Rumen Fermentation: Conversion of Cellulose Biomass to Ethanol

Nutrition Unit

Grade Level
9-12

Lesson Length
3 periods x 55 minutes

STEM Careers
- Ruminant Nutritionist
- Rumen Microbiologist
- Nutritional Biochemist
- Veterinarian
- Zoologist

Next Generation Science Standards
- MS-LS1-7
- HS-LS1-7

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These lessons aim to bring the science, skills of inquiry, critical thinking, and problem solving to life through an agricultural context.

Learning Objectives

By the end of this unit, students should be able to:

- Describe the basic difference between ruminant and non-ruminant digestive systems.
- Discuss why cellulosic materials can be digested by ruminants, but not non-ruminant animals.
- Propose and describe processing methods to increase energy extraction of fibrous feed materials that should improve nutrient utilization and production efficiency.

Materials List

- Biomass materials (fresh grass, hay, straw, grain, etc). Alternatively, student groups can be assigned to identify and locate their own biomass material to test.
- Equipment to “process” biomass samples. May include grinder and/or scissors, etc. (shared amongst groups).
- Alcohol test strips (2/group)
- 50mL falcon centrifuge tubes (1/group)
- Blood glucose meters (2, to be shared amongst groups)
- Blood glucose test strips (4/group)
- Weigh boats (2/group)
- Plastic transfer pipettes (1/group)
- Cellulase enzyme (need 0.05 g/group)
- Yeast (need ¼ tsp per group)
- ¼ tsp measurer (1/group)
- 25 or 50 ml graduated cylinder (plastic or glass; 1/group)
- Gram scale (to be shared amongst groups)
- Water bath or incubator, to hold items at 37° & 50°C

Do

Introduction (Interest Approach that Aligns with the Investigation)

Student groups will make a simple rumen model (Fischer, n.d.). Into a 20-oz plastic bottle, mix 3 tablespoons (8 packets) sugar, 1 packet of dry yeast, and 10 oz of very warm water (input last). Replace cap and shake to thoroughly mix solution. Remove cap and place 9-inch latex balloon over bottle top opening and observe.

Facilitate a brief discussion to address the following:

- What does the sugar represent?
- What does the yeast represent?
- What happens to the balloon, and how is this related to ruminant nutrition?

Essential Questions

- *Why can a cow digest grass, but not humans?*
- *How can we improve nutrient utilization of structural carbohydrates?*

Learning Activity 1: [PowerPoint Discussion]

Please use the provided information and/or associated PowerPoint to introduce students to concepts related to ruminant nutritional physiology. If teachers prefer to show a recording, that is available at … https://use.vg/9XuKE0.

Ruminant animals including cattle, sheep, goats, and giraffes have four-chambered stomachs that are specially made to help them digest plants. The first part of the four-chambered stomach is called a rumen, and this is where most of the plant material is broken down. In a mature cow, the rumen can hold almost 50 gallons of food and water. Ruminants break down plant material that humans cannot eat because of microbes that live in the rumen. These microbes have special enzymes, called cellulases, that attack and digest the tough, fibrous material of plants: cellulose. As a result, ruminant animals can use plant material and other feedstuffs that humans cannot.

Principles Concepts – Nutrition
These are principal concepts that help explain observations in this project.

1. For the energy component of foods to be used by the body, carbohydrates must be broken down to constituent glucose molecules to be absorbed across the small intestinal wall.

2. Simple carbohydrates are chains of glucose molecules, connected by α-linkages. Structural carbohydrates also consist of glucose molecule chains, but they are connected by β-linkages. Humans have the enzymes needed to breakdown α-linkages, but not β-

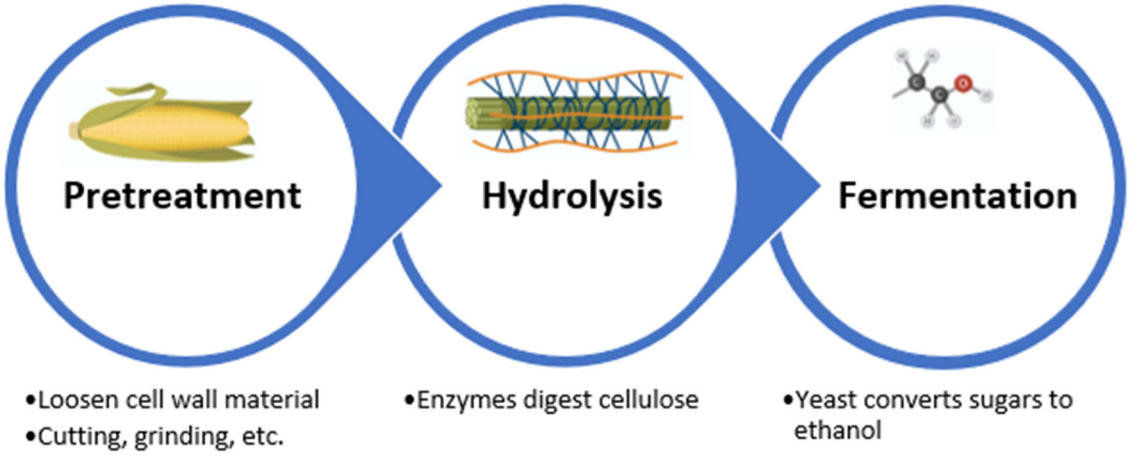
linkages. However, microbes within the ruminant stomach possess the cellulolytic enzymes required to break down cellulose to glucose through microbial fermentation.

3. The rate and extent of microbial fermentation, and thus the rate and extent of feed utilization to promote productive function (i.e., growth), is affected by numerous factors including the type, shape, and size of feed offered.

Learning Activity 2: Conversion of Cellulose Biomass to Ethanol

Students will investigate the challenge of converting cellulosic biomass into ethanol. Although ruminant animals do not necessarily convert grasses to ethanol, the concept of cellulosic biomass fermentation and how feed variables such as biomass type or processing method (i.e., grinding) affect conversion of sugars into ethanol (or other energy precursors) is foundational to ruminant nutrition and production.

The process involves three key steps: 1) pre-treatment, 2) hydrolysis associated with enzymatic digestion, and 3) fermentation.



Students will be placed into small groups to identify and test one treatment, compared to the "teacher's control". Students should receive and use experimental instructions, but it is also advised that the instructor explain (and demonstrate with the "control") each of the experimental parts to students at the appropriate time. Each group will use the provided lab report worksheets to document findings and record their hypothesis.

Overview of Experimental Activities:

Day	Stages	Activities
1	Experimental Design and Planning	• Choose biomass type and/or processing options (cutting, grinding, etc.)
	Sample Preparation	• Set up experiment • Process biomass as needed • Measure initial glucose & ethanol concentration • Hot water treatment of treatment sample. • Measure glucose concentration, after boiling
	Hydrolysis (Enzyme Digestion**)**	• Add cellulase, incubate (50°C) for 24 hrs
2	Hydrolysis cont.	• Measure glucose concentration
	Fermentation	• Add yeast, incubate (37°C) for 24 hrs.
	• NOTE – The Day 2 activity will take little time, so may use excess time to provide educational background information or to conduct other lessons	
3	Fermentation cont.	• Measure final glucose & ethanol concentration
	Data Analysis and Conclusions	• Graph results • Summarize conclusions and communicate findings to class • Write up results based on evidence collected from your treatment and those of other groups

<u>Part 1 – Experimental Design and Planning</u>

1. Students, with teacher input, should identify a "control" treatment, the treatment to which all other results will be compared. The teacher will conduct experimental processes on the control and may use the control to "demonstrate" instructions for students.
    a. Suggested control = a specific substrate biomass (i.e., grass hay), no processing.
2. Within each student group, discuss and decide what biomass type (different hay types, straw, wood chips, grain, etc.) and/or processing method (i.e., cutting, grinding, etc.) should be tested.
3. Each student group should develop and write down their hypothesis. For example ...
    a. A more mature forage will have lower rates of fermentation and produce less glucose and ethanol than a less mature forage.
    b. Processing the forage (i.e., grinding, cutting) will increase the rate of fermentation and produce more glucose and ethanol.
    c. Cellulase enzyme is needed to ferment forages, but not simple carbohydrates (grains).
    d. Grains are more fermentable and will produce more glucose and ethanol than forages.

4. Each student group should write down reasons for development of their hypothesis. For example …
    a. As forages mature, they are less preferred by animals and less digested.
    b. Grinding will increase surface area for enzymes to act.
    c. Because grains support higher levels of gain, they must have more energy which should produce more glucose and ethanol.
5. Each student group should write down and describe evidence to be collected to prove or disprove their hypothesis. For example …
    a. Glucose and ethanol concentrations will be measured upon conclusion of the experimental protocol and compared to results of the whole-class control.

Part 2 – Conduct the Experiment

Step 1: Sample Preparation

1. Label one 50mL tube and cap with your team initials, date, and sample description.
2. Gather biomass (hay, straw, grain, etc.) to be tested.
3. Process (i.e., grinding, cutting, etc.) biomass as required per your hypothesis.
4. Measure 1 gram of your biomass and put the 1-gram sample into the 50mL tube.
5. Test initial glucose concentration using the blood glucose meter and a glucose test strip. Record this data.
6. Test initial ethanol concentration using an ethanol test strip. Record this data.
7. Describe the biomass (appearance, scent, etc.). Record this data.

Step 2: Hot Water Pre-Treatment

1. Hot Water Treatment Station
    a. To reduce the number of beakers with boiling water, it is suggested that a "hot water treatment station" be developed to be used by all student groups.
    b. Up to 4 tubes (3 student groups plus 1 teacher control) may be placed in 1 beaker. For larger classes with 4-7 groups, 2 hot plates and 2 beakers are suggested.
    c. One may wish to set-up a "tube holder" for each beaker. A small piece of chicken-wire screen or aluminum foil can be used for this.
    d. Be sure to have tongs available that will allow students to safely remove samples from boiling water.
    e. Bring 400 mL of water in each 500-mL glass beaker to a boil.
2. Add 25 mL of water to your labeled 50-mL tube containing the biomass material.
3. Swirl to mix biomass and water. Let stand for 1 minute.
4. Loosely screw cap onto the tube.
5. Once the water is boiling at the Hot Water Treatment Station, gently place your tube into the beaker with boiling water through the aluminum foil or wire screen.
    a. Make sure biomass samples and liquid are completely submerged.
6. Leave tubes in boiling water for 10-25 minutes, depending on how much time is available. The longer the time, the higher the prospective yield of ethanol.

7. Turn off hot plate, remove samples, and allow them to cool to room temperature. A cold-water bath may be used to make tubes cool more quickly.
8. Test the glucose concentration using the blood glucose meter and a glucose test strip. Record data.
9. Describe any detectable changes in the biomass (appearance, scent, etc.). Record data.
10. If samples will not be used within the next 2 days to complete experiment, refrigerate or freeze immediately to limit microbial growth.

Step 3: Enzyme Digestion (Hydrolysis)

1. Teacher Note
    a. Remove samples from storage prior to start of class.
    b. Allow samples to warm to room temperature, prior to start of class.
    c. Set-up water bath or incubator to 50°C (122°F).
2. Add 1.0 mL of Celluclast™ or 0.05 gram of Carolina Biological cellulase enzyme product to tube with biomass material undergoing digestion.
3. Screw cap on tightly. Mix gently.
4. Place tube in water bath or incubator, set to 50°C (122°F).
5. Leave tube in water bath or incubator for 24 hrs (1 day).
6. After 24-hr digestion, use the blood glucose meter and a blood glucose test strip to test post-enzyme glucose concentration of your sample. Record data.
7. Describe any detectable changes in the biomass (appearance, scent, etc.). Record data.
8. If cannot start fermentation (Step 3) at this time, freeze or refrigerate sample to prevent microbial contamination.

Step 4: Fermentation

1. Teacher Note
    a. If samples were refrigerated or frozen after Step 3 (hydrolysis), remove from storage and allow to warm to room temperature prior to start of class.
    b. Set-up water bath or incubator to 37°C (98.6°F).
    c. After further preparation, students will need to STAND tubes in the water bath or incubator for 24 hours. A test tube rack or other device will be needed to keep tubes upright.
2. Add 0.25 teaspoon or 1 gram (either measurement will work) of yeast to the tube.
3. Gently mix in the yeast. The yeast will grow more quickly if evenly mixed throughout.
4. Loosely score cap onto tube. It is important that cap is NOT air-tight for the fermentation. Yeast will produce carbon dioxide and will build up pressure in the tube unless gas is allowed to escape.
5. STAND tube in the 37°C (98.6°F) water bath or incubator for 24 hours (1 day).
6. After 24 hrs, remove tube from the 37°C (98.6°F) water bath or incubator
    a. Note – If the 24-hr measurement does not fit the class schedule, the teacher can remove samples from the water bath or incubator and refrigerate or freeze until the final measurements can be completed.
7. Take final glucose measurements. Use the blood glucose meter and a glucose test strip to test the post-fermentation glucose concentration of your sample. Record data.

8. Take final ethanol measurements. Test the ethanol concentration using the ethanol test strip. Record data.
    a. Note – For more accurate ethanol readings, allow sample to reach room temperature before taking measurements.
9. Describe any detectable changes in the biomass (appearance, scent, etc.). Record data.

Learning Activity 3: Industry & Career Video – Animal Nutrition

Show the industry and career video that discusses "real world" application of these basic scientific concepts, while also introducing students to career possibilities that function to bridge science and agriculture.

Reflection

Using the prompts below to facilitate reflection, allow each student to respond in writing to the prompts and then facilitate a whole class discussion.

1. Did you observe any changes in glucose concentrations after the enzyme hydrolysis stage? Please explain.
2. Did your results match what you expected would occur? Please explain.
3. Among all the samples used and tested within the class, what biomass treatment produced the most glucose and ethanol? Explain why you think they were the most effective.
4. Among all the samples used and tested within the class, what biomass treatment produced the least glucose and ethanol? Explain why you think they were the least effective.
5. If you were to try this experiment again to produce a greater amount of ethanol, what would you do differently? Please explain why.

Apply

Use the prompts below to facilitate small group and whole class discussion.

1. What basic scientific principles are associated with rumen fermentation and nutrient utilization?
2. Given your results, what are the production and potential economic impacts of feed processing on nutrient utilization, feed efficiency, and cost of gain? Would this be the same for all animal types, or just ruminants?
3. What are the pros and cons of harvesting and feeding forages of greater maturity to ruminant animals?

References:

- Fisher, A.E. "Making a Rumen", University of Tennessee, Extension Publication W897. https://extension.tennessee.edu/publications/Documents/W897.pdf, Accessed 05.17.22.

Rumen Fermentation: Conversion of Cellulose Biomass to Ethanol
Student Instructions

Instructions

Part 1 – Experimental Design and Planning

1. Students, with teacher input, should identify a "control" treatment, the treatment to which all other results will be compared. The teacher will conduct experimental processes on the control and may use the control to "demonstrate" instructions for students.

2. Within each student group, discuss and decide what biomass type (different hay types, straw, wood chips, grain, etc.) and/or processing method (i.e., cutting, grinding, etc.) should be tested.

3. Each student group should develop and write down their hypothesis.

4. Each student group should write down reasons for development of their hypothesis.

5. Each student group should write down and describe evidence to be collected to prove or disprove their hypothesis.

Part 2 – Conduct the Experiment

Step 1: Sample Preparation

1. Label one 50mL tube and cap with your team initials, date, and sample description.

2. Gather biomass (hay, straw, grain, etc.) to be tested.

3. Process (i.e., grinding, cutting, etc.) biomass as required per your hypothesis.

4. Measure 1 gram of your biomass and put the 1-gram sample into the 50mL tube.

5. Test initial glucose concentration using blood glucose meter and a glucose test strip. Record this data.

6. Test initial ethanol concentration using an ethanol test strip. Record this data.

7. Describe the biomass (appearance, scent, etc.). Record this data.

Step 2: Hot Water Pre-Treatment

1. There should be a "Hot Water Treatment Station" set up by the teacher in the classroom.
2. Add 25 mL of water to your labeled 50-mL tube containing the biomass material.
3. Swirl to mix biomass and water. Let stand for 1 minute.
4. Loosely screw cap onto the tube.
5. Once the water is boiling at the Hot Water Treatment Station, gently place your tube into the beaker with boiling water through the aluminum foil or wire screen.
    a. Make sure biomass samples and liquid are completely submerged.
6. Leave tubes in boiling water for 10-25 minutes, depending on how much time is available. The longer the time, the higher the prospective yield of ethanol.
7. Turn off hot plate, remove samples, and allow them to cool to room temperature. A cold-water bath may be used to make tubes cool more quickly.
8. Test the glucose concentration using the blood glucose meter and a glucose test strip. Record data.
9. Describe any detectable changes in the biomass (appearance, scent, etc.). Record data.
10. If samples will not be used within the next 2 days to complete experiment, refrigerate or freeze immediately to limit microbial growth.

Step 3: Enzyme Digestion (Hydrolysis)

1. Your teacher will likely have already removed your samples from cold storage and allowed them to warm to room temperature.
2. Add 1.0 mL of Celluclast™ or 0.05 gram of Carolina Biological cellulase enzyme product to tube with biomass material undergoing digestion.
3. Screw cap on tightly. Mix gently.
4. Place tube in water bath or incubator, set to 50°C (122°F).
5. Leave tube in water bath or incubator for 24 hrs (1 day).
6. After 24-hr digestion, use the blood glucose meter and a blood glucose test strip to test post-enzyme glucose concentration of your sample. Record data.
7. Describe any detectable changes in the biomass (appearance, scent, etc.). Record data.

8. If cannot start fermentation (Step 3) at this time, freeze or refrigerate sample to prevent microbial contamination.

Step 4: Fermentation

1. Your teacher will likely have already removed your samples from cold storage and allowed them to warm to room temperature.

2. Add 0.25 teaspoon or 1 gram (either measurement will work) of yeast to the tube.

3. Gently mix in the yeast. The yeast will grow more quickly if evenly mixed throughout.

4. Loosely score cap onto tube. It is important that cap is NOT air-tight for the fermentation. Yeast will produce carbon dioxide and will build up pressure in the tube unless gas is allowed to escape.

5. STAND tube in the 37°C (98.6°F) water bath or incubator for 24 hours (1 day).

6. After 24 hrs, remove tube from the 37°C (98.6°F) water bath or incubator
    a. Note – If the 24-hr measurement does not fit the class schedule, the teacher can remove samples from the water bath or incubator and refrigerate or freeze until the final measurements can be completed.

7. Take final glucose measurements. Use the blood glucose meter and a glucose test strip to test the post-fermentation glucose concentration of your sample. Record data.

8. Take final ethanol measurements. Test the ethanol concentration using the ethanol test strip. Record data.
    a. Note – For more accurate ethanol readings, allow sample to reach room temperature before taking measurements.

9. Describe any detectable changes in the biomass (appearance, scent, etc.). Record data.

Name/Group:

Rumen Fermentation: Conversion of Cellulose Biomass to Ethanol

Data Collection Chart

		Control (conducted by teacher)		Experimental	
Lab Stage	Lab Stage Date	Glucose	Ethanol	Glucose	Ethanol
Initial Reading	Date: Time:				
Notes and Observations:					
Hot Water Pretreatment	Date: Time:				
Notes and Observations:					
Hydrolysis	Date: Time:				
Notes and Observations:					
Fermentation	Date: Time:				
Notes and Observations:					

Name/Group:

Rumen Fermentation: Conversion of Cellulose Biomass to Ethanol
(Student Answer Sheet)

1. Each student group should develop and write down their hypothesis.

2. Each student group should write down reasons for development of their hypothesis.

3. Each student group should write down and describe evidence to be collected to prove or disprove their hypothesis.

4. Chart your data in graph form and use your graphs and data to answer the following questions about your results. Then, each group will share their results and conclusions with the class.

    a. Did you observe any changes in glucose concentration after the enzyme hydrolysis stage? Please explain.

    b. Did your results match what you expected would occur? Please explain.

c. Among all the samples used and tested within your class, what biomass treatment produced the most glucose and ethanol? Explain why you think it was most effective.

d. Among all the samples used and tested within your class, what biomass treatment produced the least glucose and ethanol? Explain why you think it was least effective.

e. If you were to try this experiment again and desired to produce a greater amount of ethanol, what would you do differently? Please explain why.

Name:

Lab Report

Please complete the following report during the design and implementation of your experiment.

Research Problem

- Describe what you are investigating and justify why you are investigating the problem.

Hypothesis

- Formulate one or more hypotheses for your experiment.

Procedures

- Create the steps you will follow for your experiment.

Data Collection

- Describe the data that you will collect during your experiment.
- Provide graphs, tables, charts, and raw data as necessary.

Results

- Explain your results.

Conclusion

- Based on your data:
    - What can you conclude?
    - Were your hypotheses supported?
    - Were their limitations to your experiment?
    - What are new research questions that derived from this study?