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Laboratory Experiment

Modeling a Biorefinery: Converting Pineapple Waste to Bioproducts and Biofuel

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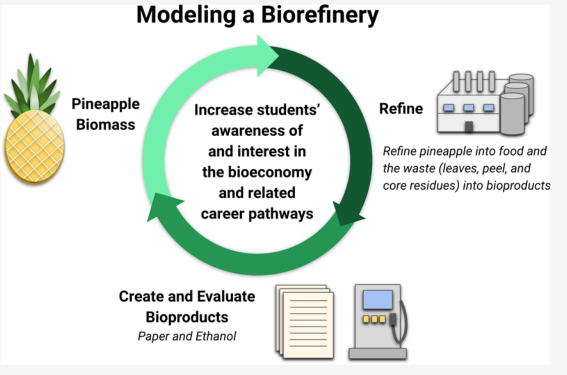
ABSTRACT: Many students may not be aware that renewable biological materials can be converted into multiple bioproducts and biofuels using a biorefinery process, a more sustainable alternative to conventional crude oil refineries. By using waste from pineapple, a plant material that most students are familiar with, a biorefinery can be modeled to demonstrate the benefits of a circular bioeconomy. Pineapple waste consists of the peel, core, and leaves that are often discarded after the fruit is processed for consumption. These "leftovers" or "residues" are rich sources of sugars and lignocellulosic biomass, which can be converted to value-added bioproducts and biofuel. In this article, the development and implementation of a high school laboratory activity that simulates a pineapple biorefinery is described. It was field tested with an Environmental Science class, in which students converted pineapple leaves into paper, and they fermented the sugars from the core and peel into bioethanol for fuel. Students investigated how different process variables influenced the tensile strength of their paper and the quantity of bioethanol produced. This lab introduces students to the potential of a circular bioeconomy and challenges them to integrate prior chemistry and biology knowledge to generate solutions to real-world sustainability problems. It can be used in chemistry classes to demonstrate stoichiometry, chemical reaction yield, chemical bonds, and the effect of reactant concentration on the rate of product formation.



KEYWORDS: *High School/Introductory Chemistry, First-Year Undergraduate/General, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Alcohols, Applications of Chemistry, Carbohydrates*

What if waste material from one process could be used as starting material for another? If waste was considered a valuable resource, could landfills be diminished and pollution be reduced? A circular bioeconomy is an economy that uses renewable, biological resources and waste streams and converts them to sustainable, value-added products for society.[1] Biorefineries are facilities where different components of one biomass source are converted into multiple products.[2] For example, biomass from trees, agricultural and crop waste, and municipal solid waste can be used as starting materials to create bioproducts and bioenergy such as biofuels, bioplastics, biopharmaceuticals, and wood and paper products.

Bioproducts and bioenergy offer the potential to replace fossil fuel-based products and reduce environmental impacts and carbon emissions.[1] Additionally, bioproducts contribute substantially to the U.S economy; the biobased products industry (excluding biofuels) added $459 billion to the U.S. economy and employed 4.65 million people in 2016.[3] The ethanol biofuel industry is another major sector in the bioeconomy, as it produces 10% of motor fuel in the U.S.[4]

For a transition to a more sustainable society and to enhance innovative solutions, it is necessary to develop a skilled and diverse workforce[5,6] and prepare the next generation of bioeconomy and STEM professionals. The American Chemical Society and other professional organizations emphasize the importance of adopting green chemistry principles (e.g., using renewable feedstocks, reducing wasteful inputs and outputs, and applying life cycle thinking) in research and industry settings as well as promoting teachers' and students' understanding of these topics to strengthen the bioeconomy and create a more sustainable future.[7] To promote students' understanding of and interest in the bioeconomy, instructors have incorporated classroom activities and laboratory investigations related to bioproducts, biofuels, and biorefineries into

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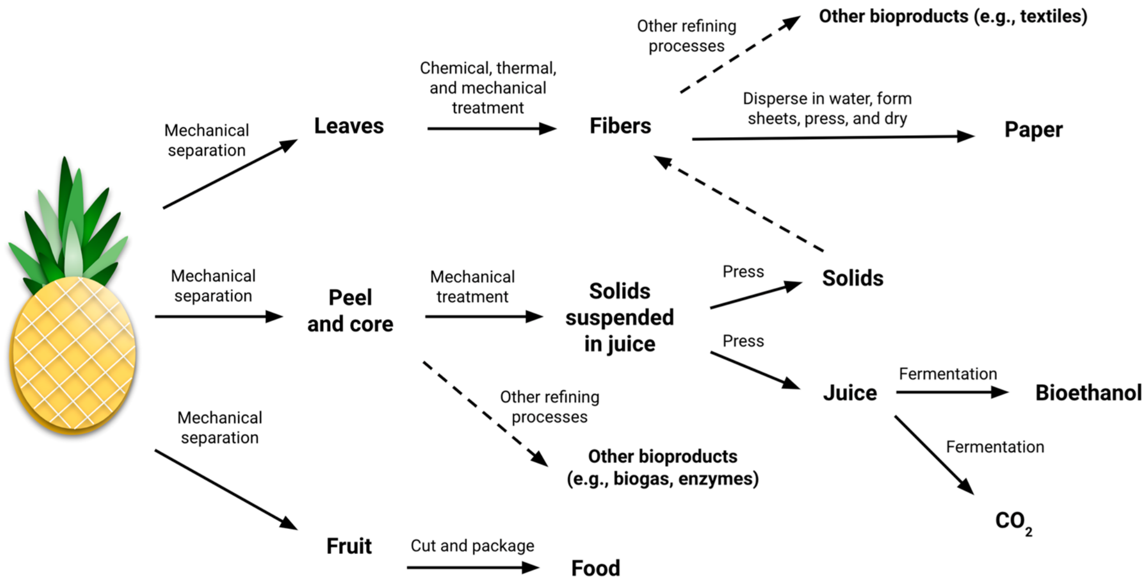

Figure 1. Simplified example of the biorefinery process, using pineapple as the biomass source. Solid arrows represent processes that were focused on in this lab. Dashed arrows indicate other possible uses of pineapple waste to produce various bioproducts.

their teaching, typically in undergraduate courses,[8−10] but also at the primary and high school levels.[10−12] Converting used vegetable oil to biodiesel is a common laboratory exercise conducted with students across all educational levels to improve students' understanding of biorefineries and underlying chemical concepts, such as transesterification, percent yield, viscosity, and combustion.[11−13] Students have demonstrated greater interest and engagement, as well as good or excellent scores on content knowledge and satisfaction surveys, following biodiesel and biorefinery laboratories.[10−12] Zhou and colleagues[10] developed an undergraduate lab for creating biodiesel, ethanol, and sunscreen from lignocellulosic components of corn. However, implementation of this integrated biorefinery concept, whereby multiple bioproducts are produced from a biomass source, has rarely been reported at the high school level.

In this article, the development and implementation of a high school laboratory activity that simulates a biorefinery is described. Students have the opportunity to explore a potential solution for processing food waste: using pineapple waste as a biomass source to produce multiple bioproducts, paper and bioethanol. By using pineapples, a plant material that many students are familiar with, students learn about the benefits of a circular bioeconomy in a classroom setting. Pineapples are one of the most popular tropical fruits worldwide, with more than 28 million metric tons produced globally in 2019.[14] Pineapple processing facilities generate large amounts of waste, as the peel, core, stem, crown, and leaves comprise about 50% of a pineapple's weight.[15] The waste generally goes to landfills or is used to make animal feed. However, these low-value agricultural "leftovers" or "residues" can also be converted into useful bioproducts. The organic matter can be broken into nutrient-rich compost or used to make biogas in an anaerobic digestion process. Alternatively, companies like Lifepack[16] and Piñatex[17] harness the potential of pineapple waste to make commercial bioproducts such as biodegradable plates and sustainable textiles, respectively. As demonstrated in this article, pineapple fibers can be used to make paper products, and the sugars can be used to make ethanol by fermentation. This lab activity introduces students to the potential of a circular bioeconomy and integrates students' STEM knowledge and skills to investigate a real-world problem.

Biorefineries

Biorefineries play a critical role in the circular bioeconomy. A biorefinery is a facility where a variety of conversion methods (e.g., biological, chemical, mechanical, and thermal) are used to process renewable biomass resources into multiple products, thus maximizing the value of biomass.[18] To be truly sustainable, biorefineries must utilize biomass as completely as possible by reusing wastes and byproducts generated by the conversion processes. In this lab, a biorefinery is modeled, and pineapple is the biomass being processed; the fruit can be processed and packaged for food, and the waste (e.g., leaves, peel, and core) can be converted to new products. Paper and bioethanol are the focus of this lab, but biorefineries are versatile, and other bioproducts are possible. Figure 1 shows a simplified example of the biorefinery process and the diversity of products that can be created at biorefineries, using pineapple as the starting biomass.

Plant and Plant Cell Composition

Pineapple waste (i.e., leaves, peel, and core) is a source of sugars and fibers, which can be converted into value-added bioproducts and biofuels.[19] Fructose and glucose are simple sugars, often accessible as free sugars in fruit, including pineapple. Plants also store complex sugar chains in a lignocellulose structure, especially in fibrous or woody parts of plants. Cellulose, hemicellulose, and lignin are the main components of lignocellulose and give plant cell walls their rigidity. Figure 2 shows the general structures of lignocellulose and fermentable sugars.

Paper-Making

Pineapple fibers can be separated in a pulping process and reformed to make paper. Paper products are important in our daily activities. In fact, the United States is one of the top producers and consumers of paper globally.[21] To make paper, plant biomass is first mechanically broken down into small pieces. Next, cellulose fibers are separated to form pulp (a slurry of cellulose fibers in water) using a combination of

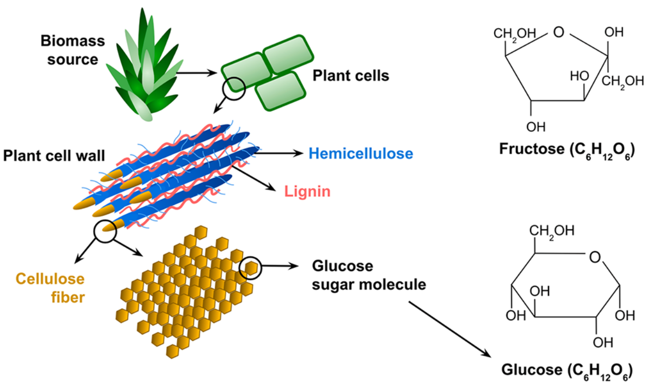

Figure 2. General structures of lignocellulose and fermentable sugars.[20]

mechanical, thermal, and/or chemical treatments. After cellulose fibers are separated and cleaned, they are pressed into the desired paper shape. As the paper dries, hydrogen bonds form between cellulose fibers, giving paper products their strength. A hydrogen bond occurs between an electronegative atom (e.g., O or N) and a hydrogen atom that is bonded to an electronegative atom (e.g., O−H or N−H). Hydrogen bonds are reversible, and adding water can reliberate fibers in paper during the recycling process.

Bioethanol Production

Simple sugars from the pineapple core and peel can provide a food source for yeast to consume and produce a valuable byproduct, ethanol. Bioethanol is the world's most common liquid biofuel.[22] It reduces our dependence on petroleum and is widely used in gasoline blends to fuel cars. Bioethanol is produced from different biomass sources, including sugar cane and corn, as well as lignocellulosic biomass such as wood and agricultural residues. Bioethanol is produced through the biological process of fermentation when microorganisms (typically yeast) metabolize sugars. Ethanol is then recovered and distilled for commercial use. The general fermentation equation is $C_6H_{12}O_6$ (glucose) → $2C_2H_5OH$ (ethanol) + $2CO_2$ (carbon dioxide). Pineapples contain a range of sugars, including glucose, sucrose, and fructose (diagrammed in Figure 2), that can undergo fermentation.

■ LAB DEVELOPMENT AND IMPLEMENTATION

The lab procedures were developed from previously established methods from the North Carolina State University Paper Science Program, in which bioethanol was produced from sugar cane.[23] The papermaking procedures were based on previously published results, also from this lab group, on making handmade paper from wastepaper.[24,25] This new lab is novel and builds on previous methods by (1) using pineapple as a biomass source, which is generally accessible and safe to handle in classroom settings, (2) combining the papermaking and biofuel production procedures with pineapple biomass into a single lab, and (3) adding an explicit focus on sustainability issues and the bioeconomy using engaging, hands-on methods, which are different from the prior single-product laboratories.

The lab was tested and refined with high school science teachers at a summer workshop, and it was implemented in multiple classrooms during the academic year. Ms. Davis, a high school teacher and a coauthor on this article, conducted the lab with AP Environmental Science students and shares the experiences in this article to help readers visualize performing the lab with their students.

Lesson Objectives and Next Generation Science Standards Connections

This lab aligns with the Next Generation Science Standards (NGSS)[26] and can be modified for high school chemistry, biology, and environmental science curricula. See Table 1 for potential NGSS connections. In Experiment No. 1, students produce paper from pineapple leaves and evaluate the refining process by investigating its effect on the paper's tensile strength. In Experiment No. 2, students ferment juice from the pineapple peel and core to create bioethanol, examining variables that affect fermentation and final bioethanol production. This lab can be used in chemistry classes to demonstrate stoichiometry, chemical reaction yield, chemical bonds, and the effect of reactant concentration on the rate of product formation.

■ SAFETY

The primary safety hazards in this lab are potential exposure to a strong base (sodium hydroxide in solution), burns from hot plates, and cuts from blenders. Students should wear lab-appropriate clothes and personal protective equipment. Additionally, heat resistant hot pads or gloves should be worn when touching hot glassware, hands should not be inserted into blenders, and hot plates and blenders should be unplugged when not in use.

■ LAB PLANNING AND PREPARATION

At least three 50 min class periods or two block periods are required to complete Experiments No. 1 and No. 2. When planning, note that the paper takes about 48 h to air-dry, and

Table 1. Potential Connections with the NGSS.[26] Other Valid Connections with the NGSS Are Possible

Performance Expectations	Classroom Connections
HS-PS1-3. Plan and conduct an investigation to gather evidence to compare the structure of substances at the bulk scale to infer the strength of electrical forces between particles.	Experiment 1: Students produce paper from pineapple waste and test the paper's tensile strength to learn about chemical bonds and their relationship to paper strength.
HS-PS1-6. Refine the design of a chemical system by specifying a change in conditions that would produce increased amounts of products at equilibrium.	Experiment 2: Students ferment sugars in pineapple juice, modify the reaction conditions (e.g., perform dilutions to test different concentrations of pineapple juice), and quantify the ethanol that is produced.
HS-PS1-7. Use mathematical representations to support the claim that atoms, and therefore mass, are conserved during a chemical reaction.	Experiment 2: Students perform calculations, analyze data, and apply the law of conservation of mass to determine the amount of ethanol that is produced during fermentation.
HS-ESS3-4. Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.	Experiments 1 and 2: Students create bioproducts as a possible sustainable solution to reducing the impact of food waste.
HS-ETS1.2. Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.	Experiments 1 and 2: Students investigate a real-world challenge of reducing and reusing waste through biorefining processes and creating multiple bioproducts from a single biomass waste source.

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the fermentation should react at least 24−48 h to detect change in mass. To prepare for Experiment No. 1, teachers should wash the pineapple and remove the crown and leaves. Each group of students will receive half of one pineapple crown, and teachers or students can carefully cut the leaves using scissors into small pieces (Figure 3). Teachers should also gather necessary supplies for the class, such as baking soda, glassware, hot plates, cheesecloth, handsheet molds, and blenders. To prepare for Experiment No. 2, teachers should remove the pineapple peel and core and cut into small pieces (Figure 3). A 1 M NaOH solution should also be prepared in advance. Teachers should gather enough supplies for the class, such as glassware, blenders, cheesecloth, yeast, and fermentation flasks and stoppers. Instructions for how teachers should prepare for the lab and a full list of materials are included as Supporting Information (see materials and standard procedures for Experiments No. 1 and No. 2).

Figure 3. Pineapple leaves cut into 2.5 cm pieces (left). Lab supplies, including pineapple peel and core placed in the blender (right).

Experiment No. 1: Creating and Testing Pineapple Paper

In this experiment, students refined pineapple leaves to create paper and tested the paper's tensile strength (maximum force applied to the sample of paper before breaking). A full list of procedures and materials are available in the Supporting Information section.

To engage students, Ms. Davis showed the class a piece of pineapple that still had its peel. She took a bite, held up the peel, and asked, "What do you think we can do with the waste that's left over after eating pineapple?" A few students called out answers. Then, it was explained they would use the pineapple waste to make paper and bioethanol bioproducts, and this led into a brief class discussion about value-added products.

In this particular class of nine students, students selected their lab partners. Each group received half a pineapple crown, and students cut the leaves into small pieces while they watched a short video about biorefineries (see Supporting Information, Additional Online Resources "Biorefinery Sustainable Solutions" and "Intro Video"). While cutting, students observed the leaves' characteristics, which sparked conversation: "It feels like aloe vera," and, "It reminds me of a corn husk." Ms. Davis asked students which characteristics of pineapple leaves and corn husks might affect the final paper product. She emphasized the connection to food waste by saying, "We eat a lot of corn and fruit, so imagine all the waste that can be used to make bioproducts."

Part 1: Producing Pulp from Pineapple Leaves. Students worked in groups of two or three. Each group tested a different variable; for example, one group used the standard procedure, one group used additional pineapple leaves, and one group used additional baking soda. Once groups defined their experimental conditions and prepared their beakers, the pineapple leaves were treated in boiling water with baking soda for 30 min. Cooking the pineapple leaves and adding baking soda to the boiling water simulated thermal and chemical treatment processes to liberate the cellulose fibers. The baking soda served as a chemical treatment to improve fiber separation by increasing the pH of the liquid that was used for pulping the pineapple leaves. Raising the pH promoted the swelling of the biomass structure, allowing for a more effective liberation of the fibers during the subsequent mechanical action (i.e., blending in the next two steps).

Part 2: Washing and Storing Pulp Samples. After the pineapple leaves cooked for 30 min, students strained and rinsed them in the sink. If conducting this lab across multiple days, the rinsed leaves can be stored in a Ziplock bag in a refrigerator or at room temperature up to a few days until the class is ready to proceed with the following step.

Part 3: Refining and Papermaking Process. Ms. Davis set up the handsheet mold stations before class (see Supporting Information for a schematic of setting up the handsheet mold; see Supporting Information, Additional Online Resources "Handsheet Mold Construction Video" to build your own handsheet molds). Students pulped their cooked leaves using kitchen blenders to simulate mechanical treatment. To avoid damaging the blenders, the pineapple leaves should be cut into 2.5 cm pieces and cooked for the full 30 min, and blender blades should be cleaned after each use or each class period.

The papermaking process was new to students, and they were excited to see their paper form as they poured their pineapple pulp into the handsheet molds (Figures 4 and 5).

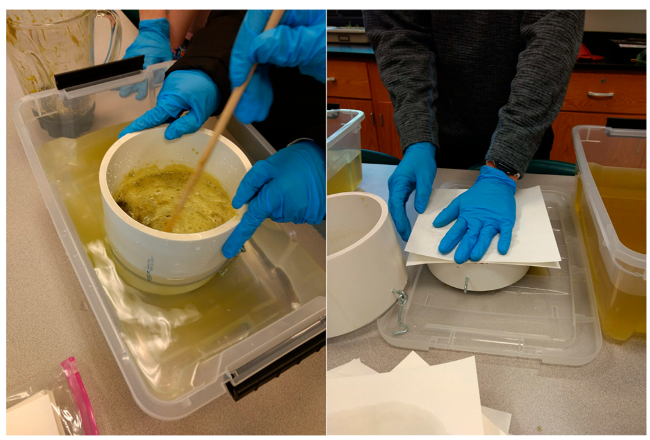

Figure 4. Pouring pineapple pulp into the handsheet mold and stirring it to evenly distribute fibers (left). Pressing handsheet with blotter paper to remove excess water (right).

Students observed the color and thickness of their paper as they pressed out the water. The group that used more pineapple leaves created a thicker handsheet that required more effort to press out the water. This provided an opportunity to discuss trade-offs involved in making paper. Students labeled their handsheets and air-dried them (2 days minimum). A hair dryer or iron can shorten the drying time.

Part 4. Testing Paper Strength. Students cut their pineapple paper into strips measuring 1 cm wide by 6 cm long and tested tensile strength using a fish scale (Figure 6). If classes do not have fish scales, paper strength can also be measured by hanging weights on the paper strips. Clear

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Figure 5. Wet handsheet (standard procedure) (left). Dry handsheet (standard procedure) (right).

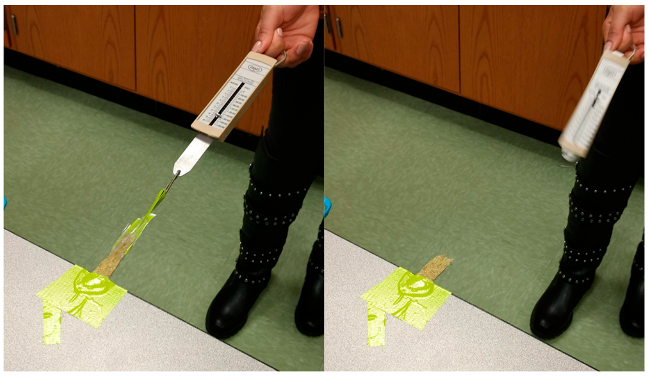

Figure 6. Testing tensile strength of pineapple paper using a fish scale before (left) and after (right) the paper broke.

packing tape and duct tape were strong enough to securely hold the pineapple paper for testing. A suggested technique to tape the paper to the table and attach the fish scale is provided in the Supporting Information.

Students tested the tensile strength and enhanced the accuracy of their measurements by slowly pulling the fish scale until the paper broke, by working in groups and having multiple students read the scale, and by conducting multiple trials and calculating the average and range. Groups recorded their data and discussed their results (see Supporting Information for an example of class data and calculations). Students practiced analyzing and interpreting data by calculating the tensile strength in Newtons/cm.

It should be noted that the tensile strength of paper is proportional to its width and thickness, and samples with similar thicknesses are appropriate to compare. The simplified handsheet making method used in this lab was not optimized for making exactly formed handsheets. Paper thickness was not measured as part of this lab but can easily be determined using calipers. In this lab, students qualitatively compared paper thickness and considered how this dimension impacted paper strength. For added inquiry and discussion, students can test the strength of other paper products for comparison (e.g., tissue, writing paper, paper towels). When conducting this lab in a chemistry class, this would also be an appropriate point for the class to discuss the relationship between the paper-making conditions, hydrogen bonding between the pineapple paper fibers, and the resulting paper strength.

Experiment No. 2: Fermenting Pineapple Juice and Quantifying Bioethanol

In this experiment, students recovered juice from the pineapple peel and core, fermented the juice using yeast, and quantified the bioethanol production. A full list of procedures and materials are available in the Supporting Information section.

Part 1: Fermenting Pineapple Peel Juice. Students blended their pineapple peel and core with water. Then, they strained and squeezed the pulp with cheesecloth and collected the juice in a beaker (Figure 7).

Figure 7. Straining and squeezing the pulp with cheesecloth and collecting juice in a beaker.

When the lab was originally developed, 1 M NaOH was added to the pineapple juice (initial pH 3−4) until it reached pH 5, the optimal pH for Baker's yeast. However, the juice in Ms. Davis' class was already at pH 5. The class discussed that perhaps their pineapples were riper and less acidic than the pineapple used when developing the lab.

All groups in Ms. Davis' class used the same fermentation conditions and juice dilution (100% juice vs 50% juice; pH 5; Figure 8) to investigate the effect of sugar concentration on bioethanol production. Students measured the initial mass of their juice-filled flasks with airlocks and stoppers on them.

Figure 8. Fermentation flasks containing 100% juice (left) and 50% juice/50% water (right).

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During fermentation, students observed bubbling in the airlock from CO_2 production.

Part 2. Quantifying Bioethanol Production. Ms. Davis' class ran the fermentation reaction for 3 weeks, recording their flasks' masses every few days, until CO_2 bubbles no longer appeared, indicating the fermentation had ended. However, most of the fermentation should have taken place within the first few days. After collecting data, students applied the law of conservation of mass to estimate the amount of CO_2 and ethanol produced and the ethanol concentration (see Supporting Information for an example of student data and calculations). Students can also graph the change in mass; mass decreases over time as carbon dioxide escapes the fermentation setup.

■ DISCUSSION

Assessing Learning

Frequent student-centered discussions were important to formatively assess student learning during this lab. Student engagement and interest were high and were reflected in their conversations during and after the lab (e.g., a student stayed after class to talk with Ms. Davis about further refining the process to produce pineapple paper that would be more similar to writing grade paper). Facilitating in-class discussions also helped students better understand the role of science and engineering in sustainability and in addressing real-world problems.

A short, ungraded quiz was given before and after the lab (see Supporting Information), as a type of assessment. Overall, students (n = 9) answered an average of four out of six questions correctly on the postquiz. The questions that all or most students answered correctly were those related to what biorefineries were (e.g., Which definition best describes a biorefinery?). The questions that students tended to answer incorrectly included a distillation/fermentation question and a chemical bonds question. Results from this pilot quiz led to modifying the questions to improve their clarity (the Supporting Information contains the modified questions). In addition, students completed short online surveys that measured their beliefs about bioproducts/bioenergy (BABB) and their career interest in bioproducts/bioenergy (CIBB).[27] Students took the BABB and CIBB surveys at the end of the first day of the lab. For the BABB survey, students had mostly positive beliefs about bioproducts/bioenergy, and the average score was 3.6 out of 5 (5-point Likert scale; 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree), with a range of 3.2 to 4.2. For the CIBB survey, the average score was 2.6 out of 5, with a range of 1.25 to 4; about half of the students expressed interest in bioproduct/bioenergy careers (average score of 3 or more), but the overall average indicated students were somewhat disinterested in bioproduct/bioenergy careers. Additional data will be collected across multiple classrooms, and results will be reported in a separate publication.

Students also completed an open-ended exit ticket on the final day of the lab to reflect on their learning by answering two questions: (1) What was your favorite part of the lab this week? and, (2) What is one thing that you learned or thought was most interesting? Students' comments revealed a range of learning and interests. Almost all of the students' favorite part of the lab was making the pineapple paper. One student commented, "The process of mixing, blending & straining was fun," and another student said, "The process of making the paper and refining the materials used in the paper was my favorite." Several students commented that they learned more about the properties of paper and were surprised by the tensile strength: "You can produce some pretty decently strong paper using pineapples." Another student wrote, "One cool thing to me was learning how to test how much force it would take to tear [break] the paper. I had never seen that before." A few students also mentioned learning more about anaerobic fermentation, dilutions, and adjusting the pH of solutions.

Extension Ideas

Digital probes can be used for collecting and analyzing data in real-time. For instance, a Vernier force probe to measure tensile strength, a pH meter to measure the pineapple juice's pH, and an ethanol probe to measure bioethanol production have been used during this lab.

Ms. Davis incorporated an extension activity based on pineapple enzymes. Bromelain, a protease enzyme in pineapple, catalyzes the degradation of gelatin proteins (see Supporting Information, Additional Online Resources "Pineapple Enzymes and Gelatin"). This allowed students to discuss the function of enzymes as well as their potential applications as bioproducts and connections within the bioeconomy.

This lab can spark discussion about bioeconomy-related careers and pathways, such as research and development, pulp and paper engineering, microbial fermentation development, and bioproduct design. Students can research careers that interest them and learn about the skills and education needed to pursue them (see Supporting Information, Additional Online Resources "The Sustainable Bioproducts and Bioenergy Program's collection of educational resources").

Limitations

Implementing this lab might be limited by teachers' lack of access to some specialized materials, such as handsheet molds. The handsheet molds in this lab were assembled and provided to Ms. Davis and other high school science teachers as part of a professional development program that the authors facilitated.[28] Instructions for constructing handsheet molds are available as Supporting Information (see Additional Online Resources "Handsheet Mold Construction Video"). Premade papermaking molds are also commercially available online and at craft stores.

■ CONCLUSION

This lab models a biorefinery and uses pineapple, a biomass source that is familiar to many students, to demonstrate the potential of the bioeconomy and engage high school students in investigating a solution to a real-world problem. Pineapple leaves were refined to create paper, sugars from the peel and core were fermented to produce ethanol, and the final value-added bioproducts were evaluated. Through this lab, students learned about broad, interdisciplinary topics such as biorefineries, bioproducts, and biofuels. They also had the opportunity to further their understanding of underlying chemical and biological concepts, such as chemical bonds and fermentation, and apply scientific practices. By introducing high school students to biorefineries and bioproducts, this lab has the potential to stimulate students' interests and help them become more aware of the growing bioeconomy and its STEM connections.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00020.

Materials and standard procedures for Experiments No. 1 and No. 2 (PDF, DOCX)
Example calculations for Experiments No. 1 and No. 2 (PDF, DOCX)
Example content knowledge quiz (PDF, DOCX)
Complete teacher and student lab guides (PDF, DOCX)
Additional online resources (PDF, DOCX)

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Notes

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