under drying lamps or in drying oven. Time needed: 1 hour.
Week 7: (Procedure A: Steps 11 & 12). Weigh plant materials. Make graphs. Time needed: 1 hour.
(Procedure B: All steps): Time needed: 2 hours. Note that some students may have difficulty with the calculations.

List of materials:

- Rapid-cycling *Brassica rapa* seeds.
  Seeds are sold under the brand names Rapid Radishes™ (Ward's Natural Science, PO Box 92912, Rochester, NY 14692. (800) 962-2660. http://www.wardsci.com) and Wisconsin Fast Plants™ (Carolina Biological, 2700 York Road, Burlington, NC 27215-3398. (800) 334-5551. http://www.carolina.com.

- Small pots.
  One of the advantages of rapid cycling *Brassica rapa* is that they can be grown in a confined space. Thus, 2-inch diameter plastic cups with a pencil size hole punched through the bottom is sufficient to grow the plants to maturity. To aid in watering, a short segment of string can be passed through the hole. The string acts as a wick and draws water from the drip pan back into the pot. As a result, the plants do not need to

be watered as often. Plants are irrigated by adding water to the reservoir, not on top of the pots to prevent disturbing the potting soil.

- Pan to hold pots and to collect dripping water from the pots. A plastic dishwashing pan works well.

- Potting soil.

- General purpose fertilizer.
  If the potting soil is not already fertilized, use a general purpose fertilizer. Apply at the rate recommended by the manufacturer. Pellet fertilizer works well and is not messy to use.

- Desk lamp with fluorescent bulbs. Compact fluorescent lights work well.
  Warning: Fluorescent light bulbs contain mercury, a toxic chemical. If the bulb breaks, follow the cleanup procedure outlined by the Environmental Protection Agency (2015). The instructions can be viewed at http://www.epa.gov/cfl/cleaning-broken-cfl. Do not discard fluorescent bulbs in municipal trash. They should be placed in closable plastic bags and taken to a recycling center. A list of recycling centers can be found at http://search.earth911.com/. Details regarding fluorescent bulb disposal can be downloaded from http://www.epa.gov/cfl/recycling-and-disposal-after-cfl-burns-out.

- Aluminum foil.

- Laboratory scale or balance that can measure weights within 0.01 g.
  If your students have access to an analytical balance then individual seeds, dried leaves, and dried stems can be measured. If you only have access to a standard scientific scale (+0.01 g), your students can estimate the mass of seeds, dried leaves, and dried stems by weighing several at the same time then calculate the average.

- Forceps.

- Weighing paper or weighing boats.

- Metric rulers.

- Graph paper.

- Popped popcorn.
  It is best to use popcorn where additional oil has not been added. An easy way to make the popcorn is to place a few dozen popcorn kernels in a brown paper lunch bag and

heat in a microwave until popped. Do not overcook the popcorn or it will burn. An alternative method of making popcorn is to use a hot air corn popper.

- Sample holders made by alligator clips attached to bent paper clips (Fig. 3A). Alligator clips can be purchased at hardware stores.
- 2 wire gauze alcohol burner stands per calorimeter.
  Stands can be purchased from Cynmar Corporation, 21709 Route 4 North, PO Box 530, Carlinville, IL 62626. Phone: (800) 223-3517, FAX: (800) 754-5154. http://www.cynmar.com; Carolina Biological, 2700 York Road, Burlington, NC 27215-3398. Phone: (800) 334-5551, FAX: (800) 222-7112. http://www.carolina.com; Ward's Natural Science, PO Box 92912, Rochester, NY 14692. Phone: (800) 962-2660, FAX: (800) 635-8439. http://www.wardsci.com.
- Aluminum mailing canister, foil cupcake wrappers, or aluminum catch bucket.
  2.5-inch aluminum catch buckets used with "overflow cans" can be purchased from Cynmar Corporation. The address is above.
- Small, light, non-mercury, metric thermometers.
  Inexpensive thermometers can be purchased from Cynmar Corporation (address above).
- Small tin cans with both ends removed. Tomato paste cans work well.
  Tin snips to cut vents into tin cans (Fig. 3B). Leather gloves should be worn while cutting the vent hole.
- Distilled water or tap water.
- 50 mL graduated cylinders.
- Wooden matches or butane lighters designed to light barbecue grills.
- Fire extinguisher.
- Chemical goggles.

Procedure and general instructions (for instructor).

Safety

In this investigation, students will use calorimetry to measure the energy content of different plant parts. The activity involves using home-made calorimeters made from metal cans and the burning of plant materials. Thus, safety precautions should be followed. If the instructor decides to have the students make the chimney shown in Figure 3, the students should be provided leather gloves to wear when they are cutting out the base hole with tin snips. When the students ignite their plant material, they should be supervised. The calorimeter should be placed on a non-flammable surface. The workspace should be clear of flammable materials. Make sure that the students are not wearing loose fitting clothing and long hair is tied back. Students should NOT wear plastic gloves because they are flammable. Fire safety procedures should be established in advance, such as having an emergency exit plan and accessibility of fire extinguishers, etcetera. Though the chemical splash hazard is low, the instructor should have the students wear chemical splash goggles. The instructor may want to review the National Science Teacher Association Safety Advisory Board (2013) recommendations on laboratory safety practices and procedures.

For the most part, the students are not using toxic chemicals in the experiment. However, issues with mercury should be noted. Use non-mercury thermometers. If the thermometer breaks, you will not need to worry about mercury cleanup procedures. Unfortunately, fluorescent light bulbs contain mercury. If a bulb breaks, follow the cleanup procedures described in the list of materials (above). If you have additional questions, contact your local poison control center at 1-800-222-1222.

Preparation and Procedure

The procedure is completely described in the students' activity sheet. The preparation of the apparatus is described in the "List of materials" section. Keys to the questions to be answered by the students are below. Additionally, a short answer assessment, (not found in the student pages) and answer key are printed below.

Answers to Pre-activity Questions:

1) Where does the potential energy found in plants come from?
Sunlight. See Figure 1.

2) Where does the carbon found in plants come from?
Atmospheric CO_2 captured by photosynthesis.

3) Why do plants make carbohydrates and oils?
To store energy captured by photosynthesis and to make structures like stems and leaves.

4) If all methods of making biofuels cost the same, then what plant materials should be used to make liquid biofuel?
It is not desirable to use food for fuel because there will be less food for people. Thus, the non-food parts of crops would be the most desirable for biofuel. Unfortunatley, food portions of corn and soybean are used to make biofuels. Additionally, most of the potential energy stored in plants is found in the structural carbohydrates plants use to make stems and leaves.

Procedure-A: Answers to Post-activity Questions

1. Look at your graph of plant growth. At the beginning of the experiment, which seedlings grew the fastest, the seedlings in the light, or the seedlings kept in the dark? Propose a hypothesis (i.e., an explanation) to explain your results.
The result that surprises most students is the seedlings kept in the dark grow the fastest. However, they look very different than light grown seedling. The dark-grown seedlings are highly elongated and not green. These are etiolated seedlings. Etiolation is a seed adaptation. Light is the signal that plant seedlings used to detect where they are, under the soil or above the ground. If the seedlings are under the soil, resources are placed towards elongation so the plant can grow out of the soil into sunlight. Once out of the soil, the seedling places resources into starting photosynthesis and turns green. Thus, the light-grown seedlings look different than the dark-grown seedlings and exhibit slower elongatation.

2. Why were the seedlings able to grow in the dark? Where did the energy come from? Where did the matter come from? [Hint: Look at the data in Table I.]
Seeds contain storage materials (starches, oils and proteins) to help the emerging seedling grow out of the soil. The energy and matter of the etiolated seedling come from the seeds itself. Thus, Table I should show no net gain in biomass. A net loss in biomass is expected because the plant releases energy through the process of respiration, which produces CO_2*. However, your students may not be able to detect this if they do not have access to sensitive scales.*

3. Where is the majority of the matter in a plant, in the seeds or in the leaves and stems? Based on this data, where do you think the majority of the potential energy of a plant is located?
The vast majority of the biomass (dry weight) of plants is in the form of stems and leaves. Though seeds can contain large amounts of oils, which are more energy dense than carbohydrates, there is so much more biomass in the stems and leaves that the vast

majority of the potential energy is found there.

Procedure B: Answers to Post-activities Questions

1. Where is the majority of the potential energy stored in a plant, the seeds or in the leaves/stems?
   There is 5 to 10 times more energy stored in the leaves/stems than in the seeds.

2. What form is most of the potential energy stored as, oils, starches or cellulose? What is the basis for this conclusion?
   Cellulose is the main carbohydrate in leaves and stems. Since the vast majority of the energy is stored in stems and leaves, the potential energy is mostly in the form of cellulose.

3. If the goal of making biofuels is to make the United States of America energy independent, what is the best liquid biofuel, biodiesel or ethanol? What is the basis of your answer?
   Ethanol. Biodiesel is made from vegetable oil found in seeds. Ethanol can be made from cellulose.

4. If the goal of making biofuels is to reduce the impact automobiles have on global warming, what would be the best liquid fuel? What is the basis of your answer?
   Since there are agricultural inputs that use fossil fuels, it is important to get the greatest return on the "energy fossil fuel investment." Thus, the parts of the plants that contain the greatest amount of energy should be used to make liquid biofuel. The colorimeter experiment should show that there is 5 to 10 times more energy in the stems/leaves than in the seeds. Thus, stems and leaves should be the feedstock for making ethanol.

5. If you want to reduce the impact that liquid biofuels will have on food supplies, what would be the best method of making liquid biofuels?
   Corn starch and vegetable oils are used to make corn ethanol and biodiesel, respectively. Both of these materials are made from seeds used for food. The leaves and stem of corn, wheat, and many other plants are not used for food. Thus, corn leaves and stems can be used in the production of ethanol without reducing food production.

6. Puffed popcorn was used in the calorimetry experiment because it is easier to handle and ignite than *Brassica rapa* seeds. Is this a fair comparison? The following nutritional information will help. 100 g of popcorn contains 350 kilocalories, 8.6 g of protein, 5.0 g

of fat, and 70.6 g of carbohydrate. 100 g of rapeseed (a type of *Brassica rapa*) contains 35 kilocalories, 4.1 g of protein, 0.4 g of fat, and 6 g of carbohydrate. How should you change the calculation in Table III to take into account for the difference between popcorn and *Brassica rapa*?

The popcorn overestimates the amount of energy stored in the Brassica rapa seeds. The popcorn stores 10 times more energy (350 kilocalories/100g / 35 kilocalories/100g = 10). Thus, the seed energy value estimated in Table III should be divided by 10.

Post activity assessment:

1. How can biofuels help prevent global warming?
   The carbon released when burning biofuels is the carbon captured from the atmosphere by photosynthesis. Thus, there is no net gain in atmospheric carbon if fossil fuels are not used to grow the crops or process the ethanol. (Learning objective 2).

2. Why does corn-based E85 fuel not prevent global warming?
   A large quantity of fossil fuel is used to grow, harvest and process corn. Thus, corn-based biofuels produce as much CO_2 *as burning petroleum gasoline.* (Learning objective 5)

3. How is potential energy measured?
   Igniting dry matter and measuring how much heat is released by observing the change in temperature of a known mass of water. One calorie of energy is the amount of heat needed to raise the temperature of 1 gram of water 1 ^{o}C. *The instrument used to measure potential energy is called the calorimeter.* (Learning objective 1)

4. Based on your experimental results, where is most of the chemical potential energy stored in plants? Explain how your data supports your conclusion?
   Both the biomass (Table I) and total potential energy (Table III) showed the greatest amount of matter and energy in the stems + leaves. (Learning objective 4)

5. What would be the best source of feed material to make E85 ethanol? Explain why.
   Stems and leaves. They contain the greatest amount of potential energy. (Learning objectives 5)

6. Do your experiments provide evidence that the energy in biofuel comes from light? Explain.
   Yes. The plants that grew in the dark produced much less biomass. To create biomass,

plants need to capture light energy. Biomass is a measure of chemical potential energy. (Learning objective 2)

7. Where does the energy plants need to germinate come from?
Chemical potential energy stored in the seeds, primarily in the form of starches, oils and proteins. (Learning objectives 2 and 3)

8. Why did you need to transfer pollen from the anther to the stigma?
Seeds cannot be produced without pollination. The anther is the pollen producing structure of the flower (Figure 2). Seeds are produced when the egg in the ovary is fertilized by sperm nuclei in the pollen grain. When the pollen lands on a receptive stigma, it produces a pollen tube that the sperm nuclei travel through. When the pollen tube reaches the embryo sac, it can fertilize the egg in the ovary. (Learning objective 3)

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