

The Fun in Fermentation

by

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Figure 1. George's "vineyard."

Part I – Making Wine

George Polsky was returning home after a long day's work. Like many people in the village, George was a modest farmer who owned a small vineyard and winery that he inherited from his grandfather. When he left work for the day, he thought about how, in his grandfather's day, their winery was the only vineyard for miles around. However, the increasing population had since tempted many newcomers to take advantage of the fertile soil to start their own wineries. This competition had forced George to put in long hours just to get half the profits made by his father and grandfather. Nonetheless, he fondly looked back once more at his vineyard and mentally made notes of what he would need to do the next day to prepare his grapes for the coming season.

When he reached home, George's favorite nephew Stanley was waiting for him. Stanley, a ninth grade honor student, had always done very well with his studies. That Friday afternoon, he came to see his uncle after school about doing a science project on fermentation. Uncle George was only too happy to help. Although Uncle George had not gone to college, he had a sense of pride about the fact that he could help his nephew with his project. "Come back early tomorrow morning," he told Stanley. "I'll give you a tour of the vineyard and show you how to prepare a fresh batch of grapes for the stainless steel batch fermenter."

The next morning, George showed Stanley how the grapes were picked and how the juice was extracted from them and added to the sterile fermenter. "The juice must be sanitized, then inoculated with actively growing yeast from a fresh yeast culture to ensure a good yield of wine," George explained. He then closed the door of the fermenter to begin the fermentation process. "The wine-making process will continue for a few days before wine is ready for harvesting. After the sugars in the fruit juice are converted to alcohol by the yeast and the alcohol content is about 15%, the process will be complete. This alcohol level will kill all the remaining yeast. We will then proceed to the next step of the wine-making process. But that's enough of a lesson for one day."

Stanley was fascinated. He thought, "Hey, this looks simple. I can do the same thing at home on a smaller scale." That night, he found a packet of his mother's baker's yeast in the refrigerator. He mixed the yeast with warm water until it looked like his uncle's inoculum. He then added the yeast to some grape juice that he poured into his favorite drinking glass, left the mixture in a warm corner of the kitchen, and went to bed.

The next morning Stanley saw bubbles covering the top of mixture in the glass. Excitedly, he grabbed his concoction and ran to his uncle's house. After taking one look and a quick smell, George declared, "Yes Stanley, the yeast grew very nicely. But, you did not make wine."

Questions

1. What chemical process is needed to make wine?
2. What is an inoculum?
3. Why did George tell Stanley that the wine-making process requires a "fresh yeast culture"?
4. Was the yeast that Stanley used living or non-living? Explain your answer.

Part II – Why Yeast?

Stanley was stumped. “Does that mean that the yeast is dead, Uncle George?”

“Not at all, Stanley, this was a good try,” said George. “These bubbles on top of the glass mean that your yeast is alive and growing. But you cannot produce alcohol using an open container. Cutting off the air supply is a key step in fermentation. By leaving the grape juice and yeast mixture in the glass without covering it, you exposed the yeast to air. The large batch fermenters we use in the vineyard are air tight.” He also told Stanley that the juice in the fermenter must first be sanitized to eliminate bacteria either by autoclaving the fermenter with steam at high pressure or by cleaning the inside of the fermenter with boiling water or with chemicals, such as potassium metabisulfite. Also, the yeast that is added must be uncontaminated. “If the juice or the yeast are contaminated, the wine that is made will have a sour taste and will not be fit for marketing. It would be a waste of entire batches of grapes.”

George could not properly explain why these two conditions for making wine were essential, but Stanley was determined to find the answers. When he returned to school the following Monday, he asked his biology teacher, Ms. Cheng, to explain.

“Yeast can grow in the presence of air or in the absence of air,” said Ms. Cheng. “However, alcohol will only be made in the absence of air by a special process called fermentation. Fermentation does not take place when air is present and so it did not take place in your open container. This is one reason why you didn’t make any wine.”

Ms. Cheng also explained that a pure yeast culture is important to avoid contamination with bacteria or other microorganisms. “Certain bacteria can convert pyruvate to acetic acid instead of alcohol. The acid will give a sour taste to the wine. If the yeast is pure, this will not happen. So, as you could see from your uncle’s vineyard, the wine-making process is more complicated than just adding baker’s yeast to a glass filled with grape juice. But you can experiment with fermentation in other ways. We will be learning about the processes of cell respiration and fermentation in our class today. That should clear things up so that you can design a good science project.”

Questions

1. How are yeast and bacteria different?
2. Why will contaminating bacteria make the wine sour while pure yeast will not?
3. What is the difference between an obligate aerobe, an obligate anaerobe, and a facultative anaerobe? Which kind of organism is yeast?
4. Why didn’t Stanley make any wine?

Part III – Aerobic Respiration and Fermentation

Ms. Cheng introduced her lesson for that day by discussing how all organisms need energy for survival. “Living things obtain energy from foods through cell respiration. In this process, the energy stored in certain compounds is transferred into a small molecule called ATP, or adenosine triphosphate. Cells can then use the ATP to fuel their cellular activities.”

Ms. Cheng went on to explain that cellular respiration can take place either aerobically (with oxygen) or anaerobically (without oxygen) (Figure 2). “When oxygen is present, sugar is completely broken down and releases large amounts of stored energy. Most of this energy is transformed into heat, but the remainder is stored in molecules of ATP. In this complex process, CO_2 and H_2O are also produced as byproducts. When oxygen is not present, cells can use an alternative chemical pathway called fermentation to quickly produce much smaller amounts of ATP that will ensure survival.”

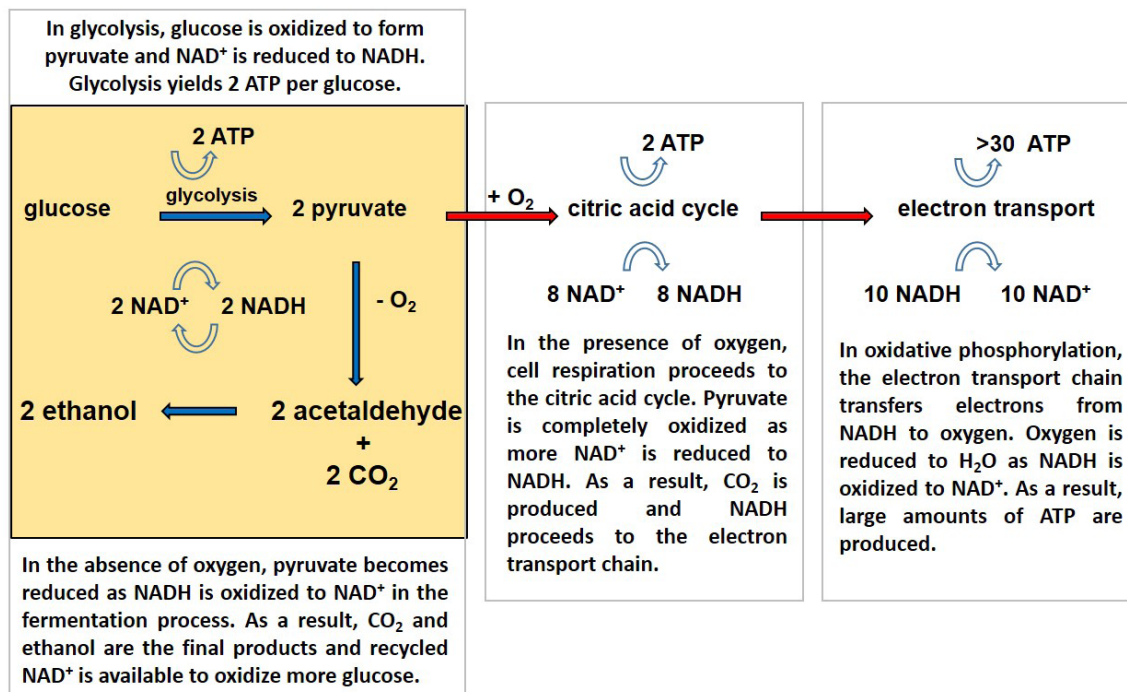


Figure 2. Ms. Cheng explains cell respiration and alcoholic fermentation.

Ms Cheng next described different fermentation processes. “In one type of fermentation, sugar is broken down to produce lactic acid. Muscle cells will undergo lactic acid fermentation when they are depleted of available oxygen. Also, certain bacteria can ferment milk sugar into lactic acid to make yogurt. In alcoholic fermentation, sugar is converted to an alcohol called ethanol and CO_2 gas is produced as a byproduct. Yeast can undergo this type of fermentation. Some bacteria can further convert the ethanol from fermentation to acetic acid. This is how vinegar is made. Today we will talk about the reactions of aerobic cell respiration and fermentation.”

Questions

1. Write a summary equation for cell respiration (refer to your textbook).
2. What are the stages of aerobic respiration?
3. What are the major differences between aerobic respiration and fermentation?
4. What is the significance of NAD^+ in fermentation and cell respiration?
5. Why do organisms undergo fermentation in the absence of oxygen?
6. What does Stanley need to do to redesign his original experiment?

Part IV – Stanley Plans His Experiment

That evening, Stanley visited his uncle George to tell him what he learned in school about fermentation. “Now I understand how you make wine from your grapes. But I’m still not sure how to use fermentation in a science project.

“Well Stanley,” said Uncle George, “I have some apple trees and a lot of strawberries. I wonder whether they can be fermented like grapes. What do you think?”

Stanley answered, “I think it’s a great idea for my science project!”

“So do I,” said George. “Why don’t you go talk to Richard? Perhaps he could help you plan your project.”

Stanley’s cousin Richard was an undergraduate at the local college pursuing a major in biology. Stanley told Richard about his idea. “I’m going to test different kinds of fruit juices to see which ones ferment the same way that grape juice does.”

“That sounds like a good experiment, Stanley. But don’t forget that a successful science experiment begins with a good hypothesis. Also, the experiment must be controlled, so only one variable can be changed. Your juices should all have the same sugar content as your grape juice. You can try cranberry, orange, apple, or strawberry juices. You can also borrow special fermentation tubes from your science lab in school. These tubes will make it easy for you to observe and measure carbon dioxide produced by yeast fermentation.”

Determined, Stanley went home to plan his experiment.

Questions

Answer the following questions to help Stanley design a proper experiment.

1. State a hypothesis for Stanley’s experiment.
2. List what materials Stanley will need to carry out his experiment.
3. Write an experimental protocol that includes a control experiment.
4. Identify the following components of your experiment:
 - Independent variable
 - Dependent variable
 - Controlled variables

Part V – Stanley’s Experiment

Richard helped Stanley write a protocol to test different fruit juices for their ability to undergo fermentation. Read through and carry out Stanley’s fermentation experiment. Notice his hypothesis. If your hypothesis was different, think about how it was different.

Stanley’s Hypothesis: All juices will ferment at the same rate if they all have the same sugar concentration.

Materials:

- Juices to be tested (preheated to 42°C)
- Yeast suspension (one package active dry baker’s yeast in 200 mL of lukewarm distilled water)
- 10 mL disposable pipettes and pumps
- 25 mL graduated cylinder
- 100 mL beakers (one for each juice plus two for controls)
- Fermentation tubes (one for each juice plus two for controls)
- Sucrose powder
- Metric scale
- Weighing paper
- Paraffin wrap
- Metric ruler

Procedure:

1. Calculate the volume of juice you will need for your experiment. You will need 2 grams of sugar. The amount of sugar will vary in each juice, so you will have to calculate how much juice you need in order to have a total of 2 grams of sugar. The concentration of sugar in your juice can be obtained from the nutritional label on the container. Use the following formula to calculate the volume of juice that you need to obtain 2 grams for your experiment: $2 \text{ grams} \div (\# \text{ grams/mL in juice}) = \# \text{ mL of juice for your experiment}$.
2. For each juice, add the volume of your juice calculated in Step 1 to a small beaker. Bring the volume in the beaker to 38 mL with distilled water.
3. For control experiments, one group will set up two additional small beakers as follows.
 - a. Weigh out 2 grams of sucrose on a metric scale and dissolve in a total volume of 38 mL of water. Add to one beaker labelled “sucrose.”
 - b. Add 38 mL of water to the second beaker labeled “no sugar.”
4. To each beaker of juice and to the control beakers, add 2 mL of yeast suspension and mix gently.
5. Pour the contents of each beaker into separate fermentation tubes (Figure 3). Cover the mouth of each tube with paraffin wrap to make the tube air tight. Carefully invert the fermentation tubes to fill the arms of the tubes completely with liquid. Make sure there are no air bubbles in the arms.
6. Place the tubes upright in a 42°C incubator or 42°C water bath for 1.5 hours. *Do not allow the tubes to tilt. The arm of the tube must remain upright at all times.*
7. After the incubation, proceed to Part VI to measure gas displacement and record results.

Questions

1. Make a chart that compares your experimental design from Part IV with Stanley’s design. Describe the similarities and differences in the hypothesis, materials, procedures, variables (independent, dependent, and controlled), and the control experiment(s).
2. For Stanley’s experiment, was the same amount of juice added for each fermentation tube that was prepared? Explain your answer.
3. What are the positive and negative controls for Stanley’s experiment?
4. What will you measure to determine whether fermentation took place?

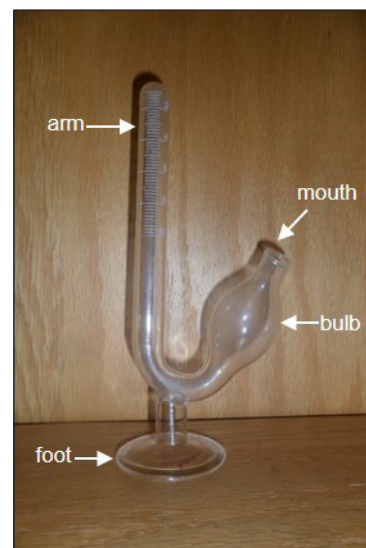


Figure 3. Fermentation tube.

Part VI – Recording the Results

Measure the amount of CO₂ gas produced: A fermentation reaction proceeds as follows: $C_6H_{12}O_6 \rightarrow 2 C_2H_5OH + 2 CO_2$. This chemical equation shows that for every molecule of glucose that is fermented, two molecules of ethanol and two molecules of CO₂ are produced. Therefore, a juice that generates more CO₂ also generates more alcohol. As CO₂ is produced, it will bubble through the liquid, collect at the top of the fermentation tube arm, and displace the liquid downward toward the bulb. If the fermentation tubes are calibrated, the displaced volume can be measured directly. If not, you will have to measure the displacement of liquid in centimeters to determine the volume of CO₂ produced. This volume will be proportional to the amount of ethanol produced and to the rate of fermentation.

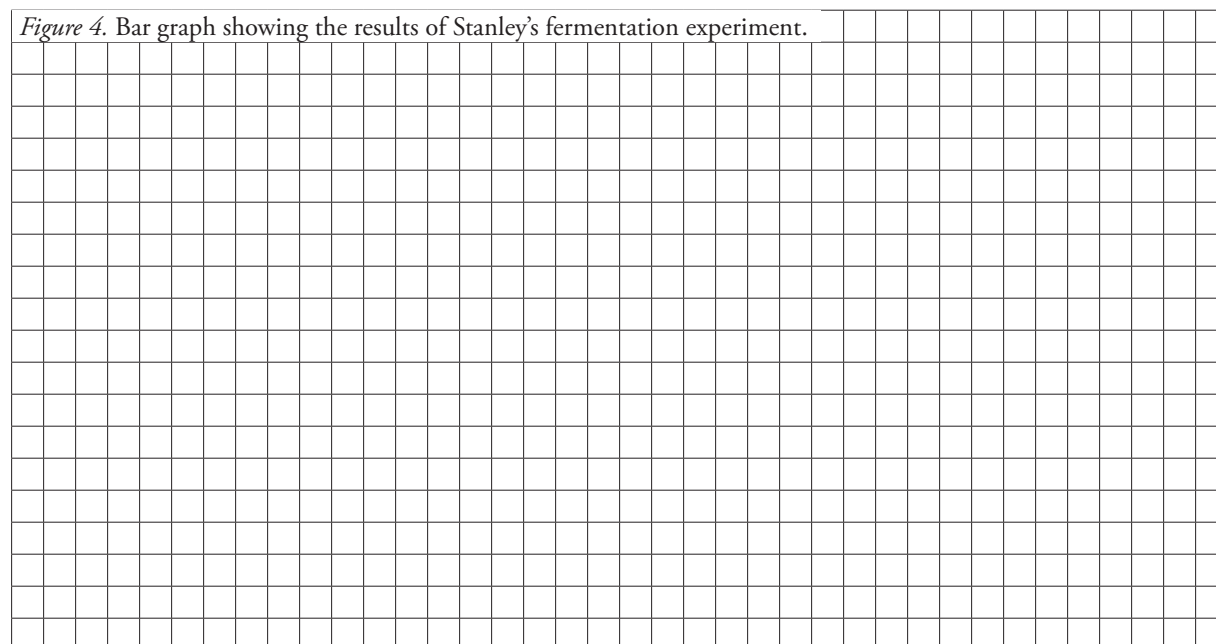
To calculate the volume of CO₂ produced in the arm of the fermentation tube, use the formula for the volume (V) of a cylinder, $V = \pi r^2 h$, where π is 3.14, r is the radius of the arm, and h is the height of the displaced liquid (in cm).

Tabulate the data: Enter the names of the juices used by each group. Enter the data for displacement of liquid (in cm), and volume of CO₂ produced in Table 1. Compile the results for each group.

Table 1. Volume of carbon dioxide gas produced by yeast.

Sample (write in name of juice)	Displacement of liquid (cm)	Volume of CO ₂ produced (cm ³)
Water		
Sucrose		
Juice 1 =		
Juice 2 =		
Juice 3 =		
Juice 4 =		
Juice 5 =		

Graph your results: To illustrate your results, prepare a bar graph in Figure 4 based on the information in you recorded in Table 1. Give your graph a title and label your axes.



Questions

1. What is the title of your graph?
2. What variable did you put on your x-axis?
3. What variable did you put on your y-axis?

Part VII – Analysis and Interpretation

Analyze and compare your data with other groups in your class. Prepare a lab report that summarizes Stanley's experiment.

Questions

1. What do the results of the control experiments tell you?
2. Discuss the results of the experiment and determine if they support Stanley's hypothesis.
3. List the factors that you think influenced the rate of fermentation.
4. Based on your results, which fruit is preferred by yeast for fermentation? Explain your answer.