Enzyme inhibition (poisoning of enzymes) with Cobra4 (Item No.: P4120560)

Curricular Relevance

Area of Expertise: Biology
Education Level: University
Topic: Biochemistry
Subtopic: Biochemistry
Experiment: Enzyme inhibition (poisoning of enzymes) with Cobra4

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

Additional Requirements:

- Android tablet or iPad
- PHYWE measure App

Experiment Variations:

- with Computer with USB port, Windows

Keywords:

Poisoning of enzymes, Enzymatic hydrolysis of urea, Silver nitrate inhibition of urease, Conductivity measurement

Information for teachers

Principle

The enzymatic hydrolysis of urea in aqueous solution liberates carbon dioxide and ammonia. The ions of these compounds increase the conductivity of the solution. Conductivity measurements can therefore be used to determine the rate of hydrolysis of urea by the enzyme urease. The enzyme can be poisoned by the addition of an inhibitor, whereby no further conversion of substrate takes place.

Fig. 1: Experiment set-up

Equipment

Experiment with Cobra4 Wireless/USB-Link with android tablet or iPad

Position No.	Material	Order No.	Quantity
2	Cobra4 Wireless/USB-Link incl. USB cable	12601-10	1
3	Cobra4 Sensor-Unit Conductivity+	12632-00	1
4	Conductivity temperature probe Pt1000	13701-01	1
5	digital magnetic stirrer with heating, stainless steel, 280 °C, 100-1500 rpm	FHO-RSM10HS	1
6	Magnetic stirring bar 30 mm, cylindrical	46299-02	1
7	Separator for magnetic bars	35680-03	1
8	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
9	Retort stand, h = 750 mm	37694-00	1
10	Boss head	02043-00	1
11	Universal clamp	37715-00	1
12	Beaker, high, BORO 3.3, 100 ml	46026-00	8
13	Beaker, low, BORO 3.3, 250 ml	46054-00	1
14	Volumetric pipette, 1 ml	36575-00	1
15	Volumetric pipette, 20 ml	36579-00	1
16	Pipettor	36592-00	1
17	Micro-l syringe, 100 micro-l	02606-00	1
18	Microspoon, steel	33393-00	1
19	Wash bottle, plastic, 500 ml	33931-00	1
20	Urea, 250 g	30086-25	1
21	Urease soln.in 50% glycerol,10ml	31924-03	1
22	Silver nitrate, cryst. 15 g	30222-00	1
23	Water, distilled 5 l	31246-81	1
24	USB charger for Cobra4 Mobile-Link 2 and Wireless/USB-Link	07932-99	1
Additional material:			
	Android tablet or iPad		
	PHYWE measure App		

Android iPad

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Experiment with Cobra4 Wireless/USB-Link and PC

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Tasks

- Use conductivity measurements to determine the rate of hydrolysis of urea by the enzyme urease.
- The enzyme can be poisoned by the addition of an inhibitor, whereby no further conversion of substrate takes place.

Safety information

Safety precautions

Silver nitrate ("lapis infernalis") is a solid with a highly corrosive action. Dust from it irritates the respiratory system. Silver nitrate liberates nitric acid under the influence of moisture and light. The silver that is then also liberated causes typical black stains. In the case of fire, silver nitrate acts as fire promoter.

Do not breathe dust. Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye protection. Store dry and closed to light. Keep out of reach of children.

First aid: In the case of contact with skin, eyes, rinse skin and/or open eyes immediately with plenty of water. In the case of harm to eyes seek medical advice immediately. In the case of accidents or if you feel unwell seek medical advice immediately. After inhalation: Fresh air, keep respiratory tract free. On difficult breathing: Transport to doctor in semi-sitting position.

Waste disposal: Collect solutions that contain silver ions in a correspondingly labelled, separate container and pass for recycling or suitable waste disposal.

Enzyme inhibition (poisoning of enzymes) with Cobra4 **(Item No.: P4120560)**

Overview

Tasks

- Use conductivity measurements to determine the rate of hydrolysis of urea by the enzyme urease.
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Fig. 1: Experiment set-up

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Set-up and procedure

Set-up

Preparatory work

Prepare the solutions required for the experiment as follows:

- 6% Urea solution: Weigh 2.00 g of urea in a 250 ml beaker and dissolve it in 123.00 g of distilled water (always prepare this fresh before starting the experiment)
- 1% Silver nitrate solution: Weigh 0.50 g of silver nitrate and dissolve it in 49.50 g of distilled water.

Note: The urease solution must be kept stored in a refrigerator.

Set-up and procedure

- Set up as in Fig. 1.
- Fasten the universal clamp to the retort stand with the right angle clamp. Fix the conductivity probe by means of the universal clamp.
- Connect the conductivity/temperature probe to the appropriate input of the Cobra4 Sensor-Unit "Conductivity+". Connect the Cobra4 Sensor-Unit "Conductivity+" with the Wireless/USB-link and switch it on .

  Connect your tablet via WiFi with the Wireless/USB-link (maximum range 50m).
  Open the PHYWE measure App and select the **Sensor** "Conductivity+".

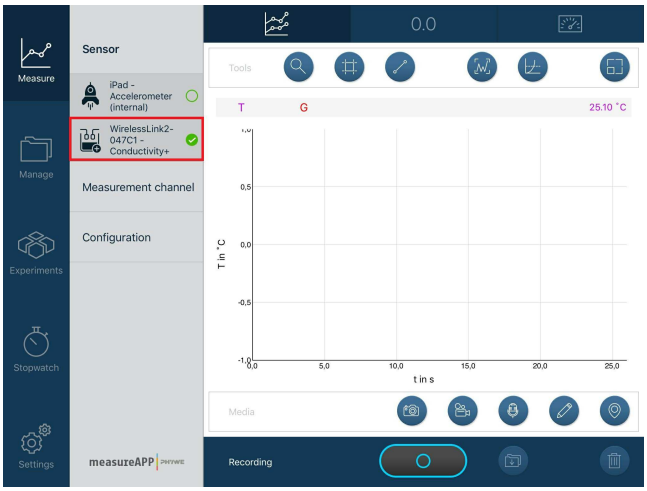

Fig. 2: Select the sensor "Conductivity+"

- The electrical conductance will be measured.

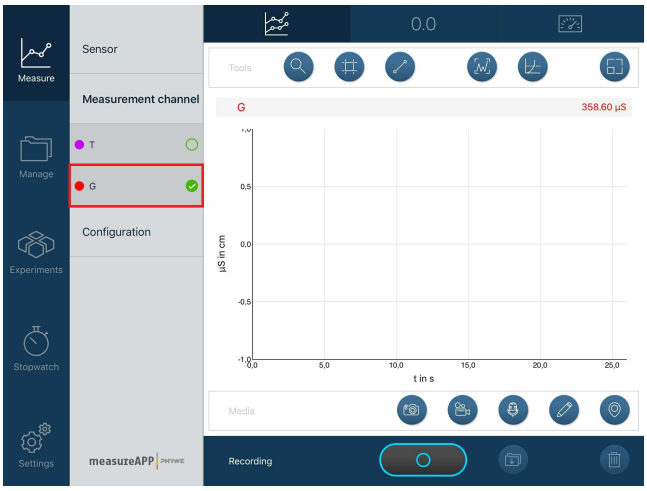

Fig. 3: Measurement of electrical conductance

Procedure

- Use the 20 ml volumetric pipette twice to pipette 40 ml of the 1.6% urea solution into a 100 ml beaker and add a magnetic stirrer bar.
- Place the beaker on the magnetic stirrer and immerse the conductivity electrode in the solution.
- Regulate the stirrer to a middle stirring speed (*Caution:* Do not let the magnetic stirrer bar hit against the conductivity electrode!).
- Add 50 µl of the urease solution with the microsyringe and start measurement without delay .
- Watch the course of the reaction over time on the monitor.
- After 300 seconds reaction time, add 1 ml of the 1% silver nitrate solution.
- Wait 600 seconds and stop the measurement .
- When measurement has stopped, save the data in the measure menu under .

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Result and evaluation

Results and evaluation

Figure 4 shows an example of a graph of conductivity against time that was recorded during the course of measurement.

Fig. 4: Conductivity-time graph of the hydrolysis of urea by urease before and after poisoning

Urease acts on urea to decompose the substrate into carbon dioxide and ammonia. Because of the ions then formed in the aqueous solution, the course in time of the reaction as it proceeds can be followed by making conductivity measurements. During the first 300 seconds the conductivity continually increases due to the enzymatic activity. The addition of silver nitrate solution results in a drastic increase in the conductivity because of the silver and nitrate ions. Following this, however, the graph shows no further increase. The reason for this is the irreversible inhibition of the urease by the silver ions, i.e. the enzyme has been poisoned. There is so no further substrate conversion.

Overview

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- Use conductivity measurements to determine the rate of hydrolysis of urea by the enzyme urease.
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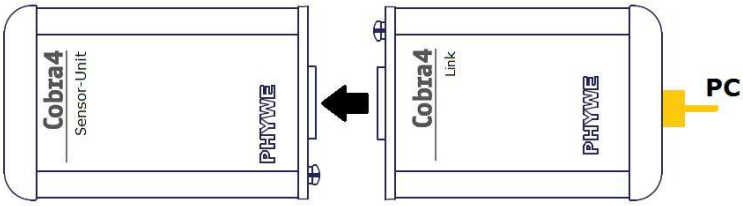

Fig. 2: Cobra4 Sensor-Unit with Cobra4 Link in USB mode

- Connect your PC with the Wireless/USB-link (via WiFi or via cabel in USB-mode).
  Start PHYWE measureLAB and switch on the Cobra4 Wireless-Link . The sensor is detected automatically.

  Boot the experiment “P4120560” (experiment > open experiment). The measurement parameters for this experiment are loaded now.

For calibration: Pour some standard solution into a beaker and immerse the well-rinsed probe into the solution (*Advice:* Both platinum electrodes of the probe have to be covered completely with the solution).
Go to and then click on Sensors/channels and select "Conductivity σ". To perform the calibration, click on the calibration button (cf. Fig. 3).
Enter the corrected value for the conductivity at a given temperature (cf. Fig. 4). You can find this value on the label of the standard solution (at 25 °C with $C = 1413\ \mu S/\mathrm{cm}$) Click the “Apply corrected value” button and finish the calibration with “OK”.

Fig. 3: Conductivity+ Settings

Fig. 4: Calibration of the Conductivity+ Sensor

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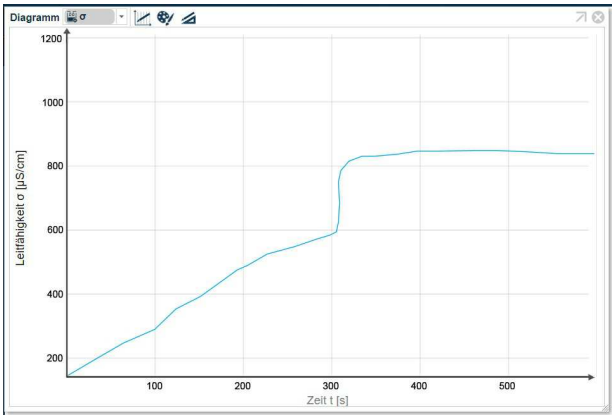

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