Teacher Guide
Bio-refinery: Producing Paper and Ethanol from Pineapple

Overview

- This laboratory activity aims to improve students' understanding of how bio-refineries operate.
- Students will produce paper from pineapple leaves and will evaluate the effect of refining on paper properties.
- Students will ferment the juice from pineapple peel to create bio-ethanol and will modify the reaction conditions to determine the influence on the reaction. Students will also apply the law of conservation of mass to determine the amount of ethanol produced.

Objectives

Students will:

- Understand the concept of bio-refinery
- Understand the process of paper production
- Understand the process of fermentation to obtain ethanol
- Evaluate the effect of yeast concentration on the final bioethanol product concentration
- Evaluate the fermentation reaction rate and reaction conditions that influence the rate
- Apply the law of conservation of mass

Content Standards

This lesson is appropriate for high school biology, chemistry, and environmental students and addresses the following standards:

North Carolina Essential Standards

Earth/Environmental	EEn.2.2; EEn.2.8
Physical Science	PSc.1.2; PSc.2.2
Chemistry	Chm.1.2; Chm.2.2; Chm.3.1
Biology	Bio.2.2; Bio.4.2

Next Generation Science Standards:

Grades 9-12, Disciplinary Core Ideas/Practices/Cross-cutting concepts:

DCI	HS-PS1 Matter and Its Interactions HS-ETS1 Engineering Design HS-ESS3 Earth and Human Activity HS-LS1 Ecosystems: Interactions, Energy, and Dynamics HS-LS2 Ecosystems: Interactions, Energy, and Dynamics
Practices	Asking Questions and Defining Problems Planning and Carrying Out Investigations

	Analyzing and Interpreting Data Using Mathematics and Computational Thinking Constructing explanations
Cross-cutting concepts	Patterns Energy and matter Stability and change Structure and function

Time Requirements
Preparation time: *60 minutes (day of or day before)*
Class-time: *2-3 class periods of 45 minutes each*
After class time: *variable*

Materials
The materials in this kit support 10 groups of students working collectively

Included in the kit:
- Cheese cloth (40 ft long)
- Baking soda, 40g
- Heat resistant gloves, 10 pairs
- Blender, 5
- Handsheet mold, 10
- Blotter paper, 80
- Roller, 10
- 24-hour Rapid Rise yeast, 50 g
- Fishscale, 10

Needed, but not supplied:
- Pineapple, 5
- Metal weighing tray, 10
- Balance
- Hot plate, 10
- Erlenmeyer flask (500 mL), 10
- Graduated cylinder (100 mL), 10
- Erlenmeyer flask (250 mL), 20
- Airlock, 20
- Airlock stopper, 20
- DI water
- 40 mm handheld strainer,10
- Plastic tub for papermaking, 10

Safety
During this experiment you will be using sodium hydroxide (strong base), hot plates and blenders. Please, make sure to:
- Wear proper PPE (lab coat, safety glasses and gloves) when handling the

sodium hydroxide and hot beakers
- Do not introduce your hands into the blender

Ensure that students understand and adhere to safe laboratory practices when performing any activity in the classroom or lab. Demonstrate the protocol for correctly using the instruments and materials necessary to complete the activities and emphasize the importance of proper usage. Use personal protective equipment such as safety glasses or goggles, gloves, and aprons when appropriate. Model proper laboratory safety practices for your students and require them to adhere to all laboratory safety rules.

Background Information

- Bio-refinery:
    - Definition: a facility designed to process biomass to generate a variety of bio-based products such as chemicals, materials or fuels.
    - Importance: development of renewable and sustainable biomaterials and biofuels to reduce the emissions and the impact on the environment; “byproducts” from one process are re-used as an input for another process.

- Paper:
    - Definition: web made of cellulose deposited from a water suspension and then dried to form inter-fiber hydrogen bonds.
    - Consistency: is the relationship between the mass of dry fiber and total mass in a pulp suspension (C=dry fiber/total mass)
    - Importance: paper and derived products are present in our daily activities (napkins, boxes, printing paper, hygienic tissue paper…) and therefore play an essential role in the development of a country. Also, in the United States, the paper industry is the fourth biggest manufacturing industry, contributing significantly to the economy of the country.

- Papermaking processes:
    - Goal: papermaking processes aims to liberate fibers from wood or plants to fabricate a suspension of cellulose in water known as pulp. This material is then transformed into paper by dehydration and further processing depending on the final desired product
    - Classification: the papermaking processes may be classified depending on the treatment applied:
        - Mechanical pulping: use of mechanical forces and thermal treatment to separate the wood fibers
        - Semi-chemical pulping: use of mechanical forces, thermal and mild chemical treatment to separate wood fibers
        - Chemical pulping: use of strong chemical treatment to separate wood fibers
            - Soda process: an alkaline pulping process that uses sodium hydroxide to remove the lignin present in wood and plants and liberate the fibers

- Fermentation
    - Definition: the anaerobic transformation of organic substrates by the action of microorganisms, such as yeasts. Glucose fermentation is the current pathway to produce bio-ethanol as a biofuel
    - Reaction: the alcoholic fermentation of glucose may be defined as the breaking down of the glucose molecule to produce ethanol and carbon dioxide. The reaction is described by the equation shown below:

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} \rightarrow 2\ \underset{\text{(Ethanol)}}{C_2H_5OH} + 2\ \underset{\text{(Carbon dioxide)}}{CO_2}$$

- Importance of bio-ethanol: ethanol is a renewable fuel that plays a vital role in the reduction of greenhouse gas emissions. This fuel has a lower impact on the environment and thus helps to reduce the use of non-renewable combustibles. Also, its production generates direct and indirect jobs, boosting the economy of the country.

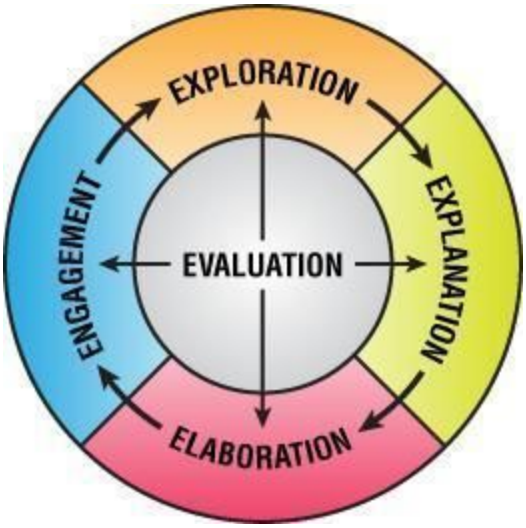
Preparation (day of or day before lab)

a. Pineapple peeling
    1. Wash, peel, and core five pineapples
    2. Save the peels and core from pineapple and cut into small pieces
    3. Wash and cut the pineapple crowns into small pieces
    4. Store the peel and leaves separately in a dry place

Note: The fruity part of the pineapple will not be used in this lab.

b. Prepare 1 L of a NaOH 1 M
    a. Weigh 40 grams of sodium hydroxide
    b. Add the sodium hydroxide into a 500 mL beaker
    c. Add 500 mL of DI water into the beaker
    d. Mix the solution until it is homogeneous
    e. Transfer the solution to a 1000 mL volumetric balloon
    f. Add DI water until the limit is reached

Guiding the lesson using the 5E Learning Cycle



Engage (5-10 minutes)

1. (5 minutes) Show a pineapple to students and ask them to visualize how this raw material could be transformed into high-value products. “What bioproducts do you think we can make from a pineapple? What do you already know about biorefining? How are a biorefinery and a petroleum refinery similar/different?” (whole-group discussion; or, give students a minute to write down their ideas before sharing with a partner/group discussion)
2. (3 minutes) Provide a brief description of the activity: show video clip to introduce biorefinery activity
3. Highlight safety concerns.

Explore/Elaborate (2 x 45 minute sessions)

1. Day 1 (45 minutes): students conduct cooking step for pineapple leaves; design and set-up for fermentation; see full procedure
2. Day 2 (45 minutes): run the fermentation reaction, make paper, and test properties; see full procedure
3. Teacher floats between groups to ensure student participation and understanding

Explain (10-15 minutes, after each explore/elaborate)

1. Day 1: students define and explain core concepts (e.g., yeast, fermentation reactions, reasons for each step (why is the cooking step necessary? Why does the pH need to be adjusted? etc.)
2. Day 2: students define and explain core concepts (e.g., discuss weight loss during fermentation, role of refining/biorefineries, discuss paper-making and paper properties)

Elaborate/Evaluate (15 minutes)

1. Groups discuss final results and how the process depends on the manipulated variables.

2. Students should be able to relate biorefining to three pillars of sustainability and the circular bioeconomy.
3. Students can pose additional questions and design additional ways to optimize and further refine the paper and ethanol production processes.

Answers to Questions in the Student Guide

1. What is a bio-refinery and what is its relevance?

A biorefinery is a facility designed to process biomass and generate a variety of bio-based products such as chemicals, materials or fuels.

2. What is paper?

Web made of cellulose deposited from a water suspension and then dried to form inter-fiber hydrogen bonds

3. What is the goal of papermaking processes?

The papermaking processes aim to liberate fibers from wood or plants to fabricate a suspension of cellulose in water known as pulp. This material is then transformed into paper by dehydration and further processing depending on the final desired product

4. What is the classification of papermaking processes? Please indicate the overall difference between them.

- *Mechanical pulping: use of mechanical forces and thermal treatment to separate the wood fibers*
- *Semi-chemical pulping: use of mechanical forces, thermal and mild chemical treatment to separate wood fibers*
- *Chemical pulping: use of strong chemical treatment to separate wood fibers*

5. What is fermentation? Please write the reaction of glucose fermentation.

Fermentation is the anaerobic transformation of organic substrates by the action of microorganisms, such as yeasts. Glucose fermentation is the current pathway to produce bio-ethanol as a biofuel

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

(Glucose) (Ethanol) (Carbon dioxide)

6. What is the function of yeast during glucose fermentation?

Yeast are microorganisms that converts sugars into ethanol and carbon dioxide (fermentation reaction).

7. What is bio-ethanol and why is it important?

Bio-ethanol is a renewable fuel that plays a vital role in the reduction of greenhouse gas emissions. This fuel has a lower impact on the environment and thus helps to reduce the use of non-renewable combustibles

Note: Please see the background section for more details.

Supplemental Resources

We'd like to acknowledge NC State's Department of Forest Biomaterials for the original "Paper and Ethanol from Sugarcane" procedure from which this lab was modified (available upon request).

Video: Bio-refinery definition	https://www.youtube.com/watch?v=92Xj0ex58g0
Fermentation definition and additional information	https://www.britannica.com/science/fermentation

Name _____ **Student Guide**
Date _____

Bio-refinery: Producing Paper and Ethanol from Pineapple

Overview

During this activity, you will use the concept of bio-refining to produce paper and bio-ethanol from pineapple. You will also evaluate the effect of yeast concentration on the fermentation process and the final ethanol production. To determine the amount of product, you will apply the law of conservation of mass. Finally, you will study the effect of refining on paper properties.

Background

- Bio-refinery:
    - Definition: facility destined to process biomass to generate bio-based products such as chemicals, materials or fuels.
    - Importance: development of renewable and sustainable biomaterials and biofuels to reduce the emissions and the impact in the environment

- Paper:
    - Definition: web made of cellulose deposited from a water suspension and then dried to form inter-fiber hydrogen bonds.
    - Importance: paper and derived are present in our daily activities (napkins, boxes, printing paper, hygienic tissue paper…) and therefore play an essential role in the development of a country. Also, in the United States, the paper industry is the fourth biggest manufacturing industry, contributing significantly to the economy of the country.

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    - Goal: papermaking processes aims to liberate fibers from wood or plants to fabricate a suspension of cellulose in water known as pulp. This material is then transformed into paper by dehydration and further processing depending on the final desired product
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            - Soda process: an alkaline pulping process that uses sodium hydroxide to remove the lignin present in wood and plants and liberate the fibers

- Fermentation

- Definition: the anaerobic transformation of organic substrates by the action of microorganism such as yeasts. Glucose fermentation is the current pathway to produce bio-ethanol as a biofuel
- Reaction: the alcoholic fermentation of glucose may be defined as the breaking down of the molecule to produce ethanol and carbon dioxide. The reaction is described by the equation shown below:

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(Glucose) (Ethanol) (Carbon dioxide)

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Materials

Experiment 1: papermaking from pineapple

- Pineapple leaves from 1 pineapple (40 g approximately)
- Balance, 1
- Hot plate, 1
- Beaker (500 mL), 1
- Graduate cylinder (250 mL), 1
- Heat resistant gloves
- Cheesecloth (2 ft)
- Blender, 1
- Handsheet mold, 1
- Blotter paper, 10
- Roller pin, 1
- Tensile strength scale, 1
- Strainer, 1
- Duct Tape
- Scissor, 1

Experiment 2: bio-ethanol production from pineapple

- Pineapple peel from 1 pineapple
- Graduated cylinder (100 mL), 1
- Erlenmeyer flask (250 mL), 2
- Balance, 1
- Weighing tray, 1
- Airlock stopper, 2
- Airlock, 2
- Plastic dropper, 1
- Beaker (250 mL), 1
- Rapid Rise Yeast, 1 g
- pH indicator strips

Safety
During this experiment you will be using sodium hydroxide (strong base), hot plates and blenders. Please, make sure to:

- Wear proper PPE (lab coat, safety glasses and gloves) when handling the sodium hydroxide and hot beakers
- Do not introduce your hands into the blender

Procedure
Session 1

Part 1: Producing pulp from pineapple leaves

1. Collect 40 g of pineapple leaves
2. Obtain a 500 mL beaker and put the leaves into it
3. Weigh the amount necessary to charge the pineapple leaves with 18% of baking soda on dry weight. Use the chart below to calculate the amount of baking soda needed
4. Charge the pineapple leaves with baking soda on dry weight and add 350 mL of DI water to the 500 mL beaker.

A. **Dry weight of leaves**	**6**
B. **% Baking soda charge**	**18%**
C. **g of baking soda (A*B/100)**	

5. Mix the water, baking soda and pineapple leaves
6. Turn on the hot plate and bring the slurry to a boil.
7. Boil for 30 minutes.
8. While pineapple leaves are cooking, prepare pineapple peel juice for fermentation (part 2)

Part 2: Production of bio-ethanol from pineapple peel juice

1. Add pineapple peel and core into a blender with 250 mL of DI water and blend on high for 3 minutes (the total volume in the blender should be 500 mL approximately)

2. Collect the pineapple juice using cheesecloth and measure the pH using pH indicator strips

3. Using a plastic dropper, add sodium hydroxide 1 M until the pH is 5. The mix must be in agitation during the pH adjustment

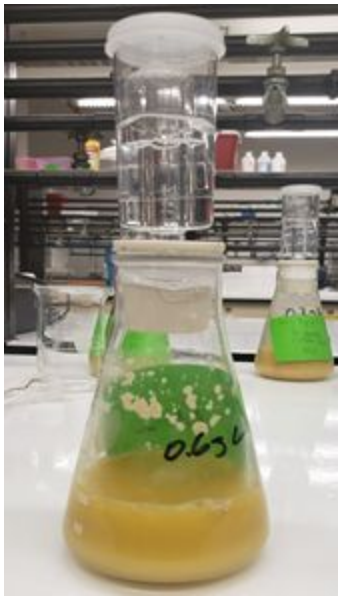
4. Add 100 mL of neutralized pineapple juice to a 250 mL flask, and 50 mL of juice and 50 mL of water into a second flask

5. Measure 0.5 g of Rapid Rise Yeast into a small pan (make sure you tare the pan)

6. Add the yeast to the pineapple juice in each of the 250 mL flasks (2 different flasks)

7. Stir the mixture by moving the fermentation flask

8. Place the stopper on top of the flask that contains the airlock stopper; ensure the stopper is tight and that there is water in the assembly.



Fermentation assembly

9. Weigh the flask assembly and record weight in the table below

10. Leave the juice to ferment for 24 hours

	100 mL Juice	Juice/Water Mix
A. **Initial weight of flask assembly, g @ Day 0**		
B. **Final weight of flask assembly, g @ Day 1**		
C. **CO2 produced, (B-A), g**		
D. **Ethanol produced, (C*46/44) (what are 46 and 44?)**		
E. **Estimated ethanol concentration, D/(100-C), %**		

Part 3: Washing and storing pulp samples

1. After 30 minutes, carefully remove the beaker containing the pineapple leaves from the hot plate with heat resistant gloves

2. Wash pulp in a cheesecloth thoroughly

3. Store the pulp samples

Session 2

Part 1: Pefining and papermaking process

1. Retrieve cooked pineapple leaves and add to blender

2. Dilute each sample with 350 mL tap water and blend pulp in a blender on high for either 30 seconds or 3 minutes (two different times of blending will be studied)

3. Fill a plastic tub with enough water to submerge the handsheet mold

4. Submerge the handsheet mold in the bath of water

5. Pour the pulp samples into the handsheet mold and shake it carefully

6. Lift out the handsheet mold and let the water drains

7. Open the handsheet mold and place two sheets of blotter paper onto the pulp mat and press with your hands to dewater.

8. Remove top wet blotter paper, add another dry sheet of blotter paper and press again, then remove pulp mat from mold (still attached to blotter paper)

9. Put a dry blotter paper on the top of the sheet

10. Compress the sheet using a roller

11. Let the sheet of paper dries

12. Separate the sheet of paper from the blotter papers

13. Use the fish scale to measure the tensile strength of each piece of paper:

    a. Carefully cut a strip of paper so that it is 1 cm wide. The test strip length should be ~6 cm. Avoid wrinkling or tearing the strip. When cutting try to make the edges as smooth as possible.

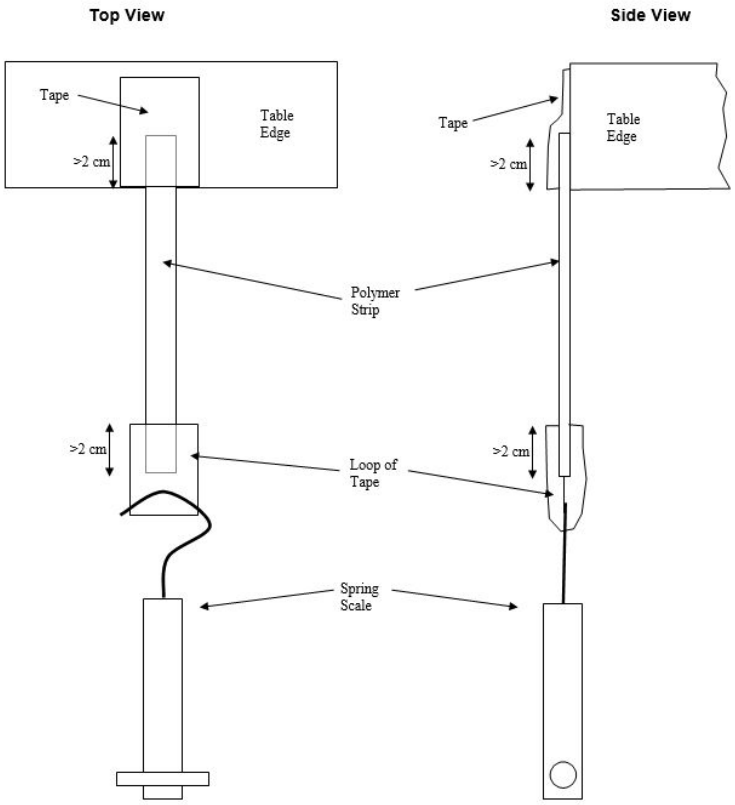
    b. Using a 10 cm length of strong Duct tape, tape at least 2 cm of length of the strip to the table edge. See illustration.

    c. Using about 20 cm length of strong Duct tape, attach a spring scale to the other end of the film strip. The tape should loop around the scale hook and then sandwich the bottom part of the paper strip. See illustration. Again make sure to tape at least 2 cm of the bottom of the paper strip.

    d. Check the spring scale to be certain that the tell tale is set zero. Now, pull on the spring scale so as to apply force along the length of the strip. Apply force to the scale at a uniform rate until the filmstrip breaks. DO NOT twist the filmstrip. Make sure that the strip has not slipped out from the tape. If it has, the experiment is not useful.

    e. Record the force applied to break the piece of paper paper using the tell tale on the spring scale.

    f. Calculate the tensile strength (in lbs/in) by dividing the average force to break the strip by the strip width. Remember to divide the paper width by 2.54 to convert the strip width from cm to inches.



Part 2: Determining the bio-ethanol production

1. Weigh the flask assembly of sugar fermentation and record weight in the table of Part 2 – Session 1. Complete the calculations showed in that table

Questions

1. What is a bio-refinery and what is its relevance?
2. What is the paper?
3. What is the goal of papermaking processes?
4. What is the classification of papermaking processes? Please indicate the overall difference between them.
5. What is fermentation? Please write the reaction of glucose fermentation
6. What is the function of yeast during glucose fermentation?
7. What is bio-ethanol and why is it important?