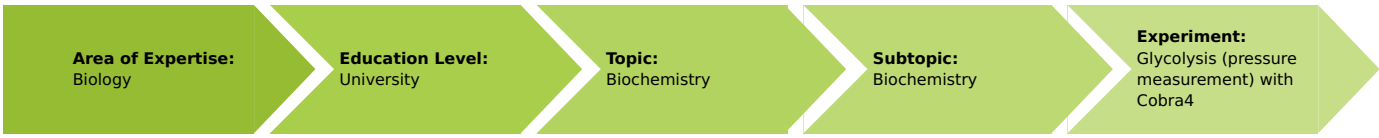
Glycolysis (pressure measurement) with Cobra4

(Item No.: P4110460)

Curricular Relevance



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

Additional Requirements:

- Grape juice
- Fresh baking yeast (Saccharomyces cerevisiae)
- Ice cubes
- Android tablet or iPad
- PHYWE measure App

Experiment Variations:

- with Computer with USB port, Windows

Keywords:

Glycolysis, Yeast fermentation of sugar, CO_2 pressure measurement, Influence of temperature and pH

Information for teachers

Principle

The aim of this experiment is to prove glycolysis by means of measuring the CO_2 production under various experimental conditions (temperature, pH).

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 Pressure, 7 bar absolute	12647-00	1
3	USB charger for Cobra4 Mobile-Link 2 and Wireless/USB-Link	07932-99	1
4	Holder for Cobra4 with support rod	12680-00	1
5	Support base, variable	02001-00	1
6	Support rod, stainless steel, 500 mm	02032-00	1
7	Boss head	02043-00	2
8	Universal clamp with joint	37716-00	1
9	digital magnetic stirrer with heating, stainless steel, 280 °C, 100-1500 rpm	FHO-RSM10HS	1
10	PT-1000 for RSM-10HS/HP	FHO-RSME320	1
11	Magnetic stirring bar, 50 mm, cylindrical	46299-03	1
12	Erlenmeyer flask, narrow neck, PN 29	36424-00	1
13	Beaker, low, BORO 3.3, 1000 ml	46057-00	1
14	Beaker, high, BORO 3.3, 250 ml	46027-00	2
15	Graduated pipette 10 ml	36600-00	1
16	Rubber stopper 26/32, 1 hole 7 mm	39258-01	1
17	Glass tube, straight, l=80 mm, 10/pkg.	36701-65	1
18	Rubber tubing, i.d. 6 mm	39282-00	1
19	Glass rod, boro 3.3, l=200mm, d=6mm	40485-04	1
20	Universal clamp	37715-00	1
21	Buffer solution tablets pH4, 100	30281-10	1
22	Buffer solution tablets pH10, 100	30283-10	1
23	Glycerol 99% 100 ml	30084-10	1
24	Dropping bottle,plastic,50ml	33920-00	1
25	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
Additional material:			
	Android tablet or iPad		
	PHYWE measure App		
	Grape juice		
	Fresh baking yeast (Saccharomyces cerevisiae)		
	Ice cubes		

Android iPad

GET IT ON Google Play Download on the App Store

Experiment with Cobra4 Wireless/USB-Link and PC

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25	Dropping bottle,plastic,50ml	33920-00	1
26	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
Additional material:			
	Computer with USB port, Windows		
	Grape juice		
	Fresh baking yeast (Saccharomyces cerevisiae)		
	Ice cubes		

Tasks

1. To identify glycolysis by measuring the production of CO_2 and plotting graphs.
2. To investigate the influence of temperature and pH on metabolic activity.

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Overview

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Fig. 1: Experiment set-up

Equipment

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Additional material:			
	Android tablet or iPad		
	PHYWE measure App		
	Grape juice		
	Fresh baking yeast (Saccharomyces cerevisiae)		
	Ice cubes		

Android iPad

Set-up and procedure

Set-up

- Set up the equipment as shown in Fig. 1.
- Connect the Cobra4 Sensor-Unit "Pressure" with the Wireless/USB-link.
  Fasten the Cobra4 Wireless Link to the support rod of the holder.
- 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.
- Switch Cobra4 Wireless/USB-link on ( ).
  Connect your tablet via WiFi with the Wireless/USB-link (maximum range 50m).
  Open the PHYWE measure App and select the sensor "Pressure" to measure pressure.

Fig. 2: Select the sensor "Pressure"

Procedure

Experiment 1

- Heat 150 ml of grape juice to 30-35°C.
- Weigh 10 g of baking yeast and fill it into the 250 ml beaker. Top it up with warm tap water up to the 100 ml mark and stir it with the glass rod.
- Pour the heated grape juice and 10 ml of the yeast suspension into the 250 ml Erlenmeyer flask and insert the stirring rod.
- Seal the Erlenmeyer flask with the rubber stopper, place it on the magnetic stirrer, and secure it in place with the universal clamp. Adjust a low stirring level and connect it to the Sensor-Unit Thermodynamics. Make sure the air intake nozzle of the Sensor-Unit is located above the flask.
- Start the measurement ( ) (runtime limited to 5000 s; Stop:).

Experiment 2: (for the procedure, see experiment 1)

- Fill the 1000 ml beaker half full with tap water. Place the Erlenmeyer flask into the beaker and add some ice cubes. Seal the Erlenmeyer flask with a stopper and start the measurement (runtime limited to 5000 s).

Experiment 3: (for the procedure, see experiment 1)

- Fill the 1000 ml beaker half full with hot tap water (try out different temperatures, e.g. 50/70/90°C).
- Place the Erlenmeyer flask into the beaker, seal it with a stopper, and start the measurement (runtime limited to 5000 s).

Experiment 4: (for the procedure, see experiment 1)

- Add different buffer solutions (e.g. 20 ml of a buffer solution with pH 4.01 or pH 10.01). To do so, put one buffer solution tablet into 20 ml of water.
- Seal the Erlenmeyer flask with a stopper and start the measurement (runtime limited to 5000 s).

Observation and results

- **Experiment 1 (normal conditions):** The curve rises significantly. After approximately 40 minutes, at a pressure of approximately 1150 hPa, the rubber stopper was pushed out of the Erlenmeyer flask (Fig. 3).

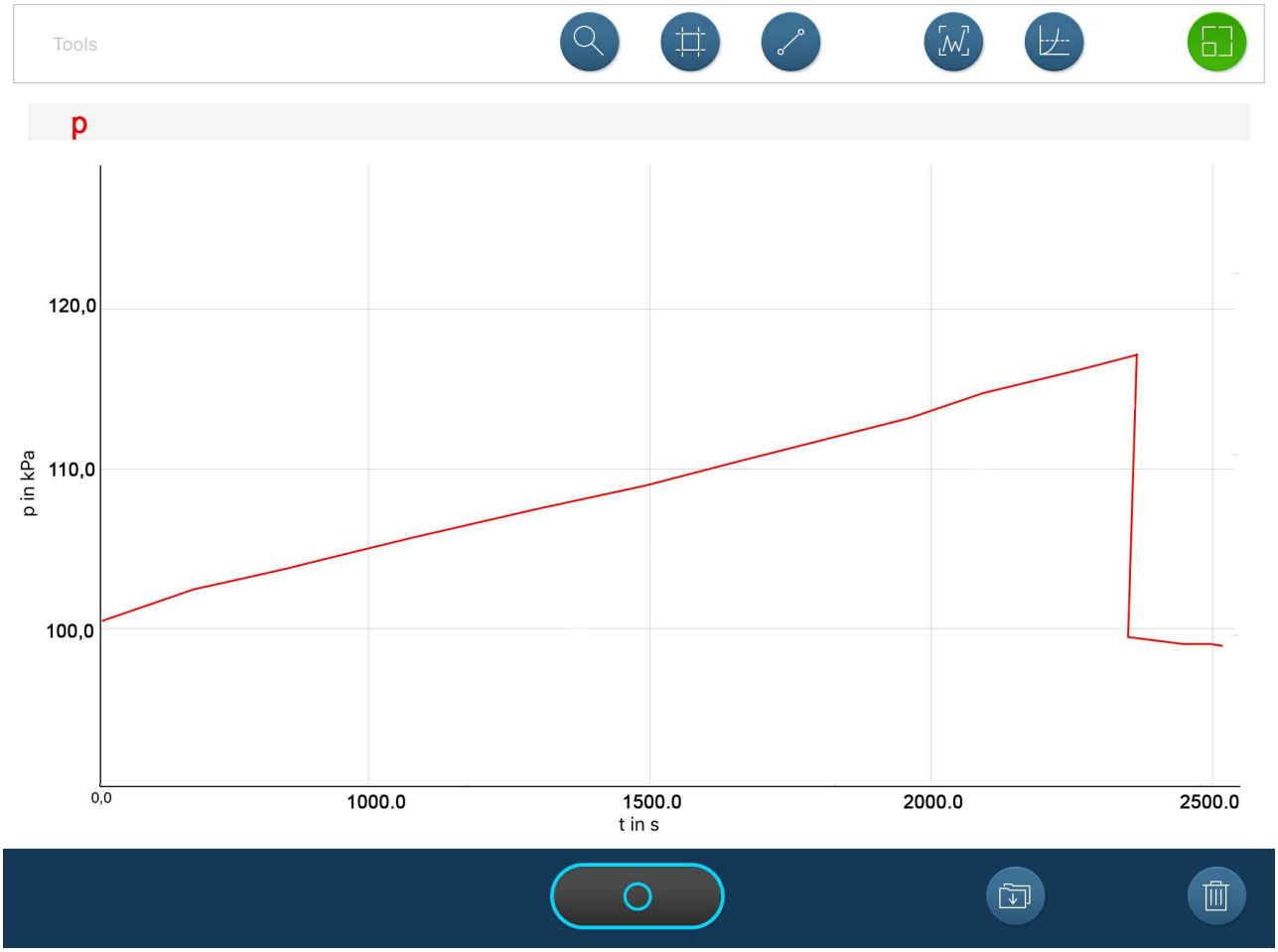

Fig. 3: Measurement result at room temperature

- **Experiment 2 (lowered temperature):** At first, the curve hardly increases, and then it increases more significantly, but not as pronounced as during the measurement at room temperature. After approximately 74 minutes, at a pressure of 1200 hPa, the rubber stopper was pushed out of the Erlenmeyer flask (Fig. 4).

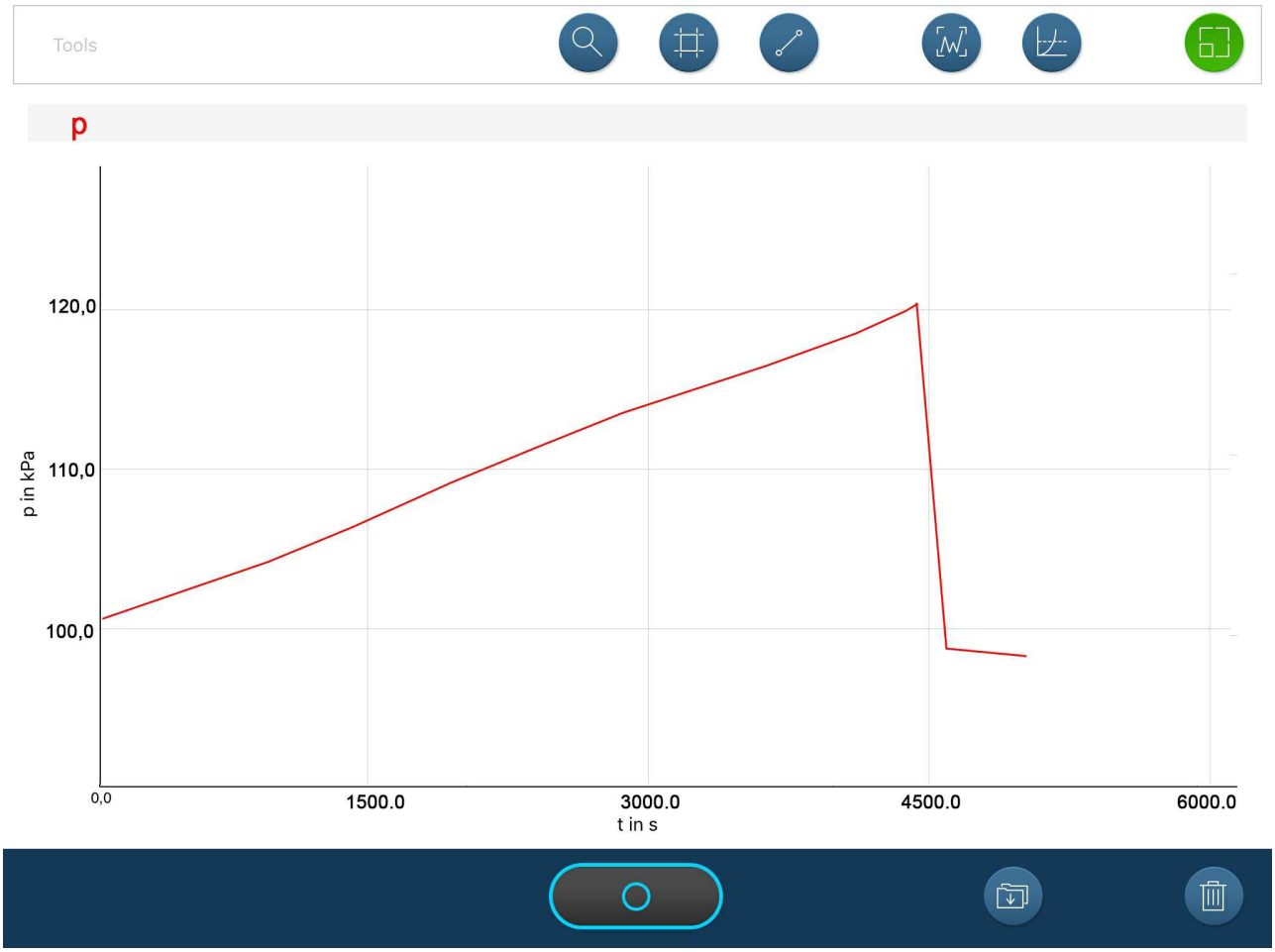

Fig. 4: Measurement result of the ice bath

- **Experiment 3 (raised temperature):** At first, the curve shows a negative slope before it recovers after approximately 17 minutes, which is followed by a strong rise (Fig. 5).

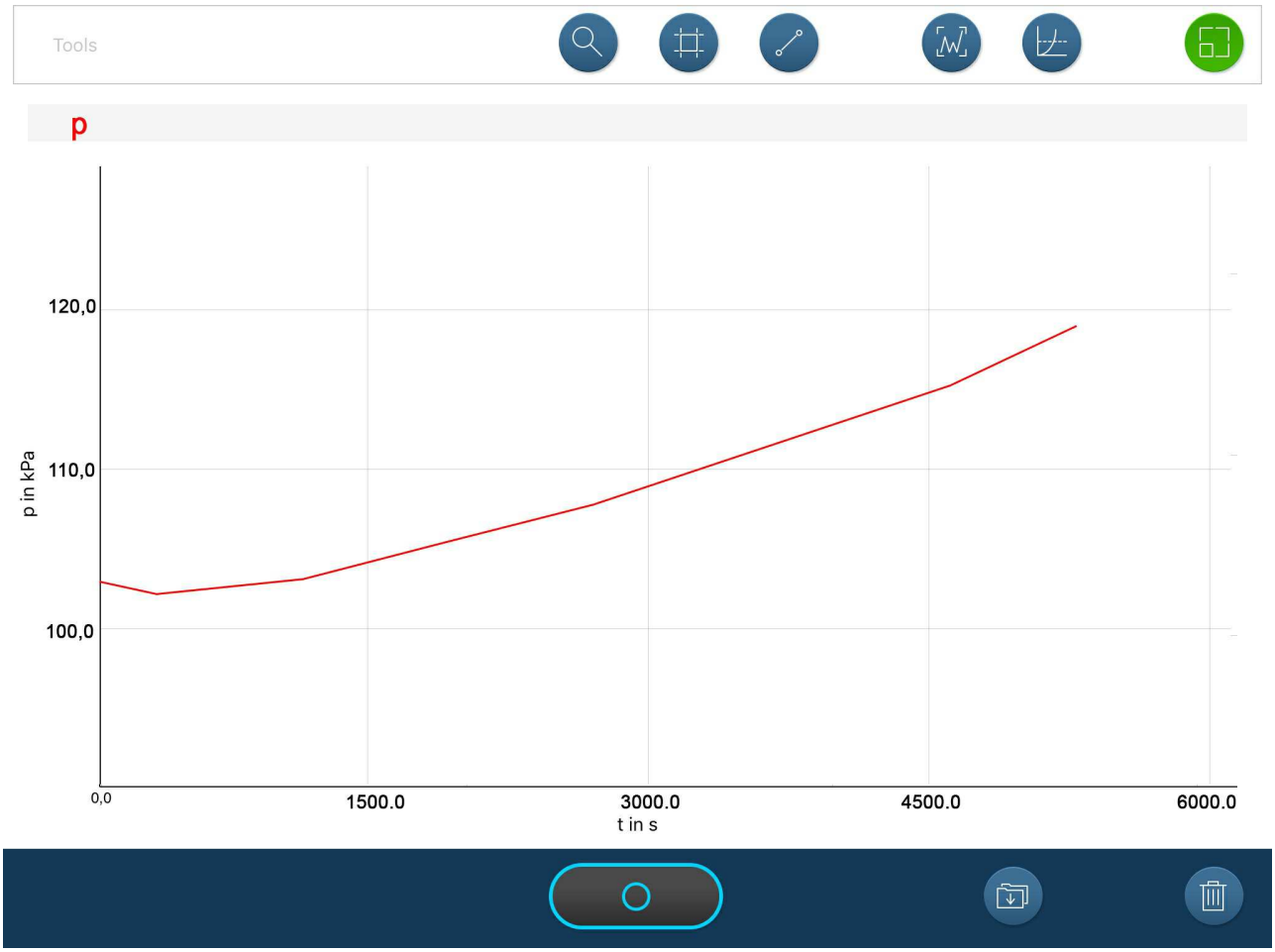

Fig. 5: Measurement result in the 70°C bath

- **Experiment 4 (pH of the nutrient medium lowered):** The curve rises drastically until the rubber stopper is pushed out of the Erlenmeyer flask after approximately 25 minutes at a pressure of approximately 1250 hPa (Fig. 6).

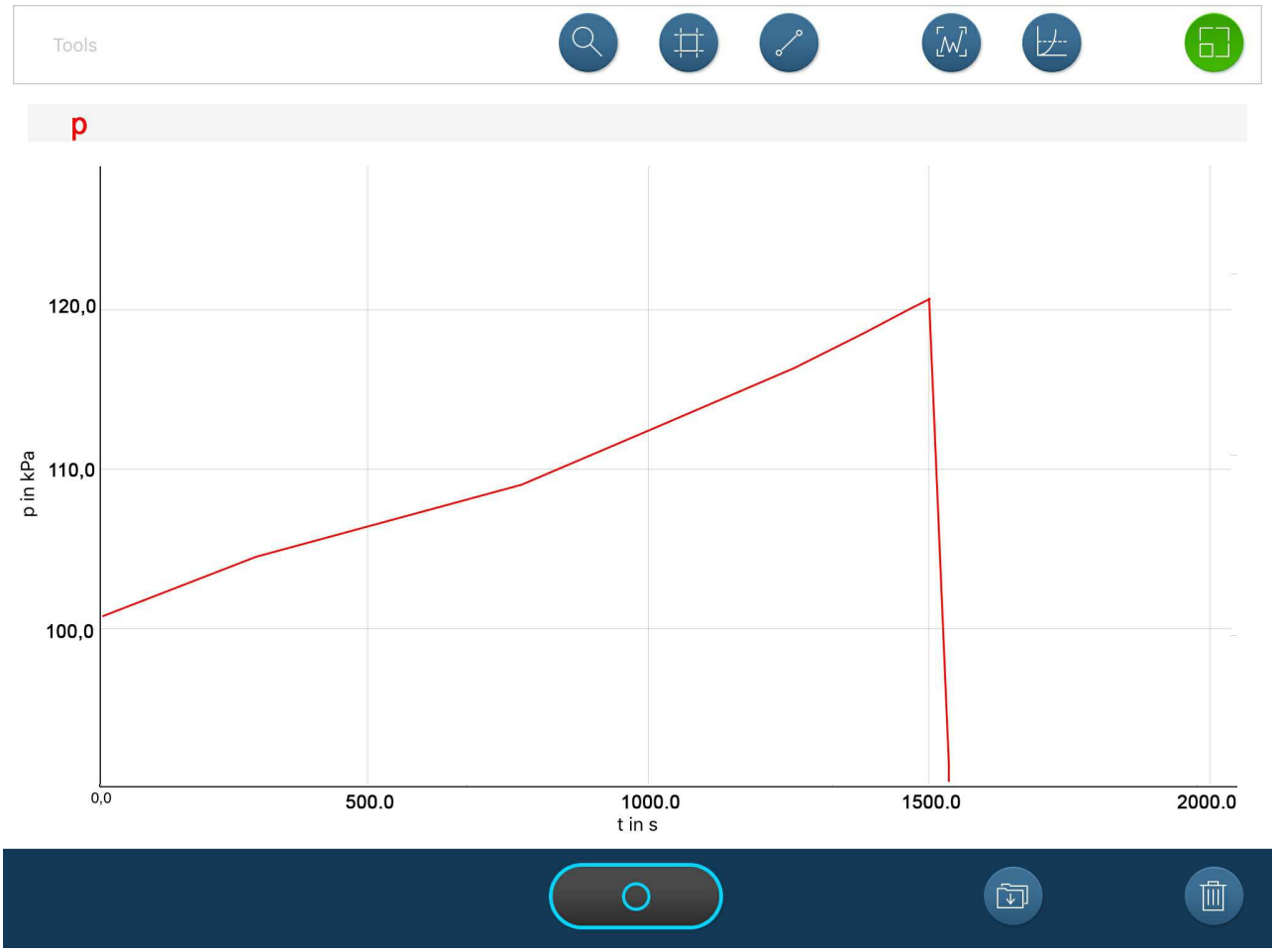

Fig. 6: Measurement result when an acid buffer solution is added

Notes

- In order to demonstrate the effects of the different experiment conditions, it is not necessary to let the partial experiments run until the rubber stopper is pushed out of the Erlenmeyer flask. An experiment duration of 5, or preferably 10, minutes for each of the partial experiments is sufficient.
- **Experiment 1:** Yeast ferments sugar. During this process, the glucose that is included in the grape juice undergoes glycolysis. During glycolysis, one glucose molecule is transformed into two pyruvate molecules in ten enzymatically catalysed reactions. A by-product of this reaction chain is CO_2.
- **Experiment 2:** Under the influence of cold, the metabolism of the yeast is slowed, which can be seen particularly clearly at the beginning of the experiment. Hardly any carbon dioxide is released. When the ice melts and the temperature rises slowly, the CO_2 production resumes. This experiment shows that glycolysis is temperature-dependent (Fig. 4).
- **Experiment 3:** When the temperature is too high, the metabolism of the yeast stops completely. If the temperature is above 45°C for a long time, the yeast dies, or the enzymes involved in glycolysis are inactivated. On the other hand, temperatures that are in the optimum range of enzymatic activity (approx. 32°C) stimulate metabolic activity.
- **Experiment 4:** Since yeast prefers a pH range of 3.8 to 5.2, the metabolic activity increases significantly when an acid buffer solution is added. If one adds a basic buffer solution instead, the conditions are no longer optimal for the yeast. As a result, the metabolic activity decreases.

Overview

Tasks

1. To identify glycolysis by measuring the production of CO_2 and plotting graphs.
2. To investigate the influence of temperature and pH on metabolic activity.

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Set-up and procedure

Set-up

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  Fasten the Cobra4 Wireless/USB-Link to the support rod of the holder.

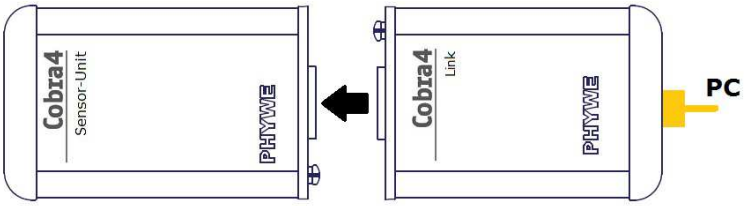

Fig. 2: Cobra4 Sensor-Unit with Cobra4 Link in USB mode

- 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.
-
  - Switch on the Cobra4 Wireless/USB-link .
  - Connect your PC with the Cobra4 Wireless/USB-link (via WiFi or via cable in USB-mode).
  - Start PHYWE measureLAB and The sensor is detected automatically.
  - Choose the experiment from the start screen by selecting `Load Experiment' .
  - Go to “PHYWE experiments”, search for “P4110460”, and select the respective folder containing the experiment. All necessary presetting will be loaded.

Procedure

Experiment 1

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- Weigh 10 g of baking yeast and fill it into the 250 ml beaker. Top it up with warm tap water up to the 100 ml mark and stir it with the glass rod.
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- Start the measurement (runtime limited to 5000 s; Stop:).

Experiment 2: (for the procedure, see experiment 1)

- Fill the 1000 ml beaker half full with tap water. Place the Erlenmeyer flask into the beaker and add some ice cubes. Seal the Erlenmeyer flask with a stopper and start the measurement (runtime limited to 5000 s).

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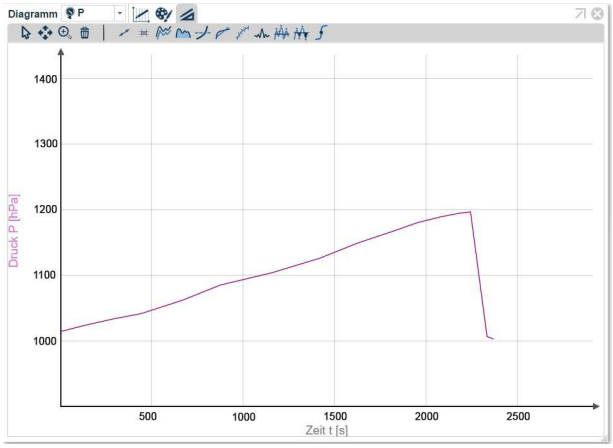

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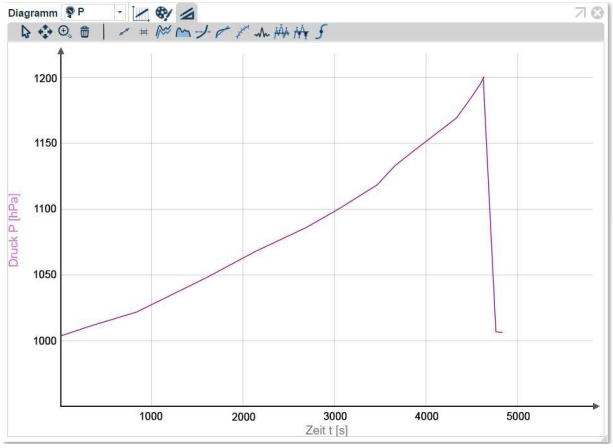

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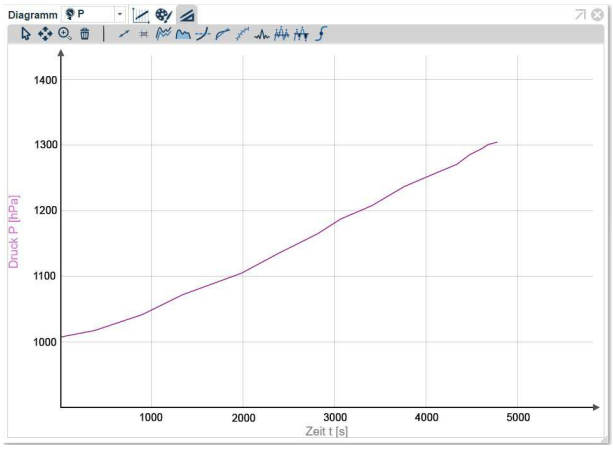

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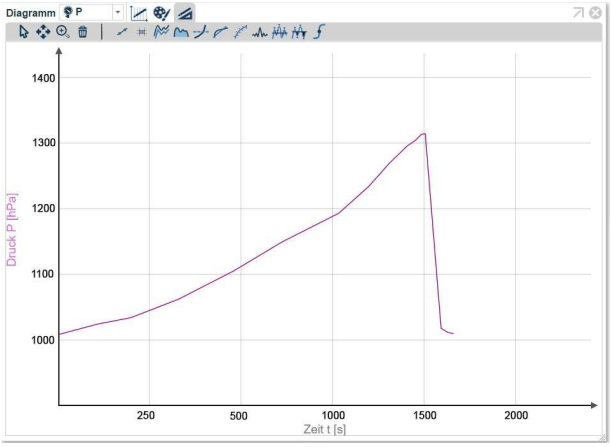

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