Experiment B-7
Enzyme Activity

Objectives

- To understand basic concepts of enzyme activity.
- To learn about the enzyme catalase and how it breaks hydrogen peroxide.
- To investigate how different quantities of an enzyme affect the enzyme activity rate.
- To investigate how the temperature affect the enzyme activity rate.

Modules and Sensors

- PC + NeuLog application
- USB-200 module
- NUL-203 Temperature logger sensor
- NUL-210 Pressure logger sensor

Equipment and Accessories

Item	Qty
▪ Utility stand	1
▪ Right angle clamp	1
▪ Extension clamp	1
▪ 50 ml beaker	4
▪ Tube rack	1
▪ Test tube	6
▪ Perforated cap	6
▪ Pasteur pipette	2
▪ Sample container	1

Materials

Material
▪ Half a teaspoon of yeast
▪ 20 ml of water (for yeast suspension)
▪ 20 ml of Hydrogen peroxide 1%
▪ 30 ml of water at room temperature (for second part)
▪ 30 ml of boiled water (for second part)

Introduction

Enzymes are proteins which speed up the rate of reactions that would happen more slowly without their activity. The enzyme is not altered by the reaction. Each cell contains hundreds of different types of enzymes and each one is responsible for a particular reaction in the cell.

The catalase enzyme is present in almost all living cells. It breaks hydrogen peroxide (which is generated during cell metabolism) in order to protect cells from oxidative damage. Human catalase has a role in preventing diseases and possibly some aging processes. This is the general reaction that is catalyzed by the enzyme:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

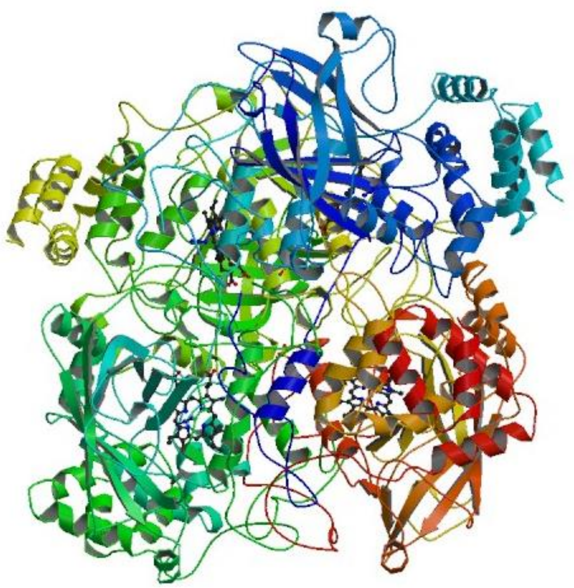
Catalase breaks millions of hydrogen peroxide molecules every second. The hydrogen peroxide is destroyed in two steps:

1. Catalase breaks down hydrogen peroxide by removing and binding one oxygen atom to itself. The rest of the molecule is released as water.

2. Catalase breaks down a second hydrogen peroxide molecule releasing another water molecule and oxygen gas (two oxygen atoms, one of them from the first reaction).

The enzyme structure includes four identical protein subunits. Each one of them contains an iron atom that binds the oxygen atom from the first step of the reaction.

In this experiment we will follow the activity of catalase from yeast. We will measure the initial rate of the reaction when different quantities of the enzyme are used. We will also compare the initial reaction between different temperatures.



Structure of yeast catalase, taken from the protein data bank website http://www.rcsb.org/pdb/explore/explore.do?structureId=1A4E

Procedure

Experiment setup

> Caution:
>
> It is recommended to wear personal protective equipment. Material Safety Data Sheets (MSDS) are available online.

1. Set up the experiment as shown in the picture below.

2. Take the sample container with the yeast (half a tea spoon) and place it in 20 ml of water.

3. Mix it with the Pasteur pipette until you get a homogeneous suspension.

4. Take 2 ml of hydrogen peroxide with the Pasteur pipette and place it in one of the test tubes.

5. Attach the test tube to the utility stand with the extension clamp and right angle clamp.

6. Make sure you also have boiled water and water at room temperature in two 50 ml beakers.

Sensor setup

3. Connect the USB-200 module to the PC.

4. Check that the pressure sensor is connected to the USB-200 module.

Note:

The following software functions are explained in short. It is recommended to practice the NeuLog application functions (as described in the user manual) beforehand.

5. Run the NeuLog application and check that the pressure sensor is identified.

Settings

6. Click on the **On-line Experiment** icon in the NeuLog main icon bar.

7. Click on the pressure **sensor's module** box.

8. Click on the **Range** button.

9. Select the kPa button to set the sensor's mode.

10. Click on the **Experiment Setup** icon and set the:

    Experiment duration to 5 minutes
    Sampling rate to 60 per minute

Testing and measurements

11. One of the products of the reaction catalyzed by the enzyme is oxygen. When oxygen accumulates in the test tube, the pressure inside it increases. This gives us an indication of how much oxygen was produced in time, which is the enzyme's initial rate.

    Before we start to work with the enzyme, we want to make sure that the enzyme itself is responsible for the pressure increase.

    Place the perforated cap in the test tube and then insert the pressure sensor's probe into the cap.

12. Click on the **Run Experiment** icon to start the measurement.

13. A minute is enough to see that the pressure is not rising because the reaction occurs very slowly without the enzyme.

    Click on the **Stop Experiment** icon after one minute.

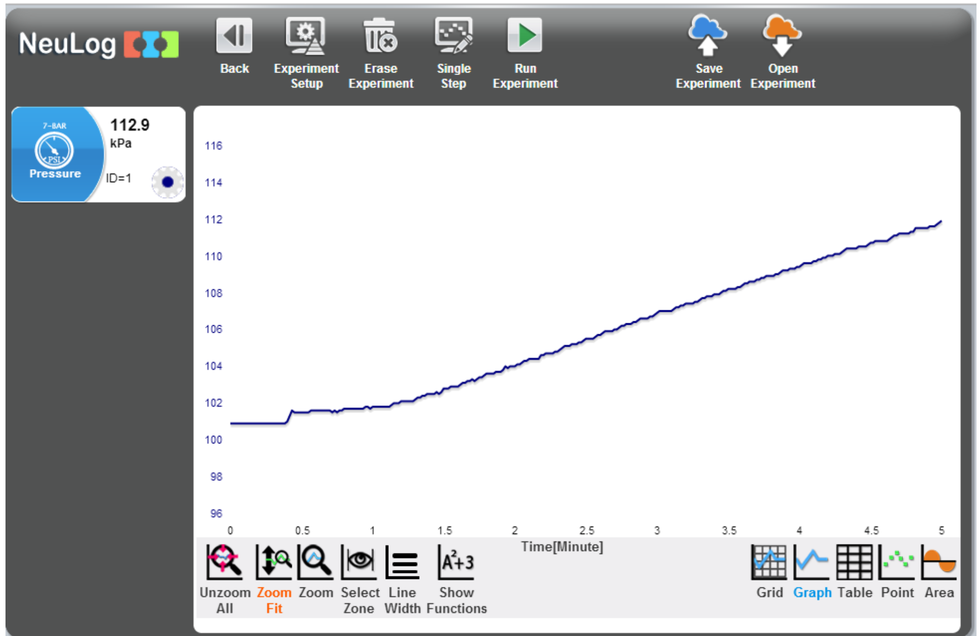
14. Click on the **Erase Graph** icon .

15. Remove the sensor, and perforated cap from the test tube and click on the **Run Experiment** icon to start the measurement (you will measure the atmospheric pressure for a few seconds).

16. With the Pasteur pipette, add 3 drops of the yeast suspension, in which the enzyme is in, to the hydrogen peroxide solution in the test tube (you do not have to replace the solution you have tested before).

17. Gently shake the test tube and immediately place the perforated cap with the sensor's probe attached to it into the test tube. Watch how the pressure increases with time. You can click on the **Zoom fit** icon during the measurement to see better how the graph changes.

18. Your graph should be similar to the following:



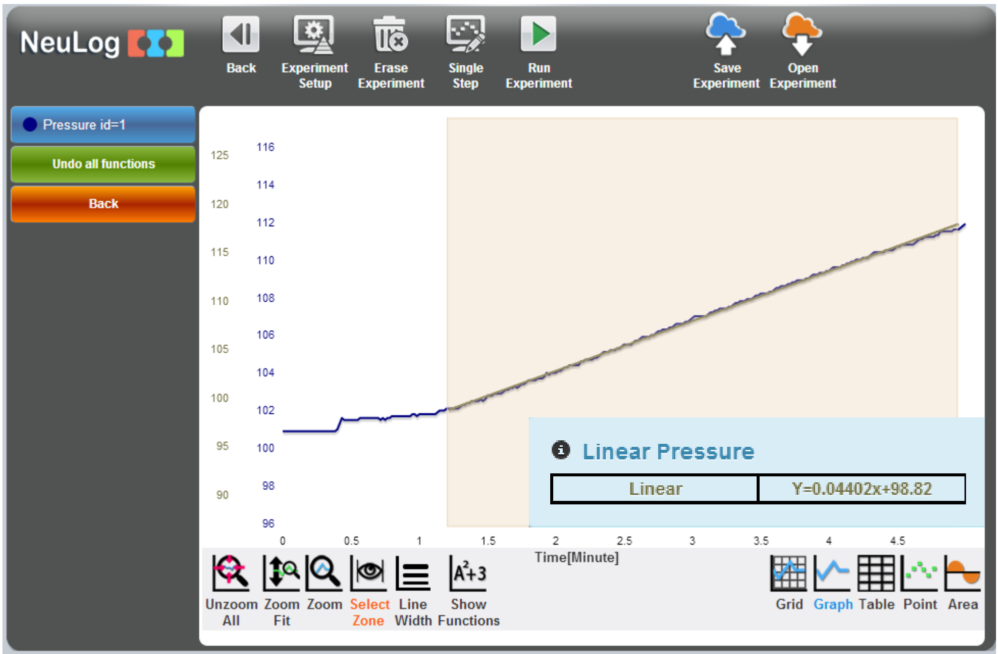
19. Save your graph.

20. You should be able to see a linear graph that represents the initial rate of the reaction.

21. Click on the **Select zone** icon and select the linear area of the graph.

22. Click on the **Show Functions** icon .

23. Click on the **Pressure** button on the left of the screen and then click on the **Linear fit** button.



This is the linear fit function we have received: Y=0.044X+99

The initial rate of the reaction is 0.044 kPa/minute for the sample experiment.

24. Fill in your results in the following table:

Temperature	Number of enzyme suspension drops	Rate of Hydrogen peroxide decomposition [kPa/m] (rate of the reaction)
Room temperature (sample experiment)	3	0.044
Room temperature	3	
Room temperature	5	
Room temperature	7	
~30 °C	3	
~40 °C	3	
~10 °C	3	

25. Repeat the measurement with a new test tube and fresh hydrogen peroxide solution. This time add 5 drops of the yeast suspension. Write the initial rate in the table.

26. Repeat the measurement with a new test tube and fresh hydrogen peroxide solution. This time add 7 drops of the yeast suspension. Write the initial rate in the table.

Challenge experiment

27. Attach the temperature sensor to the pressure sensor and click on the **Search sensors** icon .

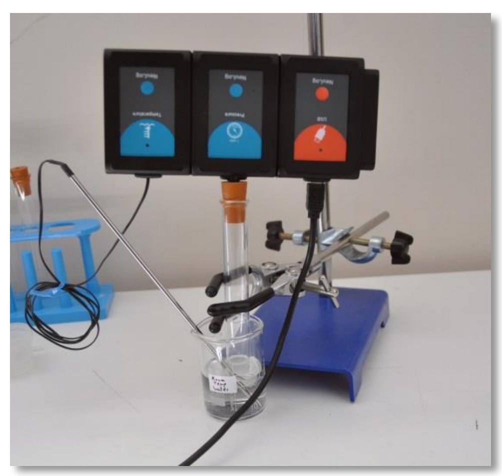
28. Click on the **Experiment Setup** icon and set the:

    Experiment duration to 5 minutes
    Sampling rate to 60 per minute

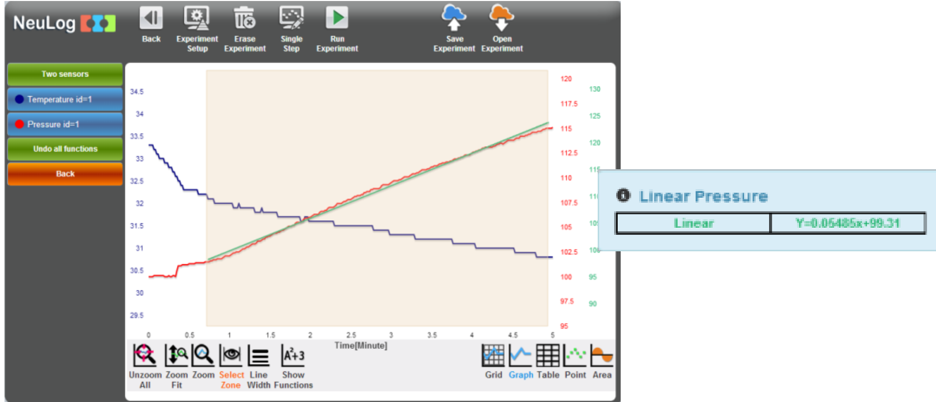
29. Place the temperature sensor probe into the beaker with the room temperature water. Add some of the hot water in it until it reached about 30-35 °C.

30. Place the test tube inside the warm water, wait for about 30 seconds and then repeat the measurements as you did before (with 3 drops of the yeast suspension).

    Keep the temperature sensor in the water during the measurement.



This is an example of an experiment conducted at ~30 °C.

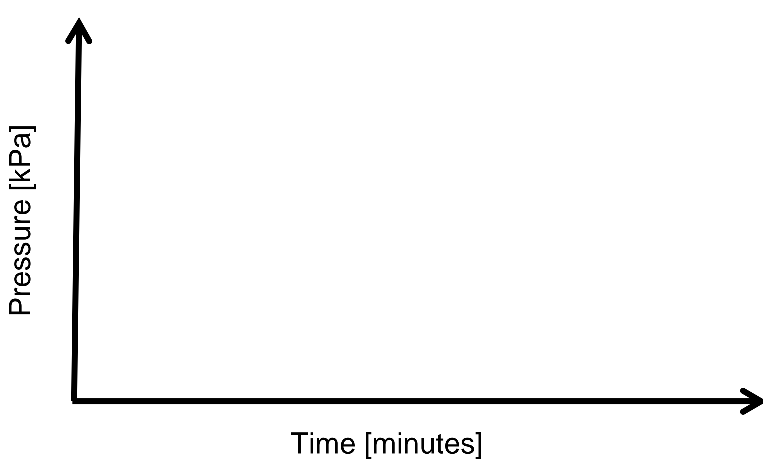


Summary questions

1. How did the number of yeast drops affect the initial rate of the reaction? Explain.

2. How did the temperature affect the initial rate of the reaction? Explain.

3. Draw a graph of pressure versus time for the reaction you have studied today. Include the part that should come after the initial rate part (after the linear part of the graph). What causes the rate to change?



4. Give an example of another enzyme and describe its functionality.