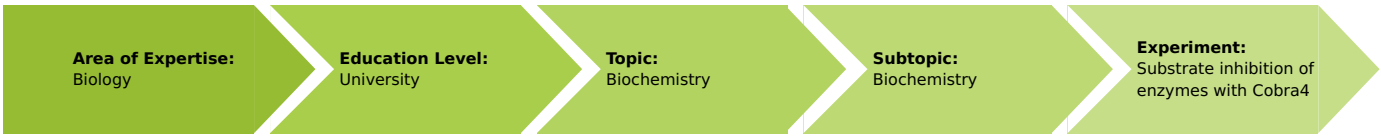
Substrate inhibition of enzymes with Cobra4

(Item No.: P4120460)

Curricular Relevance



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

Additional Requirements:

- Paper tissues
- Android tablet or iPad
- PHYWE measure App

Experiment Variations:

- with Computer with USB port, Windows

Keywords:

Substrate inhibition, Enzymolysis of urea, Conductivity-time plot, Reaction velocity of enzymatic hydrolysis

Overview

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 measure the rate of hydrolysis of urea by the enzyme urease at different substrate concentrations. Substrate inhibition occurs at excessive substrate concentrations.

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Fig. 1: Experiment set-up

Equipment

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

Position No.	Material	Order No.	Quantity
1	Cobra4 Wireless/USB-Link incl. USB cable	12601-10	1
2	Cobra4 Sensor-Unit Conductivity+	12632-00	1
3	Conductivity temperature probe Pt1000	13701-01	1
4	digital magnetic stirrer with heating, stainless steel, 280 °C, 100-1500 rpm	FHO-RSM10HS	1
5	Magnetic stirring bar 30 mm, cylindrical	46299-02	1
6	Separator for magnetic bars	35680-03	1
7	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
8	Retort stand, h = 750 mm	37694-00	1
9	Boss head	02043-00	1
10	Universal clamp	37715-00	1
11	Beaker, high, BORO 3.3, 100 ml	46026-00	8
12	Beaker, low, BORO 3.3, 250 ml	46054-00	1
13	Erlenmeyer flask 100 ml, narrow neck, PN 19	36418-00	7
14	Rubber stopper, d=22/17 mm, without hole	39255-00	7
15	Volumetric pipette, 20 ml	36579-00	1
16	Volumetric pipette, 50 ml	36581-00	1
17	Pipettor	36592-00	1
18	Micro-l syringe, 100 micro-l	02606-00	1
19	Microspoon, steel	33393-00	1
20	Wash bottle, plastic, 500 ml	33931-00	1
21	Urea, 250 g	30086-25	1
22	Urease soln.in 50% glycerol,10ml	31924-03	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		
	Paper tissues		

Android iPad

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Experiment with Cobra4 Wireless/USB-Link and PC

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Position No.	Material	Order No.	Quantity
1	curricuLAB measureLAB	14580-61	1
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
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25	USB charger for Cobra4 Mobile-Link 2 and Wireless/USB-Link	07932-99	1
Additional material:			
	Computer with USB port, Windows		
	Paper tissues		

Task

- Investigate the inhibition of the enzyme by the substrate at excessive substrate concentrations.

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Set-up and procedure

Preparatory work

Urea solutions of various concentrations are required for this experiment. They are always to be freshly made by means of a dilution series before the start of the experiment:

- 1.6% Urea solution (urea stock solution): Weigh 2.00 g of urea in a 250 ml beaker and dissolve it in 123.00 g of distilled water.
- 0.8% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 1.6% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.
- 0.4% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 0.8% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.
- 0.2% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 0.4% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.
- 0.1% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 0.2% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.
- 0.05% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 0.1% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.
- 0.025% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 0.05% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.
- 0.0125% Urea solution: Use the 50 ml pipette to pipette 50 ml of the 0.25% urea solution in a 100 ml Erlenmeyer flask and add 50 ml of distilled water.

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

Collecting the measurement data

- Set up the experiment as shown in Fig. 1.
- Fasten the universal clamp to the retort stand with the right angle clamp.
- Plug the Cobra4 Sensor-Unit Conductivity+ to the Cobra4 Wireless/USB-Link.
- Connect the conductivity/temperature probe to the appropriate input of the Cobra4 Sensor-Unit Conductivity+.
- Fix the conductivity probe with the universal clamp.
- Depending on the software you are using you can acquire the data either on a computer or on a tablet:
  - If you use the PC: Set up a connection of the Cobra4 Wireless/USB-Link to the PC either wirelessly or with the USB cable and switch it on.
  - If you use a tablet: Connect the Cobra4 Wireless/USB-Link to the tablet in the wireless WiFi mode after switching it on.
- Start the software (measureLAB or measureAPP). The Cobra4 measuring device will be automatically recognized.
- Set up the software for the measurement. In measure APP, chose the sensor Conductivity+ in the sensor list. In measureLAB, select the measurement window of your choice.
  For both software versions, it is useful to choose the measurement graph.

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Fig. 2: Choosing the sensor in measure APP

- Use the 20 ml volumetric pipette twice to pipette 40 ml of the 0.0125% urea solution into a 100 ml beaker and add a magnetic stirring 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 finishing this measurement and each of the following measurements, save the data so that it can be evaluated when all measurements have been made.
- Use this procedure to make measurement on each of the other solutions in the succession of increasing concentration.
- First, however, now and after each of the following measurements, take the beaker from the magnetic stirrer and use the removal rod to take the magnetic stirrer bar out of the solution in the beaker.
- Rinse the magnetic stirrer bar thoroughly with distilled water, briefly dry it with a paper tissue and put it into the next solution to be measured.
- Also rinse the conductivity probe thoroughly with distilled water before using it for the next solution.

Compiling the data for data analysis

Determine and record the conductivity values after reaction times of 100 s and 200 s as well as their difference for all eight measurements that were carried out. This can best be done using a spreadsheet program.

Result and evaluation

Results and evaluation

A higher excess of substrate leads to a reversible inhibition of enzymes, i.e. the reaction rate does not further increase with increasing substrate concentration but remains constant or even decreases, because the substrate molecules spatially hinder each other (“Substrate inhibition”).

The average rates of enzymolysis between times of 100 and 200 s after the start are required for evaluation. These are determined by taking the difference between the conductivity values after 100 and 200 seconds (ΔY) in each case and dividing them by 100 s. When these rates (in $\mu S \cdot cm^{-1} \cdot s^{-1}$) are plotted against the concentration of urea (in mmol/L) (see Fig. 4), the substrate concentrations c_s (in mmol/L) can be calculated from the percentage concentration values according to the equation:

$$c_s = (W \cdot 10000)/M$$

where:

W = Concentration of the urea solution in %
M = Molar mass of urea = 60.06 g/mol = 60,06 g/mol

The graphical evaluation is conducted with a suitable software, e.g. the free PHYWE software measure. It allows an estimation of the concentration at which substrate inhibition starts to occur (graph maximum). The reaction rate reaches a maximum when the urea has a concentration of about 40 mmol/L, after which substrate inhibition occurs (Fig. 3).

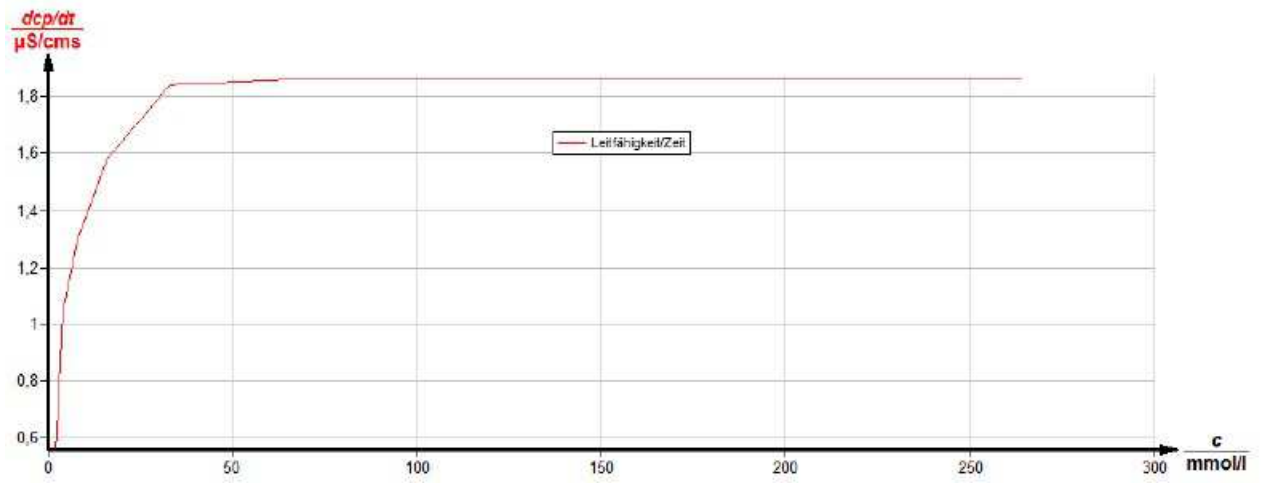

Fig. 3: The dependence of the rate of enzymolysis on the concentration

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- Connect the conductivity/temperature probe to the appropriate input of the Cobra4 Sensor-Unit Conductivity+.
- Fix the conductivity probe with the universal clamp.
- Set up a connection of the Cobra4 Wireless/USB-Link to the PC either wirelessly or with the USB cable and switch it on.
- Start the software . The Cobra4 measuring device will be automatically recognized.
- Choose the experiment from the start screen by selecting `Load Experiment`. Accordingly, choose “PHYWE experiments”, search for “P4120460”, and select desired folder containing the experiment. All necessary presetting will be loaded.
- Use the 20 ml volumetric pipette twice to pipette 40 ml of the 0.0125% urea solution into a 100 ml beaker and add a magnetic stirring 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!).
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$$c_s = (W \cdot 10000)/M$$

where:

W = Concentration of the urea solution in %
M = Molar mass of urea = 60.06 g/mol = 60,06 g/mol

The graphical evaluation can be executed in measureLAB. Go to Datapool and generate two datasets under + for 'Average enzymolysis rate' and 'substrate concentration', respectively (c.f. Fig. 3 and Fig 4).

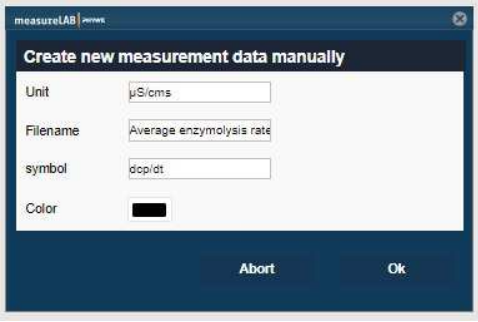

Fig. 3: Create data set for average enzymolysis rate

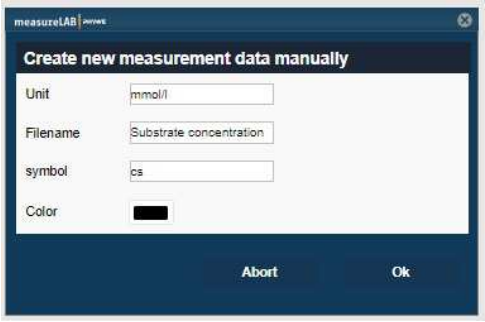

Fig. 4: Create data set for substrate concentration

After creation of data sets, close all tables and diagrams and go back to Datapool . Select both created datasets and choose the 'Diagram' option (c.f. Fig. 5). Following this, go to and select 'Substrate concentration' to be displayed as x-axis (c.f. Fig. 6).

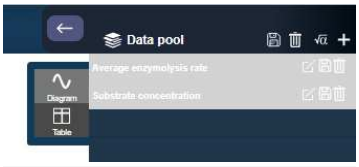

Fig. 5: Display diagram with generated data sets

Fig. 6: Select 'Substrate concentration' as X-Axis

The reaction rate reaches a maximum when the urea has a concentration of about 40 mmol/L, after which substrate inhibition occurs (Fig. 7).

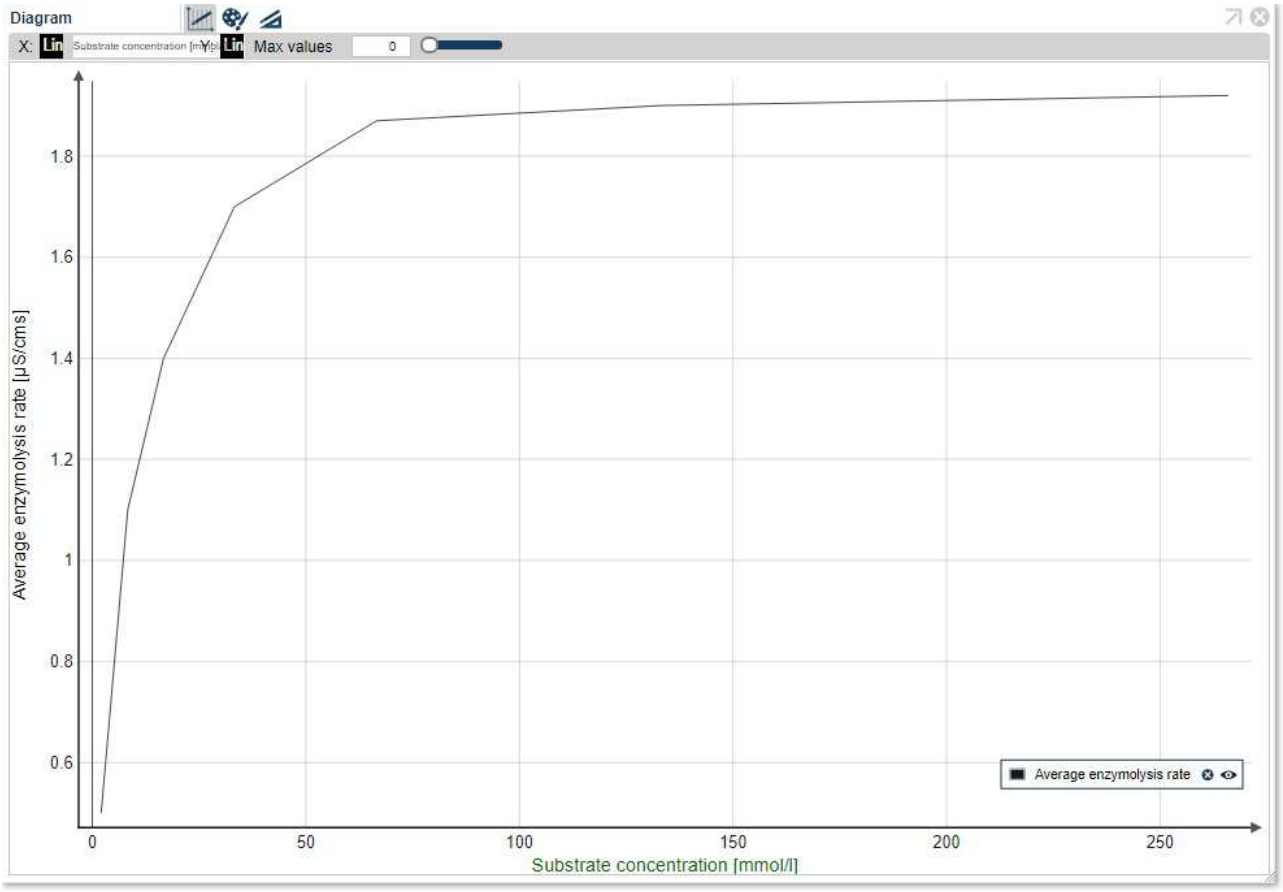

Fig. 7: The dependence of the rate of enzymolysis on the concentration