BLOW UP A BALLOON

Teacher's guide

Subject: Biology

Teaching topic: Fermentation and energy production

1. Learning outcomes

From the Syllabus - students will:

- Understand that metabolic processes in a cell involve chemical reactions in which energy and substances are transformed.
- Understandthat cells typically temporarily store the energy released during metabolic processes in the phosphate bonds of small, energy-rich molecules such as ATP.
- Learn that ATP is the direct source of energy for driving biological processes in all living organisms and understand that cells regenerate ATP during the breakdown of organic molecules (glycolysis, cellular respiration, alcoholic and lactic acid fermentation).
- Recognize that during cellular respiration, glucose in the cytoplasm breaks down through the process of glycolysis into smaller organic molecules, during which a small amount of ATP is regenerated. In anaerobic cellular fermentations, lactic acid or ethanol is produced from pyruvate.

Upon completing the practical, students will:

- Familiarize themselves with the process of alcoholic fermentation and its significance for organisms.
- Be able to describe the course of alcoholic fermentation, record reactants and products, and explain the energy aspects of the process.
- Understand how the quantity of sugar affects the fermentation process.
- Understand fundamental concepts related to the scientific research method.
- Be able to formulate one or more hypotheses and explain the dependent, independent, and controlled variables in the experiment.

2. Theoretical background

Fermentation is the process of obtaining energy from organic substances in the absence of oxygen. The difference compared to cellular respiration lies in the absence of an electron transport chain, and that the final products are still energy-rich. The first stage of fermentation is identical to the first stage of cellular respiration, which is glycolysis. There are several types of fermentation, with the two most common being alcoholic and lactic acid fermentation. During alcoholic fermentation, pyruvate, a molecule with three carbon atoms, is broken down into ethanol and carbon dioxide. The products of this process can be observed during the production of wine or the rising of bread. In this process, yeast fungi produce alcohol during the breakdown of glucose, accompanied by the production of carbon dioxide. The dough rising when preparing bread is due to the formation of carbon dioxide. During baking, yeast dies, leaving air spaces, resulting in fluffy bread. Only two ATP molecules are produced from glucose during fermentation.

Source: Dolenc Koce J., Zidar P., Ambrožič Avguštin J., Tomažič I. 2021. Biologija 1: O biologiji, celicah in genetiki. Učbenik za biologijo v gimnazijah in srednjih strokovnih šolah. Ljubljana, Mladinska knjiga.

3. Materials and methods

The practical is adapted from materials available at: https://www.biotopia.net/en/9-english/282-yeastballoon (accessed on October 10, 2023) and a video available at: https://www.youtube.com/shorts/XcTekSyhLLI (accessed on October 10, 2023).

Duration of the practical: two 45-minute class session

Materials:

- packet of dry yeast
- 3 balloons
- 3 narrow-necked bottles/jars (500 mL)
- measuring cylinder/container with markings
- sugar
- warm water
- teaspoon
- funnel (optional)
- alcohol marker
- ruler
- mobile phone (stopwatch)

Methods:

1. Prepare all the necessary materials on the table.
2. First, inflate all three balloons and keep them inflated for a few minutes (2-3 minutes) to stretch them slightly, then release the air from them.
3. Add one teaspoon of dry yeast to each bottle. Use a funnel for easier transfer into the bottle.
4. Next, add sugar as follows: add two teaspoons of sugar to the first bottle, one teaspoon to the second bottle, and do not add sugar to the third bottle.

5. Pour approximately 100 mL of warm water into each bottle. Use an alcohol marker to mark the liquid level on the bottle. The water should be at a temperature similar to what you would use for bathing or washing hands.
6. Leave the bottles open and gently swirl them a few times to dissolve the sugar and yeast. Do not mix the contents during the experiment.

7. Attach a balloon tightly to the neck of each bottle.
8. Start the stopwatch.
9. Take a photograph of the initial state and another when the experiment is completed.
10. Wait for 45 minutes and observe what happens to the balloons and the contents in the bottles. During the wait, come up with three hypotheses for each bottle.
11. Mark the level of the bottle's content (foam peak) with the alcohol marker on each bottle every 5 minutes. Pay attention to the size of the bubbles. Record the results in the table below.

12. Measure the diameter of the balloon at its widest part every 5 minutes. Record the results in the table below. An approximate measurement is acceptable as it can be challenging to measure precisely.

13. Sketch what is happening with the contents in the bottles and the balloons on each of the bottles, comment on the process, and answer the questions below.

4. Guidelines for teachers

- This practical is suitable as a motivational activity before starting the topic of fermentation. The teacher should use a dialogue and discussion method and, together with the students, debate the fermentation process, reactants, products, as well as dependent, independent, and controlled variables. Students should also come up with three hypotheses for each bottle.
- Divide the students into smaller groups of 4-5 students each.
- Each group should prepare all the necessary materials and carry out the exercise following the instructions.
- Students can perform the exercise independently or, if necessary, in steps with the teacher's guidance.
- Students should answer the questions on the worksheet as they complete the exercise.
- After the exercise, review the questions together with the teacher, and discuss the progress of fermentation and its significance for organisms.
- The teacher can also introduce the process of lactic acid fermentation as an extension.

5. Questions and tasks for students

1. Predict what will happen in the bottles and formulate a hypothesis for what is happening in each of the bottles.

2. Fill in the table below with the experiment results.

Time [min]	Foam height [mm]			Balloon diameter [mm]		
	B1	B2	B3	B1	B2	B3
beginning						
5						
10						
15						
20						
25						
30						
35						
40						
45						

3. Sketch what happens with the contents and balloons in each of the bottles. Comment on each picture, explaining the process that occurs in each bottle, including reactants and products.

4. Present the results from the table above in a graph (two separate graphs for foam height and balloon diameter). Properly label the graph and adjust the scale on both axes according to your results. Plot 3 curves in each graph (for each bottle).
5. What process is taking place in each of the bottles? What are the main products formed in this process?
6. Why did we add sugar to the bottles? Does the reaction proceed even in the absence of sugar?
7. What differences occur in the bottles? Explain why this happens.
8. How long do noticeable changes take?

   a) Foam height increase

   b) Balloon diameter change
9. How could you prove that carbon dioxide was produced during the experiment and not, for example, oxygen?
10. Summarize the key findings of the experiment.
11. What is the dependent and independent variable in the experiment? List 3 controlled variables.

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Worksheet for students

BLOW UP A BALLOON

Before starting the exercise, think about the following questions.

- Have you ever heard of alcoholic fermentation? What are the products of this process, and what is its significance?
- Do you know of examples from everyday life where this process is used?
- Which organisms carry out alcoholic fermentation?

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ACTIVITY DURATION: 2 school hours

MATERIALS

- a packet of dry yeast
- 3 balloons
- 3 narrow-necked bottles/flasks (500 mL)
- measuring cylinder/container with markings
- sugar
- warm water
- teaspoon
- funnel (optional)
- alcohol marker
- ruler
- mobile phone (stopwatch)

METHODS

1. Prepare all the necessary materials on the table.
2. First, inflate all three balloons and keep them inflated for a few minutes (2-3) to stretch them slightly, then release the air from them.
3. Add one teaspoon of dry yeast to each of the bottles. Use a funnel for easier transfer to the bottle.
4. Then add sugar as follows: put two teaspoons of sugar in the first bottle, one teaspoon in the second, and no sugar in the third bottle.
5. Pour approximately 100 mL of warm water into each bottle. Use the alcohol marker to mark the liquid level on the bottle. The water should be at a temperature similar to that used for bathing or washing hands.

6. Leave the bottles open and gently rotate them a few times to dissolve the sugar and yeast. Do not mix the contents further during the experiment.

7. Place a balloon over the neck of each bottle. The balloon should tightly cover the bottle's opening.
8. Start the stopwatch.
9. Take photos of the initial and final states.
10. Wait for 45 minutes and observe what happens to the balloons and the contents of the bottles. While waiting, make three hypotheses for each bottle.
11. Every 5 minutes, mark the liquid level (top of the foam) on the bottles using the alcohol marker. Pay attention to the size of the bubbles. Record the results in the table below.
12. Measure the diameter of the balloon at the widest part every 5 minutes. Record the results in the table below. An approximate measurement is acceptable since it can be challenging to measure precisely.
13. Sketch what happens to the contents in the bottles and the balloon on each of the bottles. Comment on the process and answer the questions below.

RESULTS AND DISCUSSION

1. Predict what will happen in the bottles and make a hypothesis for each of the bottles.

BOTTLE 1 (2 teaspoons of sugar):

_____

BOTTLE 2 (1 teaspoon of sugar):

_____

BOTTLE 3 (no sugar):

_____

2. Fill out the table below with the experiment results.

Time [min]	Foam height [mm]			Balloon diameter [mm]		
	B1	B2	B3	B1	B2	B3
beginning						
5						
10						
15						
20						
25						
30						
35						
40						
45						

3. Sketch what happens to the contents and the balloon in each of the bottles, and comment on each image to explain the process taking place in each bottle, including reactants and products.

BOTTLE 1 (2 tabelspoons of sugar)

BOTTLE 2 (1 tablespoon of sugar)

BOTTLE 3 (no sugar)

4. Present the results from the table above in a graph (two separate graphs for foam height and balloon diameter). Label the graphs appropriately, and adjust the scale on both axes to suit your results. Plot 3 curves in each graph (for each bottle).

5. What process occurs in each of the bottles? What are the main products generated in this process?

_____

_____

6. Why did we add sugar to the bottles? Does the reaction occur in the absence of sugar?

_____

_____

7. What differences occur in the bottles? Explain why this is the case.

_____

_____

8. How long do noticeable changes take to occur?

a) Rising of the bottle's content (foam): _____

b) Change in balloon diameter: _____

9. How could you demonstrate, through a simple experiment with a tea candle and the gas produced, that carbon dioxide was produced during the experiment, rather than oxygen, for example?

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10. Summarize the key findings of the experiment.

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11. What is the dependent and independent variable in the experiment? List three controlled variables as well.

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Examples of suitable answers to the questions

1) Predict what will happen in the bottles and formulate a hypothesis for what will happen in each of them.

   BOTTLE 1 (2 teaspoons of sugar): The balloon in bottle 1 will inflate the most, and the foam will rise the highest.

   BOTTLE 2 (1 teaspoon of sugar): The balloon in bottle 2 will inflate slightly less than in the first bottle, and the foam's height will be lower.

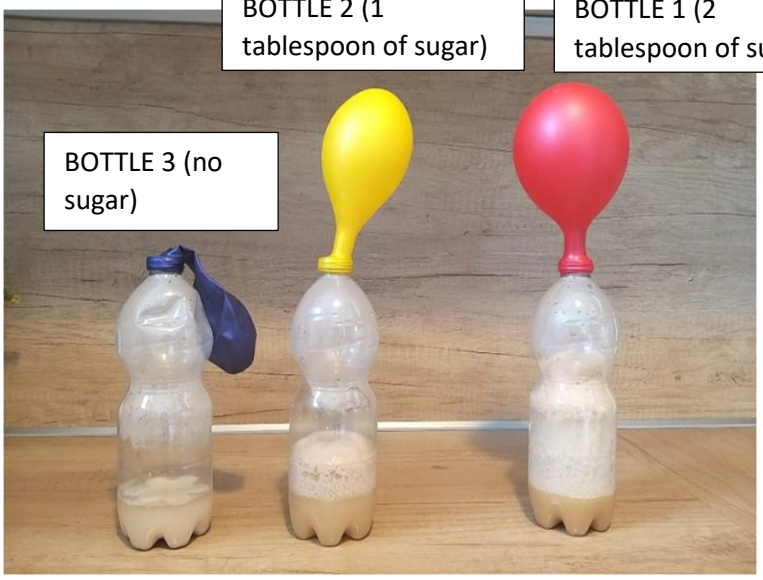
   BOTTLE 3 (no sugar): The balloon will not inflate, and no foam will form since the fermentation process will not occur.

2) Fill in the table below with the results of the experiment.

Time [min]	**Foam heght [mm]**			**Balloon diameter [mm]**		
	P1	**P2**	**P3**	**P1**	**P2**	**P3**
beginning						
5						
10						
15						
20						
25						
30						
35						
40						
45						

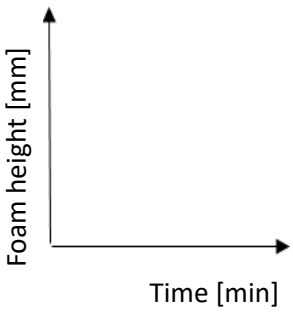
3) Sketch what happens with the content and the balloon in each of the bottles, and comment on each picture, explaining the process happening in each bottle and including reactants and products in the explanation.

Final state:



BOTTLE 1 (2 tablespoon of sugar)	BOTTLE 2 (1 tablespoon of sugar)	BOTTLE 3 (no sugar)
In the bottle, the fermentation process occurred. Yeast cells, aided by enzymes, convert sugar into ethanol and carbon dioxide. This is observed as the formation of foam and the rising of the bottle's contents. The balloon inflated due to the production of carbon dioxide. The quantity and height of the foam in this bottle are the highest, and the balloon inflated the most.	The fermentation process also took place in this bottle, but due to the smaller amount of sugar, less carbon dioxide was produced. As a result, the balloon inflated later (only towards the end of the designated time for the experiment) and to a lesser extent compared to the first case. Additionally, the foam's height is lower than in the first case, where yeast had more sugar available.	In this case, fermentation did not occur because yeast did not have sugar available, which serves as an energy source for them to carry out the fermentation process. The liquid level in the bottle remained the same, and the balloon did not inflate since there was no production of carbon dioxide.

4) Present the results from the table above with a graph (two separate graphs for foam height and balloon diameter). Properly label the graphs and adjust the scale on both axes to fit your results. Plot three curves in each graph (one for each bottle).



5) What process takes place in each of the bottles? What are the main products generated in this process?

In the first and second bottles, the process of alcoholic fermentation takes place. The primary products generated in this process are alcohol (ethanol) and carbon dioxide.

6) Why did we add sugar to the bottles? Does the reaction proceed in the absence of sugar?

Sugar serves as a food source (energy) for yeast. In the absence of sugar, the reaction does not proceed because yeast does not have an energy source for the fermentation process.

7) What differences occur in the bottles? Explain why this is the case.

Differences in foam height and balloon diameter occur in the bottles. This is due to the amount of sugar added. In the first bottle, where the most sugar was added, more carbon dioxide was produced, resulting in a larger balloon diameter (more inflation). The balloon also inflated more quickly than in the second bottle. The contents in the bottle also rose higher (more foam). In the last bottle, fermentation did not occur, resulting in no significant changes.

8) How long do noticeable changes occur?

a) Increase in bottle content (foam): during the whole time of the experiment

b) Balloon diameter change: In the first bottle, the balloon inflates quickly, and then its diameter remains more or less the same. In the second bottle, it took more time for the balloon to inflate.

9) How could you demonstrate, through a simple experiment with a tea candle and the gas produced, that carbon dioxide was produced during the experiment, rather than oxygen, for example?

You could remove the balloon from the bottle and quickly seal the bottle with your hand. Slowly "pour" the gas that has accumulated in the bottle onto a burning tea candle by placing the bottle's opening directly above the candle. Be cautious not to tilt the bottle too much, as this could spill the gas onto the candle's flame. If oxygen were being produced during the fermentation process, the candle would burn more vigorously when exposed to the gas. However, because carbon dioxide was produced, the candle will be extinguished when exposed to the gas.

10) Summarize the key findings of the experiment.

With this practical, we aimed to demonstrate the process of alcoholic fermentation carried out by yeast. Since fungi require sugar (organic substance) for their function and energy source, we added sugar to the bottles. We wanted to investigate how the amount of sugar affects the fermentation process, so we added different amounts of sugar to two bottles while keeping the third bottle as a control with no added sugar. Balloons were placed on top of the bottles because carbon dioxide is produced during fermentation, and we expected the balloons to inflate in bottles where fermentation occurred. We found that in the bottle with the most sugar, the fermentation process occurred most intensely, as indicated by the highest foam height and the fastest and largest balloon inflation. In the second bottle, the fermentation process also occurred, but the foam height and balloon diameter were smaller than in the first bottle. In the third bottle, where no sugar was added, the fermentation process did not take place, and the state remained the same compared to the initial condition. Initially, it took some time for the process to begin in the first two bottles,

but after a few minutes, the process intensified, as seen in the increased content and foam in the bottles. There is a noticeable difference between the bottles. In the third bottle, nothing happened due to the absence of sugar. In the first bottle, the content and foam height in the bottle were higher than in the second bottle, and the balloon inflated more quickly, reaching an upright position after about 15 minutes, while it took longer in the second bottle (about 40 minutes). The experiment demonstrated that the presence of sugar is necessary for the fermentation process, and the quantity of sugar also affects the process, as evident from the larger balloon in the bottle with more added sugar and the higher foam height.

11) What is the dependent and independent variable in the experiment? List three controlled variables as well.

Dependent variable in our experiment: Foam height in the bottle and balloon diameter Independent variable: The amount of sugar Controlled variables: Amount of dry yeast, amount of added warm water, bottle size, balloon size.

Prepared by Eva Šajn, biology teacher