Application of yeast in manufacturing of bioproducts or biofuels

Overview

In this series of activities, students will apply the engineering design methods along with the scientific method to explore the application of yeast for production of bio-products. First, students will first learn about biomanufacturing at various scales- lab scale, pilot scale, and production scale. Students will design a lab scale bioreactor where they will grow up a yeast culture. They will be tasked with designing and building a bioreactor from available materials and the design must allow them to quantify the fermentation rate of the yeast. After building and testing, the groups will test experimental questions about yeast optimization. Students will form hypotheses and identify variables as well as design and conduct experiments based on their questions about the yeast activity. Students will also use deduction to figure out how to optimize the yeast performance. Finally, students will evaluate their hypotheses and form conclusions after performing an analysis of their results.

Objectives

Students will…

- Design, build, and test a bioreactor prototype.
- Design and conduct a scientific investigation.
- Explore the process of fermentation for production of bio-products, e.g., types of sugars available, what yeast is, by-products of fermentation.
- Learn applications of fermentation for biotechnology.

Content Standards

This lesson is appropriate for high school biology and chemistry students and addresses the following Next Generation Science Standards:

North Carolina Essential Standards

Earth/Environmental	EEn.2.2.2; EEn.2.6.3; EEn2.8.1
Physical Science	PSc.2.2.4; PSc.3.1.2
Chemistry	Chm.2.2.1; Chm.2.2.3; Chm.2.2.4; Chm.3.1.1
Biology	Bio.1.2.1; Bio.1.2.3; Bio.2.1.1; Bio.2.1.2; Bio.2.2.1; Bio.4.2.1

Next Generation Science Standards

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

DCI	• HS-LS1 (From Molecules to Organisms: Structure and Processes) • HS-PS1 (Matter and its Interactions) ○ HS-PS1-5. Apply scientific principles and evidence to provide an explanation about the effects of changing

	the temperature or concentration of the reacting particles on the rate at which a reaction occurs
Practices	• Asking questions and defining problems • Planning and carrying out investigations • Analyzing and interpreting data • Using mathematics and computational thinking • Constructing explanations and designing solutions • Engaging in argument from evidence • Obtaining, evaluating, and communicating information
Cross-cutting concepts	• Structure and function • Stability and change • Cause and effect

Time Requirements
Class-time: 1 to 2 class periods, depending on class duration (50 or 90 minutes) and level of student inquiry-based investigations.

Day 1
Teacher Preparation: 30 minutes
Student Design Activity and Approvals: 20 minutes
Students Build and test bioreactor: 20 minutes

Day 2
Teacher Preparation: 30 minutes
Student Design Experiment: 10 min
Student perform Experiment: 20 min
Students Analyze results: 10 minutes

Materials
These materials support 30 students working cooperatively in 10 groups of 3.

Included in the kit:
CO_2 gas or ethanol sensor probe for demo
Hot pot, water heater
Sucrose, 15 g
Lactose, 10 g
Glucose, 10 g (also known as dextrose)
Commercial sweetener, 10 g

40 small (1-oz) medicine cups
10 (5-oz) plastic cups
40 10-cc syringes
40 end caps for syringes

40 balloons
40 wooden stirrers
aquarium tubing

Needed, but not supplied:
9 packets or a jar of Rapid Rise yeast (available from a grocery store – check the expiration date before purchasing)
1-L wide-mouth bottle, with cap (for yeast suspension)
1.5 L of 35°C tap water (for yeast suspension)
10 250-mL beakers or Erlenmeyer flasks
10 25-mL graduated cylinder
500 or 1000mL beaker or graduated pitcher
10 permanent markers
10 timing devices
2 thermometers
test tube racks
Tape to label syringes with sample ID
Scissors, clear tape, string, graph paper, rulers, pens, colored markers/pencils

Optional for the experiment (not supplied):
- Water baths (various temperatures)
- Balance (accurate to 0.01 g)
- Weigh boats

Safety
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: emphasize the importance of proper usage. Use personal protective equipment (PPE) such as safety glasses or goggles, gloves, and aprons when appropriate. Model proper laboratory safety practices for your students and require them to practice all laboratory safety rules.

Day 1 Preparation
1. Photocopy the Student Guide for each student or each group, as desired.
2. Organize students into 10 groups of three. If your class size differs, adjust the size of the 10 groups accordingly.
3. Set out the materials for the design/experiments in a central, accessible location so students can see them and consider how they might use them. Remember, the method that they pick will determine the basic set of materials they will need. Students should not make any permanent modifications to the bioreactor materials so that they can be rinsed and reused for Day 2.

4. Prepare a ~2% glucose solution by adding 4 g of sucrose to 200mL water. Swirl or shake the solution until the solids are completely dissolved. Aliquot 10 mL of sugar solution into 10 15-mL tubes. Each group will get 2 of these tubes.

5. Important: Prepare the yeast suspension 20-30 minutes before students are to perform their yeast observations.

    a. Add 500 mL of warm tap water (35-40°C) to a 1-L-wide-mouth bottle. **Warning:** temperatures above 40°C will kill the yeast: 36-37°C is optimal.
    b. Add 35 g of active, dry yeast to the bottle. Optimal fermentation is observed with 7 g of yeast dissolved in 100 mL of tap water (7 % wt/v).
    c. Cap the bottle. Gently invert the capped bottle 15 times to mix the yeast. At this point, the yeast should be equally distributed throughout the suspension.
    d. Allow the yeast to hydrate for at least 5 minutes.
    e. Resuspend yeast before distributing. Aliquot 20 mL into specimen cups for each group.
    f. The remaining yeast suspension can be used for the probeware demonstration.

6. Each group station should have a test tube rack, 10 mL of 2% glucose solution, 20 mL yeast suspension, and 2 pipets.

Day 2 Preparation

Prepare sugar solutions

1. Prepare 200 mL of 2% sugar solutions with each of the various sugar types as you did for the glucose in Day 1. Aliquot 10 mL of each sugar solution into 10 15-mL tubes. Each group will get 1 of each of these tubes.

2. Prepare the same yeast suspension as in Day 1 20-30 minutes before students are to perform their yeast observations.

Day 1 Procedure

1. Watch Yeast Optimization intro video and Novozymes Tour Video.
    a. Yeast Bioreactor Lab Activity Intro Video

https://www.youtube.com/watch?time_continue=2&v=FJP-QcWhiMg

b. Recommended video for Career Connections: Focus on 5:40-7:12

https://www.novozymes.tv/video/18870808/novozymes-north-america-virtual-tour-1

2. Introduce lab concepts

- yeast as a powerful tool for synthesizing valuable molecules
- basic fermentation reaction and requirements

- concept of scaling-up operation for production

3. Task students with designing their own lab-scale bioreactor that can quantify how well their yeast are performing. Show students their design tools that may include pens, paper, graph paper, rulers. Also show them the prototype building materials, naming and describing each item available to them.

4. Provide time for student groups to discuss and draw their plan for their prototype. Remind students that they must be able to compare two or more fermentation environments and they need to get quantitative data that is repeatable.

Ideas for bioreactor prototypes:

- Students may use a balloon on a tube to collect CO_2. CO_2 volume can be estimated by taking the diameter of the balloon with a string or flexible ruler. They may use displacement to measure the volume of the balloon by submerging the balloon in a large beaker. These designs are limited because the balloon doesn't quiet expand proportionally to the amount of CO_2.

- Students may use a capped syringe to quantify CO_2 production. The plunger will move proportionally to gas production.

- Students can create a calibrated bag with the wide plastic tubing to also measure the volume of CO_2.

- They may decide to manually agitate their bioreactor to simulate a shaking device.

5. Look over and approve prototype designs.

6. Have students build their prototypes with the given materials. They shouldn't make any permanent modifications to the materials. You may have them create one bioreactor or two. If they make two, then they can start thinking about comparing two reaction conditions.

7. Have students take notes about their design and setup conditions.

8. Recommend that students use all 10mL of sugar solution to maximize fermentation during their testing phase.
9. Fermentation and gas production should be visible in 10-15 minutes. As time permits, have students observe their bioreactors and make observations for 20 minutes.

10. Have student groups discuss their designs and any minor modifications they would like to make.

11. Have each group share about their prototype design and its effectiveness with the class.

Day 2

1. Help students design and conduct a yeast optimization experiments using their bioreactor prototypes. They should build 2-4 bioreactors for the experiment.

Ideas for student experiments:

- They may compare the various sugar types or concentration.
- To evaluate the effect of concentration of sugar stock solution on fermentation rates, change the proportion of sugar and water to obtain the desired concentration.
- To evaluate the effect of fermentation rate at various temperatures, adjust the temperature of the tap water to the desired value before adding the dry yeast.

To evaluate the effect of yeast pre-treatment, freeze or heat the yeast prior to hydration with tap water.

- To evaluate the effect of incubation time prepare two yeast suspensions, let one of them to hydrate for 5 minutes and the other for 30 minutes.

Examples of questions for students for formative/summative assessment:

1. What experimental question did you explore?
2. On the basis of your previous laboratory exercise, background knowledge, and research, what is the hypothesis that you tested?
3. Describe the conditions that you used in your fermentation experiment.
4. What were the independent and dependent variables?
5. What is the control group(s)?
6. What results did you see in the experiment?
7. Was the hypothesis accepted or rejected? What conclusions can you draw on the basis of the data and analysis?
8. How would you change the apparatus or what experimental conditions would you use to optimize fermentation and produce more ethanol?
9. Plot the change in volume of reaction mixture as a function of time. From this curve, determine the rate of fermentation in each of the syringes in millimeters per minute (slope of the curve once the fermentation starts).
10. How did the fermentation rate changed for the different conditions tested?
11. Did the fermentation start at the same time for each of the conditions? Was there an overlap between the different curves? Did they meet at some point, or they stayed separated throughout the data collection? How can you explain such observations?
12. How would you correlate the response of the fermentation reaction with the chemical structure of the sugar?
13. Explain why changing only one variable at a time is important when conducting a scientific investigation. Give an example to support your response.

14. What sources of error may have existed, and how might the experiment have been conducted differently? What additional questions arose from the experiment?

Supplemental Resources

Fermentation of yeast and sugar, exploratory video using balloon	https://www.youtube.com/watch?v=FYClCHVT00M
Biological approach for explaining alcoholic fermentation	https://www.youtube.com/watch?v=HNOcQKwW5ks
Fermentation for producing alcoholic beverages	https://www.youtube.com/watch?v=zP21LH3T9yQ

Name ____________________ **Student Guide**
Date _____________________

Yeast Bioreactor Design & Yeast Optimization Experiment

Overview

In this lab, you will design and build a bioreactor prototype. You will then use the bioreactor to explore the applications of yeast for production of bio-products.

You will observe yeast in a solution of sugar water in your newly designed bioreactor. Then, you will ask questions derived from the observations of the suspensions. Based on your questions, you will form a hypothesis and identify variables as well as design and conduct experiments to test the yeast activity. You will also use deduction to figure out how to optimize the yeast performance. Finally, you will evaluate your hypotheses and form conclusions after performing an analysis of your results.

Background

Sugars have been known to humankind since prehistoric times. Sugars are soluble carbohydrates commonly found in food. According to the number of molecules in their chains, sugars are classified into oligosaccharides or polysaccharides (for longer chains), and monosaccharides (simple sugars), which are the most basic units of carbohydrates. Sugars are essential for maintaining life and can be found in many different forms. For instance, the cell walls of plants are built from cellulose, a polysaccharide which makes up the plant fibers to provide their mechanical stability.

In biochemistry, fermentation refers to a catabolic process of incomplete oxidation for producing organic compounds in the absence of oxygen (anaerobic conditions). The fermentation of biomass-derived sugars, especially those containing six carbons, is the primary pathway currently used for bio-ethanol production. The fermentation in this lab will be done by yeasts. Yeasts are unicellular fungi with very simple needs for survival. In anaerobic conditions, yeasts attain energy by fermenting sugars. During fermentation, sugar molecules are broken down through a series of reactions that produce energy the yeast cell can use.

The overall chemical reaction for alcoholic fermentation of glucose, a simple sugar with the molecular formula $C_6H_{12}O_6$, is:

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

As a result of the alcoholic fermentation, one mole of glucose is converted into two moles of ethanol and two moles of carbon dioxide. The two significant by-products of fermentation are carbon dioxide and alcohol. However, by-products such as lactic acid, succinic acid, glycerol, can also be derived from alcoholic fermentation depending on the sugar stock and the reacting conditions.

Ethanol produced through alcoholic fermentation (also referred to as bio-ethanol) is a crucial part of the biofuel production. The consumption of ethanol fuel in the U.S. has increased dramatically from about 1.7 billion gallons in 2001 to 14.4 billion in 2016. Production of ethanol in the United States is based primarily on grains as a feedstock. A feedstock is usually a type of grain that is used to produce biofuel on a large scale; corn is the most commonly used feedstock because of its high ethanol yield. Approximately 2.65 gallons of ethanol can be produced from a bushel of corn. Ethanol is a renewable energy source and is domestically produced; ethanol as a fuel source can help reduce oil dependency as well as help reduce greenhouse gas emissions.

Part 1:

1. Design a yeast bioreactor from given materials. The bioreactor must allow you to quantify the fermentation rate. This design should help you figure out how to create an optimal environment for yeast health. Sketch and describe a lab-stale bioreactor that will allow you to compare fermentation rates. Get instructor approval for your design before building.

2. Build and test your design. Use the prepared yeast suspension and sugar solution for the fermentation reaction. Use all 10mL of sugar in the reaction if possible. Take notes on your design choices and the methods you used for preparing your fermentation reaction.

3. Observe and collect data about your bioreactor and the fermentation reaction over 15-25 minutes.

Part 2:
Use your bioreactor design to create an experiment to test two different conditions for fermentation. What conditions do you think would maximize fermentation? Can you test them?

1. Regarding your observations of the yeast suspension, collaborate with your group to come up with a question and testable hypotheses about the fermentation of yeast.

2. Design an experiment to test your question. Modify your experiment

3. Perform the experiment and collect data. During down-time, draw and label a diagram describing your experimental setup.

4. After you perform the experiment, analyze your data.

5. Be prepared to present the findings of your experiment to the class.