

TEACHING MATERIAL FOR HIGH-SCHOOL STUDENTS:

MICROBES CAN BE USEFUL TOO

With a total workload of four hours in two days, the course consists of introduction lesson, simple microbiology classroom experiment that can be observed over two days and data analysis at the end.

This course will provide a tool to help students understand what yeasts are and how they are used in food industry.

Keywords: experiment design, fermentation, food industry, yeast.

HANDS-ON MICROBIOLOGY EXPERIMENT

YEAST *Saccharomyces cerevisiae* BLOWS UP BALLONS

What you will need:

- 4 balloons
- 4 clean and empty plastic bottles
- 2 spoons of sugar
- 1 spoon of salt
- 200 ml water per plastic bottle
- 4 packets of dry yeast

How to make yeast balloons:

First label your empty plastic bottles: A – D.

Experiment design with different variables. Variables are parts of an experiment that can be kept the same, or changed in order to test different outcomes:

- Yeast + sugar + 200 ml warm water
- Yeast + sugar + 200 ml cold water
- Yeast + salt + 200 ml warm water
- Yeast + 200 ml warm water

Which other variables could you test and why?

- _____
- _____

* Reminder: Use warm not hot water. Hot water would kill yeast!

Carefully pour warm or cold water into the four marked plastic bottles. Add a spoonful of sugar or salt per water bottle and swirl the bottle to dissolve the sugar.

At the end, add one yeast packet into each bottle, mix the mixture, and quickly stretch a balloon instead of lid over the opening of each bottle. Leave bottles at room temperature.

In the table below, take note of the time of when experiment was started and observe when each balloon rises.

START TIME OF EXPERIMENT: _____

EXPERIMENT BOTTLE	Start of balloon inflation
A	
B	
C	
D	

Create another table on your own to record your data for next two days: the diameter of the balloon, the time passed, and any other observations.

From the collected data of your experiment create a graph (example: diameter of the balloon during these two days for every bottle) to compare your results with other students.

Questions for discussion before and after experiment:

- a) Which balloon was/will be first blown up and why?
- b) Which ingredient (salt or sugar) makes yeast growing well?
- c) Does yeast grow faster in warm or cold water?
- d) What happened/will happen to the balloon on the bottle C where salt was added instead of sugar?
- e) Which variable would you add to the experiment to get more data on yeast living conditions?
- f) Your own question: _____

Write a conclusion:

Write what was your experimental question (what you wanted to find out with this experiment) and your answer to the question.

You can also add any other findings and observations or plans for future additional experiments with yeasts in a conclusion.

Take your learning further:

If there is a microscope at the hand, students can also observe yeasts cells under it.

Students can design additional yeast experiments at home. For example, varying the amount of sugar added to the mixture, keeping experiment at different temperatures (in a fridge versus at a room temperature) or varying the amount of water. By performing a combination of these experiments, the

optimum conditions for yeast growth can be determined. In that way, students will start thinking as an experimental scientist, considering the ways that an experiment can be altered so that different experimental questions can be tested and answered.

The science behind:

Students observed how sugar and water-temperature affect the growth rate of yeast and also learned how to plan an experiment, collect data, and write a conclusion.

Yeasts are a type of microorganisms that belong to the fungus kingdom. These single-celled organisms are widely dispersed all around us, in water, in soil, on plants, on animals and in the air. Yeasts can be beneficial to us, including important industrial organisms (e.g. baker's yeast *Saccharomyces cerevisiae*), or harmful to us in the form of pathogenic yeasts (e.g. yeast infections caused by *Candida albicans*). If you look at yeast cells under the microscope, you will find simple cells shaped like balloons. Some yeast species also form multicellular strings or clusters of single cells. There are a large number of different species of yeast, but the one most commonly used for baking bread is *Saccharomyces cerevisiae*. As well as being used in the baking industry to raise dough, this yeast is also used to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages.

When you buy a packet of dry yeast in the store, the yeast cells remain dormant as long as they are kept in dry and cool conditions, but they start to grow quickly as soon as they are exposed to water and sugar at the right temperature. But what is the "right" temperature to grow yeasts? During the experiment, the yeasts are kept at different temperatures to find out how temperature affects living organisms. At temperatures above 55°C, the yeast cell reaches the thermal death point. At such a high temperature, the yeast cells have several surviving problems. Their cell membrane consists of lipids and proteins that get damaged, and the membrane organization is disrupted. Proteins called enzymes which are responsible for all chemical reactions in yeast cells, become inactive, which hinders the cells' metabolism. At 4°C (refrigerator temperature), the yeast is too cold to function properly. However, the yeast cells are still alive, they are just in a dormant state. If the environment would warm up a little, they would gradually start to grow. The ideal temperature for yeasts that are active and growing is between 40 and 45°C.

The next question that might arise is: "Can a living organism survive with a minimal amount of oxygen in a plastic bottle if we seal it with a balloon?". To understand why yeasts are able to thrive in both conditions, we need to learn the chemical reaction that takes place during the experiment. Yeasts are

simple organisms that can live in an environment with or without available oxygen. At the beginning of the experiment, there is enough oxygen in the bottles covered with a balloon. Oxygen is readily available, and as soon as the yeast is placed in the sugar solution, it begins to chemically convert the sugar in the water and the oxygen in the air into energy, water and carbon dioxide, which is known as a process called **aerobic respiration**. Towards the end of the experiment, however there is only a limited amount of oxygen left in the covered plastic bottle. At this stage, yeast simply switches from **aerobic respiration** (with oxygen) to **anaerobic respiration** (without oxygen). In the absence of oxygen, the products of this chemical reaction are different, and this process, known as **fermentation**, produces carbon dioxide and ethanol. **Fermentation is a metabolic process in which sugar is converted into gasses and alcohol in the absence of oxygen** (Fig. 1).

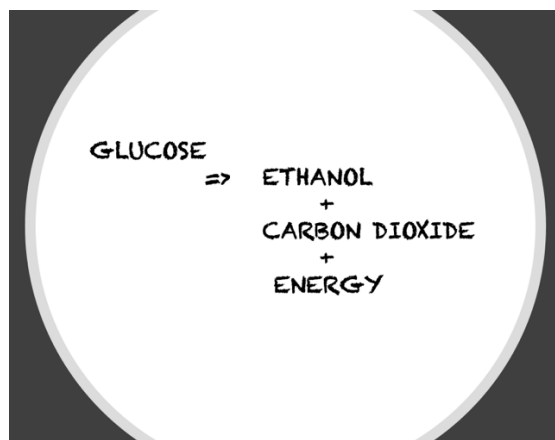


FIGURE 1. FERMENTATION PROCESS IN YEASTS.

The fermentation process is not specific to yeasts, but it also occurs in microorganisms such as bacteria as well as in oxygen-depleted human muscle cells. Under anaerobic conditions, the sugar in the yeast is converted into ethanol, while the muscle converts it into lactate (Fig. 2).

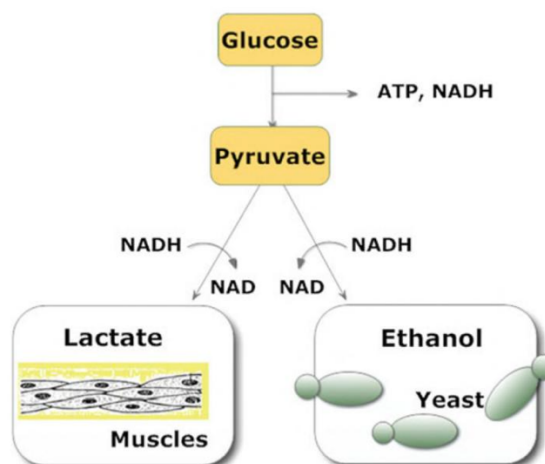


FIGURE 2. DIFFERENCE BETWEEN FERMENTATION PROCESS IN MUSCLES AND YEASTS. FIGURE TAKEN FROM REFERENCE 2.

If the experiment is observed for a longer period of time, the students will notice that the growth of the yeasts slows down. This happens for three reasons: First, anaerobic respiration produces less energy than aerobic respiration. Secondly, because the ethanol produced is toxic for the yeast in higher concentration. As the ethanol concentration in the environment increases, the yeast cells begin to get damaged, which slows down their growth. And thirdly, the yeasts consume sugar from the mixture, and after a long time there is no more sugar available for growth.

During bread making, the yeast also begins by respiring aerobically, producing the carbon dioxide that makes the bread rise. As the yeast digests the sugar, the gas it produces forms many small bubbles that cause the dough expand or rise. These bubbles burst during baking and leave tiny holes in the bread. Or in our experiment, these bubbles inflate a balloon. The balloon inflates because the gas gets trapped, and more and more gas is formed as the yeasts multiply. Eventually the available oxygen is used up, as in our experiment, and the yeast switches to anaerobic respiration, producing alcohol and carbon dioxide instead. But do not worry, this alcohol evaporates from the bread during the baking process, so you will not get drunk from eating the bread.

REFERENCES:

1. Link to The Stem Laboratory website (Retrieved 7. December 2023):
<https://thestemlaboratory.com/inflate-balloon-yeast-experiment/>
2. Piškur, Jure; Compagno, Concetta (2014). Molecular Mechanisms in Yeast Carbon Metabolism. Springer. p. 98. ISBN 978-3-642-55013-3.
3. Legras JL, Merdinoglu D, Cornuet JM, Karst F (2007). Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. Molecular Ecology. 16(10): 2091-2102. doi:10.1111/j.1365-294X.2007.03266.x.