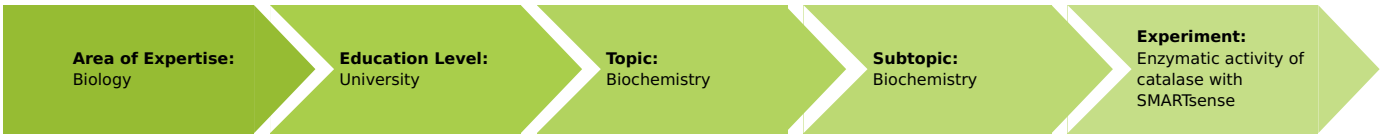
Enzymatic activity of catalase with Cobra SMARTsense (Item No.: P4120669)

Curricular Relevance



Difficulty	Preparation Time	Execution Time	Recommended Group Size
Intermediate	10 Minutes	50 Minutes	2 Students

Additional Requirements:

- Ice cubes
- Water boiler
- Distilled water
- Small piece of chicken liver
- Android tablet or iPad
- PHYWE measure App

Experiment Variations:

- with Computer with USB port, Windows

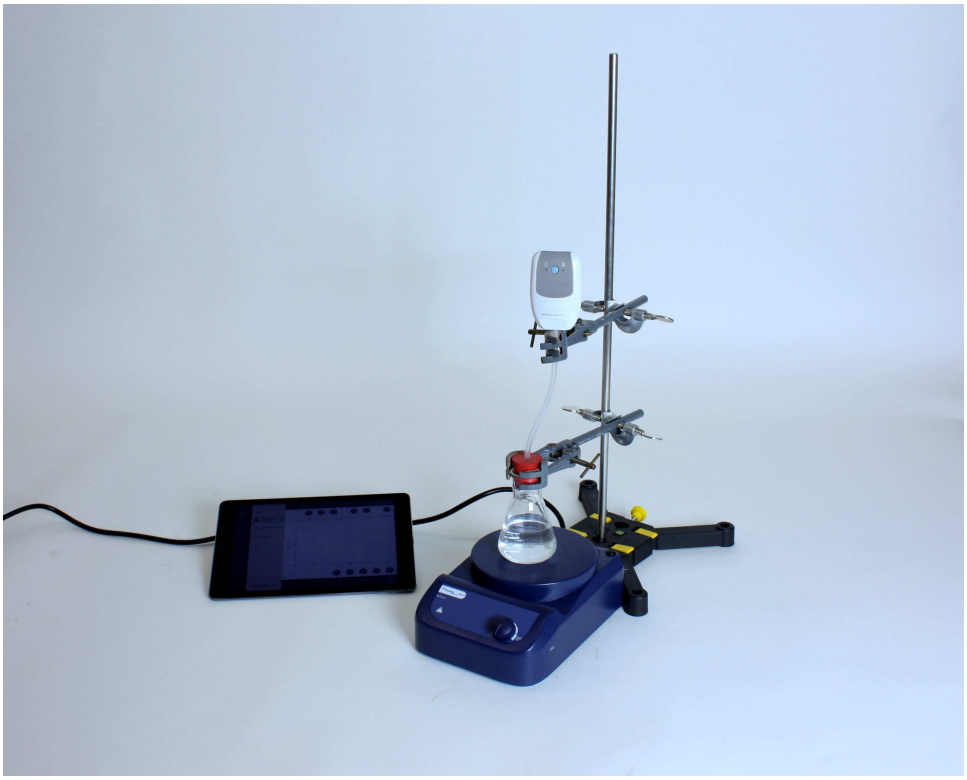
Keywords:

Enzyme catalase, Decomposition of H_2O_2, Poisonous by-product of cell respiration, Influence of temperature and pH on enzymatic activity

Information for teachers

Prinzip

Catalase is an enzyme that – in humans – is found predominantly in the liver and erythrocytes. It decomposes hydrogen peroxide (H_2O_2), which is a toxic by-product of cellular respiration, to form oxygen (and water). This allows to measure enzyme activity by measuring the increase of pressure in an air-tight reaction vessel.



Equipment

Position No.	Material	Order No.	Quantity
1	Cobra SMARTsense - Pressure, 20 ... 400 kPa	12905-00	1
4	Support base, variable	02001-00	1
5	Support rod, stainless steel, 500 mm	02032-00	1
6	Boss head	02043-00	2
7	Universal clamp with joint	37716-00	1
8	digital magnetic stirrer with heating, stainless steel, 280 °C, 100-1500 rpm	FHO-RSM10HS	1
9	Magnetic stirring bar, 50 mm, cylindrical	46299-03	1
10	Erlenmeyer flask, narrow neck, PN 29	36424-00	1
11	Rubber stopper 26/32, 1 hole 7 mm	39258-01	1
12	Glass tube, straight, l=80 mm, 10/pkg.	36701-65	1
13	Rubber tubing, i.d. 6 mm	39282-00	1
14	Graduated cylinder 100 ml	36629-00	2
15	Mortar with pestle, 150 ml, porcelain	32604-00	1
16	Sieve, fine mesh, d=60 mm	40968-00	1
17	Graduated pipette, 1 ml	36595-00	1
18	Graduated pipette 10 ml	36600-00	2
19	Beaker, high, BORO 3.3, 250 ml	46027-00	2
20	Test tubes 100x12 mm,FIOLAX,100pc	36307-10	1
21	Glycerol 99% 100 ml	30084-10	1
22	Dropping bottle,plastic,50ml	33920-00	1
23	Hydrogen peroxide, 30%, 250 ml	31710-25	1
24	Hydrochloric acid, 1.0 mol/l, 1000 ml	48454-70	1
25	Caustic soda solution, 1.0 m, 1000 ml	48329-70	1
Additional material:			
	Android tablet or iPad		
	PHYWE measure App		
	Ice cubes		
	Water boiler		
	Distilled water		
	Small piece of chicken liver		

Safety and disposal

Safety information

Depending on the concentration, sodium hydroxide solutions have a strong corrosive or irritating effect on the skin, eyes, and mucous membranes. Sodium hydroxide fog irritates the respiratory organs. Chemical burns lead to the destruction of tissue and severe pain. Keep away from children.

Depending on the concentration, hydrochloric acid has a strong corrosive or irritating effect. Hydrochloric acid fog irritates the respiratory organs, in particular the mucous membranes and upper respiratory tract. Concentrated acids destroy the skin and textiles.

Do not inhale any vapours (fog). Avoid contact with the skin. Wear suitable protective clothing, gloves, and goggles when working with these substances.

First aid: Immediately flush the skin with plenty of water. If the eyes are affected, flush them immediately with plenty of water while keeping the eyes open. In the event of eye injuries, seek medical attention immediately. In the event of an accident or if the affected person does not feel well, seek medical attention immediately. Inhalation: Provide fresh air and keep the respiratory tracts clear. If breathing proves difficult, transport the affected person in a semi-sitting position to a doctor.

Disposal: Dilute the solution with water, neutralise it (pH 6-8), and flush it away.

Since a considerable amount of pressure builds during the experiment, we recommend wearing protective goggles.

Tasks

1. To examine the enzymatic decomposition of hydrogen peroxide, a cell respiratory poison, in the liver.
2. To investigate the influence of the temperature and pH on the metabolic activity.

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Overview

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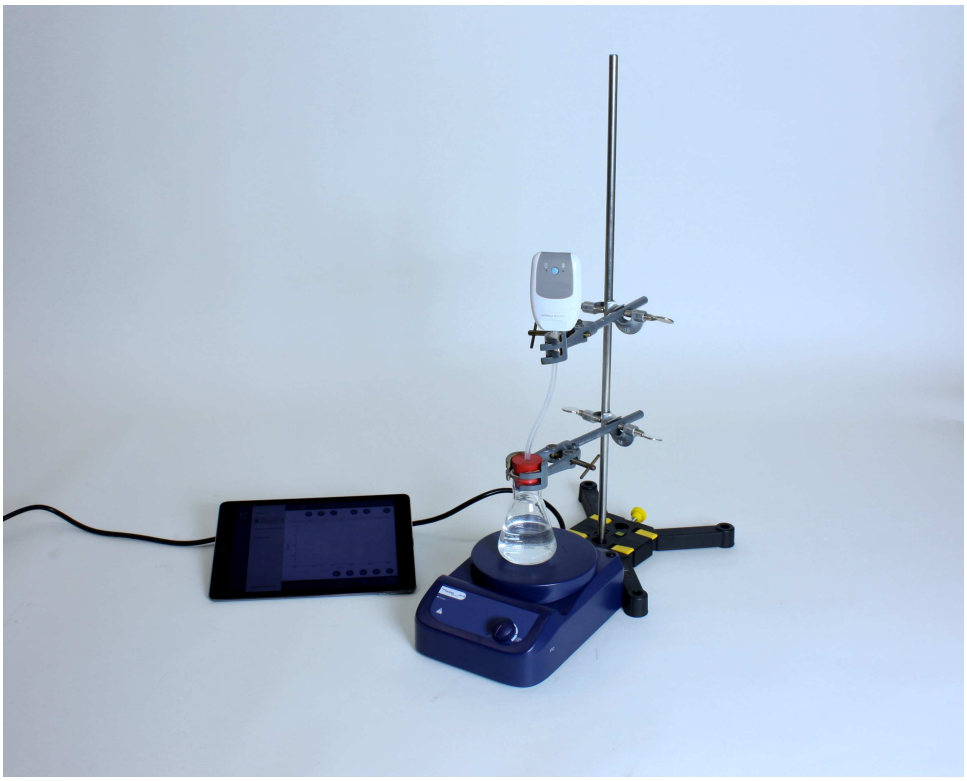

Fig. 1: Experimental Setup

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Set-up and procedure

Set-up

- Set up the equipment as shown in Fig. 1.
- Turn on the Cobra SMARTsense by pressing the power button. Ensure that Bluetooth is activated on your device.
- Place the Erlenmeyer flask on the magnetic stirrer and position it below the pressure module with the aid of the universal clamp and the bosshead. Screw the glass tube into the rubber stopper with the aid of some glycerol. Then, connect the pressure module to the glass tube. Ensure that the rubber tube that is used for the connection is as short as possible.
- Open the PHYWE measure App and select the sensor Sensor "Pressure".

Fig. 2: Selecting the sensor "Pressure" in measure APP

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Procedure

Put a small piece of liver (if necessary, chop it beforehand) into the mortar and add some distilled water. Crush it with the pestle and fill the juice via a sieve into the beaker.

Experiment 1:

- Prepare a hydrogen peroxide solution of nearly 0.5%. To do so, prepare a 3% hydrogen peroxide solution (10 ml of 30% H_2O_2solution and 90 ml of distilled water). Then, fill 15 ml of the 3% solution into a graduated 100 ml cylinder and top it up with distilled water up to the 100 ml mark.
- Pour the solution into the Erlenmeyer flask, insert the stirring bar, and place the flask on the magnetic stirrer.
- Adjust a low stirring level and start the measurement (runtime about 150 s; Stop:).
- Add 1 ml of the liver juice and immediately seal the Erlenmeyer flask with the rubber stopper.

Experiment 2a+b:

- The execution is the same as for experiment 1. This time, however, 10 ml of the sodium hydroxide solution (1 mol/l) or 10 ml of the hydrochloric acid solution (1 mol/l) must also be added.

Experiment 3a+b:

- The execution is the same as for experiment 1. This time, however, the liver juice must be filled into a test tube, which is then placed into a beaker with cold (ice cubes) or boiling water for 5 minutes prior to the experiment.

Observation and results

- **Experiment 1:** During the first experiment, the pressure curve rises drastically (Fig. 3). (In the course of the measurement, the curve suddenly drops perpendicularly, which is due to the fact that the rubber stopper was pushed out of the Erlenmeyer flask.)

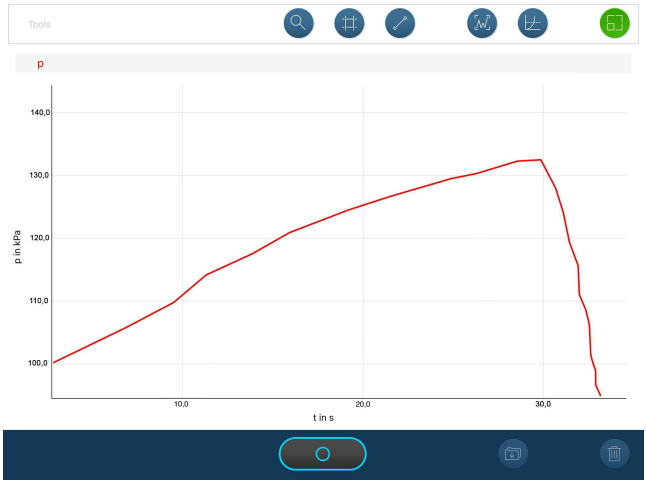

Fig. 3: Measurement result under normal conditions

- **Experiment 2a:** When sodium hydroxide is added, the curve rises more slowly compared to the curve under normal conditions (Fig. 4).

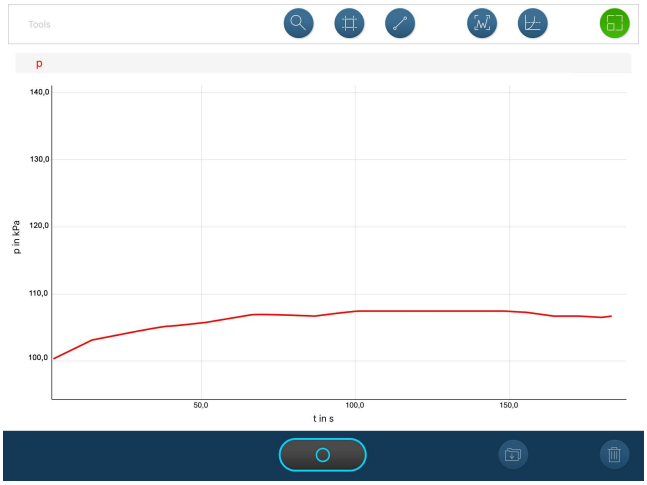

Fig. 4: Measurement result (base)

- **Experiment 2b:** When hydrochloric acid is added, the pressure curve does not rise at all (Fig. 5).

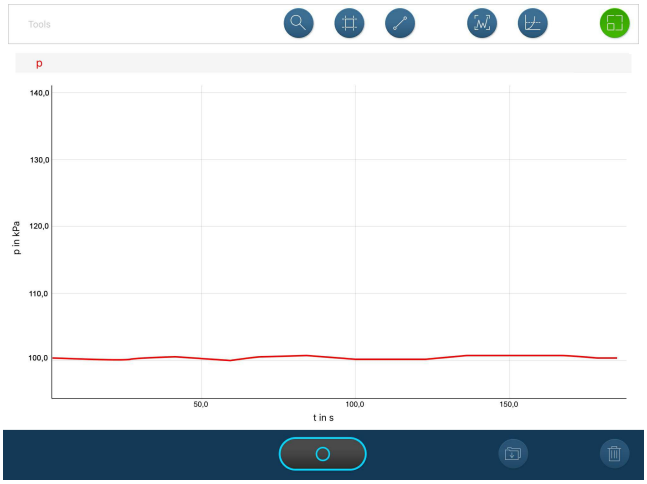

Fig. 5: Measurement result (acid)

- **Experiment 3a:** After an approximately 5-minute-long ice bath, the pressure increases nearly as quickly as when under normal conditions (Fig. 6). (In the course of the measurement, the pressure suddenly drops, which is due to the fact that the rubber stopper was pushed out of the Erlenmeyer flask.)

Fig. 6: Measurement result (cold)

- **Experiment 3b:** After an approximately 5-minute-long heat treatment, the pressure inside the Erlenmeyer flask remains constant (Fig. 7).

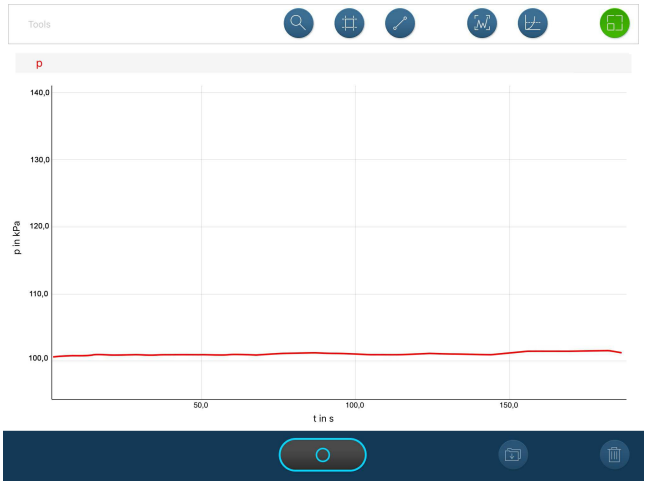

Fig. 6: Measurement result (heat)

Note

- Catalase is an enzyme that – in humans – is found predominantly in the liver and erythrocytes. It decomposes hydrogen peroxide (H_2O_2), which is a toxic by-product of cellular respiration, into water and oxygen. If one mixes, for example, blood with H_2O_2, one can see the resultant oxygen bubbles.
- Enzymes depend on the pH value. Experiment 2 shows that catalase prefers the neutral pH range rather than the alkaline or even acidic pH ranges as the enzyme is sensitive to an acid environment and stops its activity.
- Enzymes consist of proteins. Proteins denature at high temperature (catalase as of approximately 40°C). This is why the pressure does not increase any further after the 5-minute heat treatment in experiment 3b. The proteins of the enzymes were destroyed by the heat. A cold shock, on the other hand, inactivates the catalase only temporarily. Once the temperature has risen, the enzymes resume their normal activity.