Task 2
Task Sheet

Chemistry, Physics, Biology – Tied by Time and Change

EOES2025, Zagreb, Croatia
26.04. – 03.05.2025.

EOES
European Olympiad of Experimental Science

Introduction to the task:

Time and change are at the heart of everything in the natural world. Chemical reactions, whether they happen in a fraction of a second or over millions of years, reshape matter. Biological systems evolve, grow, and adapt through processes bound by time. Physical forces drive changes from the smallest atomic motions to the vast movements of galaxies. Across all sciences, time and change are threads that connect discoveries, explain patterns, and reveal the dynamic nature of life and the universe. In this task, you will glimpse how different sciences capture transformations shaped by the passage of time.

Here are the approximate times you will need to spend on each problem.
Problem 1 – Kinetics of the reaction between crystal violet and hydroxide ions - 3.5 hours (total marks: 41.5)
Problem 2 – Velocity and Drag - 3 hours (total marks: 43.5)
Problem 3 – Heat it up! Speed it up! Step it up! - 3.5 hours including 1 h of incubation time (total marks: 40)

Note: read the entire Task Sheet first and then start the experiment.

Problem 1. Kinetics of the reaction between crystal violet and hydroxide ions

Materials and equipment

- Plastic UV-vis cuvettes with caps – 4
- Stand for UV-vis cuvettes – 1
- Bottles with stock solutions (**A** = NaOH, **B** = NaCl, **C** = crystal violet = CV)
- Volumetric flasks (3×50 mL, 1×10 mL)
- Graduated pipettes (5 mL and 10 mL)
- Pipetting bulb
- Bottle with distilled water
- Dropper – 2
- Automatic pipette (100-1000 µL)
- Automatic pipette tips
- Beakers (1×100 mL, 1×50 mL, 4×25 mL)
- LEGO brick set with assembly instructions
- Power supply (phone charger)
- USB to crocodile clips – 1
- Banana-plug to crocodile clips – 2
- Double crocodile clips wire – 1
- 150 Ω resistor
- LED diodes – 2
- Multimeter
- Stopwatch
- Graphing paper
- Ruler
- Beaker for waste

Introduction

A photometer (more commonly a spectrophotometer) is an essential laboratory equipment with analytical applications. It is used to measure the light absorption of a specific wavelength in a solution, which allows to determine the concentration of the absorbing species (dissolved substance) in the sample. These instruments are expensive and often not available in schools. However, a very simple and cheap version of the instrument can be assembled from easily available parts – LEDs, wires, a phone charger, a multimeter, and LEGO bricks. That is your today's task. It is known that an LED connected to a power supply will light up, but it works also the opposite way – if one shines a light on the LED, a voltage will develop on it, which is approximately proportional to the intensity of light that reaches it. This is the basis for the light source and detector in the instrument you will assemble today (Fig. 1.1.).

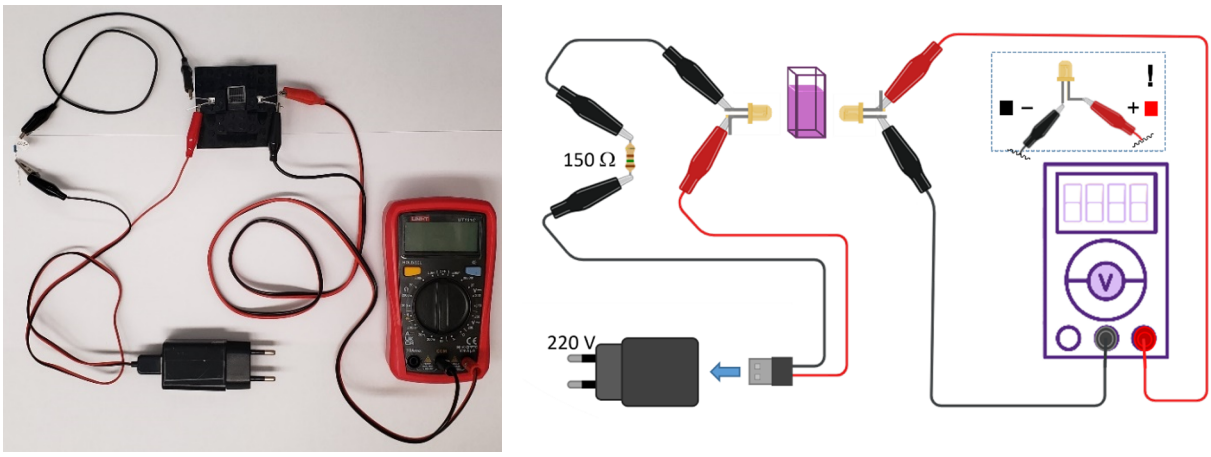

Figure 1.1. The instrument you will assemble today (left) and circuit diagram (right).

The sample solution in the cuvette between the light source and detector absorbs part of the light that enters it. In this experiment, you will use a solution of the dye crystal violet (CV), that is intensely violet in color. This means it transmits violet light while absorbing light of its complementary color—yellow. Therefore, the LED's you will use as a light source are yellow. The voltage you will read on the multimeter is approximately proportional to the intensity of light reaching the detector, which allows the measurement of the absorbance.

From the voltage measured on the multimeter, the absorbance (A) can be calculated, which is directly proportional to the concentration of the absorbing species in the solution, given by the Lambert-Beer's law equation:

$$A = \varepsilon \cdot l \cdot [\mathrm{CV}] \qquad (1)$$

where:

ε – molar absorption coefficient ($M^{-1}\ cm^{-1}$)
l – absorption optical path length (cm)
[CV] – molar concentration (M) of the dissolved species, in this case crystal violet

In this task, the term 'concentration' written in the square brackets always refers to the total molar concentration (M = mol/L).

To obtain the absorbance, both the voltage for the solvent (distilled water, U_{BL}) and the solution (U_{CV}) should be measured. According to the following equation, the absorbance can be calculated:

$$A_{CV} = log\left(\frac{U_{BL}}{U_{CV}}\right) \qquad (2)$$

From the obtained absorbance and known molar absorption coefficient (ε), the concentration of the absorbing species in the solution can be calculated:

$$[\mathrm{CV}] = \frac{A_{\mathrm{CV}}}{\varepsilon \cdot l} = \frac{log\left(\frac{U_{\mathrm{BL}}}{U_{\mathrm{CV}}}\right)}{\varepsilon \cdot l} \quad (3)$$

This opens up the possibility to monitor the concentration of the colored species during a chemical reaction and thus study the reaction rate, *e.g.* chemical kinetics, which is a very important area of chemistry with the main goal of determining the reaction mechanism.

In this experiment, you will study the kinetics of the reaction between CV and hydroxide ions in an aqueous solution (Fig. 1.2.). Crystal violet exists as a cation ($C_{25}H_{30}N_3^+$) with chloride as its counter-ion ($C_{25}H_{30}N_3Cl$). Chloride ion does not absorb in the visible spectrum and does not influence the reaction between the CV cation and OH^-, thus it can be omitted from equations like in Fig. 1.2.

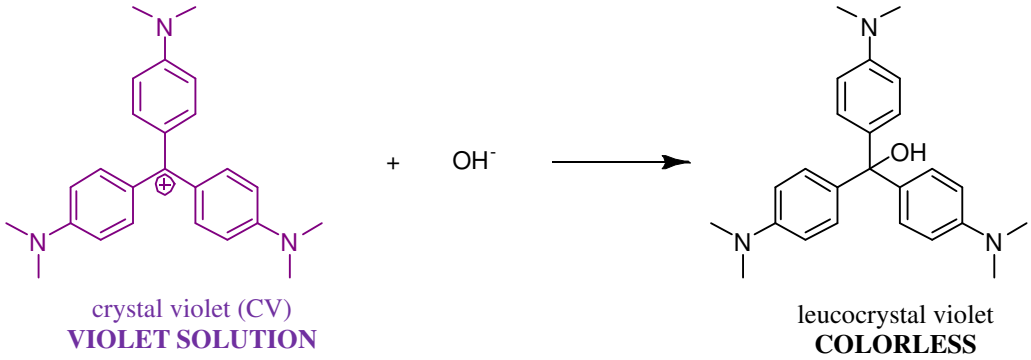

Figure 1.2. Reaction of crystal violet with hydroxide ions.

In this reaction, two species are involved (CV and OH^-), meaning the reaction rate depends on frequency of their collisions, which is directly proportional to their concentrations. In today's experiment, the concentration of OH^- is significantly higher than that of the CV (by a factor of 300-500). As a result, the concentration of OH^- can be considered constant during the reaction which leads to pseudo-first-order rate of reaction, depending only on concentration of CV. The integrated rate law can be expressed as:

$$ln\left(\frac{[\mathrm{CV}]_t}{[\mathrm{CV}]_0}\right) = -k't \quad (4)$$

where:

$$k' = k[\mathrm{OH}^-] \quad (5)$$

and where:

t – time

$[CV]_t$ – concentration of CV at time t

$[CV]_0$ – concentration of CV at time $t = 0$

k' is pseudo-first order rate coefficient

k is the second order rate coefficient

However, determining [CV] would typically require knowledge of its molar absorptivity (ε) and optical path length (l). In this experiment, this will not be necessary, as substituting the absorbance into expression (4) causes the terms ε and l to cancel out:

$$ln\left(\frac{[\mathrm{CV}]_t}{[\mathrm{CV}]_0}\right) = ln\frac{\frac{A_t}{\varepsilon \cdot l}}{\frac{A_0}{\varepsilon \cdot l}} = ln\left(\frac{A_t}{A_0}\right) = ln(A_t) - ln(A_0) \quad (6)$$

where:

A_t – absorbance of the solution at time t

A_0 – absorbance of the solution at time $t = 0$

Finally, combining eqns. (4) and (6) yields the expression that will be used to process the data from today's experiment:

$$ln(A_t) = ln(A_0) - k't \quad (7)$$

Your task is to assemble the photometer and use it to determine rate constants k' and k. The rate constant k' depends on the $[OH^-]$ (acc. to eqn. (5)), while the rate constant k is independent of the molar concentration of the reactants.

Step 1.1: Assembling the photometer (2 p)

1. Assemble the instrument from LEGO bricks according to the instructions.

2. Insert the LEDs into each of the 2×1 LEGO elements with holes. Press the diodes into the holes firmly. *Note: Make sure they are not tilted*. Space the wires a little to avoid short-circuiting later.

3. Position the photometer far from the edge of the table and connect the circuit as shown in Fig. 1.1. *Note: Take care to connect the wires with the correct polarity, otherwise, the LED will not light*. The power supply will be provided after the lab assistant checks that everything is connected correctly.

Before continuing, raise the red card so the lab assistant can check whether you have assembled the instrument correctly. If it is not assembled correctly, you will not receive points for this specific question, but the assistant will assemble the instrument correctly.

4. Set the multimeter to the 2000 mV setting before starting the experiment. Ensure that the LED and the multimeter remain ON throughout the entire experiment. *Note: The multimeter automatically turns off after a prolonged period of inactivity (you will hear a beep). Press the blue button to keep it on.*

Step 1.2: Preparation of solutions for measurement

1.2.1. Preparation of sodium hydroxide solutions

Stock solutions **A** ($[NaOH]_{ST} = [OH^-] = 0.080$ M) and **B** ($[NaCl]_{ST} = 0.30$ M) are provided for the preparation of three solutions that will be used in the experiment (**I**, **II**, and **III**). Each solution should be prepared in a 50 mL volumetric flask and will contain a mixture of NaOH and NaCl. Label flasks and beakers with **I**, **II** or **III** using a permanent marker. The concentrations of NaOH are: 1.20×10^{-2} M,9.60×10^{-3} M, 7.20×10^{-3} M for experiments **I**, **II** or **III**, respectively. The concentrations of NaCl need to be calculated, all in accordance with **Table 1.2.1** (Answer Sheet). The purpose of adding NaCl is to keep the total ion concentration constant for all three experiments. This means that the sum of NaOH and NaCl concentrations for each solution (**I**, **II**, and **III**) must be equal in each experiment:

$$[NaOH] + [NaCl] = const. = 3.00\times10^{-2} \text{ M} \qquad (8)$$

- *1.2.1. Fill in **Table 1.2.1.** and detail your calculations only for the first line on the Answer Sheet below **Table 1.2.1.**! Indicate your final result for [NaCl] using the scientific notation with 3 significant figures (example: 1.21×10^{-5}). For calculated volumes of solutions A and B, indicate the result as a number with 2 significant figures.* (4.5 p + 1.5 p)

1.2.2. Preparation of CV solution

From stock solution **C** ($[CV]_{ST} = 4.80\times10^{-4}$ M) prepare 10 mL of the CV solution with the concentration $[CV]_1 = 2.16\times10^{-5}$ M. Using a permanent marker, write corresponding labels on the flask and beakers (CV stock, CV diluted).

- *1.2.2. Show details of your calculations on the Answer Sheet. Indicate your final result for $V_{ST}(CV)$ as a number without decimal places in the μL units.* (1 p)

Step 1.3: Measurement of the reaction rate

1.3.1. Voltage measurement for the blank sample (distilled water) (1.6 p)

First, measure the voltage (light intensity) for the blank sample (pure solvent, in this case distilled water). Fill the empty UV-Vis cuvette with distilled water (≥ 2 mL) and place it in the instrument. *Note: <u>During all measurements, make sure that the cuvette is turned the right way - the clear sides must face the diodes. It is also important that there are no air bubbles in the liquid and that you do not touch the cuvette during the kinetic experiment.</u>* The value on the multimeter screen will stabilize quickly, so you can read and write it down immediately. It has been found that room lighting does not interfere with the measurements, so you do not need to cover the photometer while measuring.

Before continuing, raise up the red card for the lab assistant to check the result. If there is a large deviation from the expected value, the assistant will replace the diodes and you will NOT receive negative points for this.

Remove the cuvette from the instrument, wait for the value on the multimeter to stabilize and return the cuvette to the instrument. Repeat the measurement two more times.

- *Record the values in **Table 1.3.1.** (on the Answer Sheet).*
- *1.3.1. Calculate the average value ($\overline{U_{BL}}$) for the blank sample on the Answer Sheet. That is the value you will use in data processing. Show details of your calculations and indicate your final result for $\overline{U_{BL}}$ as a number without decimal places in mV units.*

1.3.2. Instructions for measuring reaction kinetics

When you have prepared all the solutions from parts 1.2.1. and 1.2.2., you can start the measurements. You will perform measurements for 3 samples having the same concentration of CV but different concentrations of OH^-. Each measurement lasts 21 minutes. The diode voltage must be read from the multimeter every 3:00 minutes precisely and recorded on the Answer Sheet in **Tables 1.4.1.**

Before continuing, raise the red card for the lab assistant to record the temperature in the lab. The rate constant is temperature-dependent and it is very important to record the temperature.

Method:

1. Take a clean, dry UV-Vis cuvette and use an automatic pipette to transfer 1.000 mL of solution **I** into it.
2. Using an automatic pipette, add 1.000 mL of the CV solution that you prepared in part 1.2.2. *Note: Use a new pipette tip for each experiment.*
3. Put the cap on the cuvette and turn it 3-4 times to mix the solution. *Note: Do not shake vigorously, as foam will form, which can greatly affect the measurement results.*
4. Place the cuvette in the instrument, immediately read the voltage and write it down in the **Table 1.4.1.** (Answer Sheet) (t = 0:00 min), and at the same time start the stopwatch. *Note: The stopwatch should be started at the moment of the first reading, not immediately at the moment of mixing the solution! For all samples, the delay between mixing and first reading should be as similar as possible to ensure consistency.*
5. Continue to read the voltage every 3:00 minutes until 21:00 minutes have elapsed (8 measuring points).
6. All measured voltage data should be recorded in **Tables 1.4.1.** on the Answer Sheet.

Repeat the measurements in the same way using solution **II** and **III** instead of solution **I**.

Before continuing, raise the red card for the lab assistant to record the temperature in the lab. The rate constant is temperature-dependent and it is very important to record the temperature.

Step 1.4: Data processing

1.4.1. Calculation of the absorbances and $\ln(A_t)$ (10.8 p + 1 p)

- *From the measured voltages, calculate the data needed to draw a graph and fill in **Tables 1.4.1.** on the Answer Sheet. Absorbances and ln(A) must be indicated as numbers with 4 decimal places.*

- *Detail your calculations **only for the first line** of **Table 1.4.1**/Experiment **I** on the Answer Sheet. Absorbances and ln(A) must be indicated as numbers with 4 decimal places.*

1.4.2. Calculation of the *k*' (11.1 p)

- *1.4.2.1. Draw a graph of $ln(A_t)$ versus t on the graphing paper. Data for all three experiments must be shown in the same graph. Data points must be shown as ×, points used for the calculation of the slope must be shown as ⊗. Label the paper with your team/country's sticker.*
- *1.4.2.2. Determine the reaction rate constants k' for each of the three measurements from **Graph 1.4.2.1.** Detail your calculations **only for the Experiment with solution I** on the Answer Sheet.* Express the values in units min^{-1} having 4 decimal places *and fill in the Table 1.4.2.*
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1.4.3. Calculation of the rate constant *k* (8 p)

- *1.4.3.1. Assuming the OH^- concentration remains constant during the experiment, calculate its concentration in the cuvette for all three experiments. Results must be given using the scientific notation with 1 decimal place (example: 1.2×10^{-5}).*

Determined *k*' depends on the [OH^-]. Now, you have all the necessary data to calculate the reaction rate constant *k*, which is independent of the reactant concentrations.

- *1.4.3.2. Draw the graph on the graphing paper using the data from **Table 1.4.2.** and concentrations of [OH^-] in all three experiments calculated in 1.4.3.1. Data points must be shown as ×, points used for the calculation of the slope must be shown as ⊗. Label the paper with your team/country's sticker.*
- *1.4.3.3. From **Graph 1.4.3.1.** calculate the value of the rate constant k. Show the details of your calculations. Final value of k that you report must be in M^{-1} min^{-1} units and have 2 significant figures.*

Problem 2. Velocity and Drag

Materials and equipment

- Plastic body with string (the pendulum)
- Styrofoam (larger than A3 paper)
- Plastic Styrofoam holder
- A3 paper with printed angles
- Clamp stand with three angle clamps
- Pins
- Stopwatch
- Plastic pivot holder (S-shaped shaft)
- Papers of varying dimensions (3.5 cm, 5.0 cm, 7.0 cm, 14.0 cm and 21.0 cm), with a marked line of symmetry – multiple copies
- Scotch tape
- Ruler

2.1 Introduction

In this problem, we will examine the drag that a rigid body experiences in a fluid. In general, this drag depends on many factors: the shape of the body, the viscosity of the fluid, the speed of the body relative to the fluid, etc. Here we will study the case in which the speed of the body is low and the drag is directly proportional to the speed.

For this experiment we will utilize (a physical) pendulum consisting of a cylindrical body of varying heights. The time dependence of the pendulum's angular displacement on timewhen drag is neglected, and for small displacements, is sinusoidal (see the dashed gray line in Figure 2.1), and the movement corresponds to that of a simple harmonic oscillator. However, with drag proportional to speed, the time dependence of the displacement θ decreases (Figure 2.1, solid red line), with maxima that decrease exponentially (Figure 2.1, dashed blue line). The expression used to describe the blue dashed line is:

$$\theta(t) = \theta(0)\, e^{-\gamma t} \tag{2.1}$$

where $\theta(0)$ is the deflection at time t = 0, and γ is the so-called damping constant.

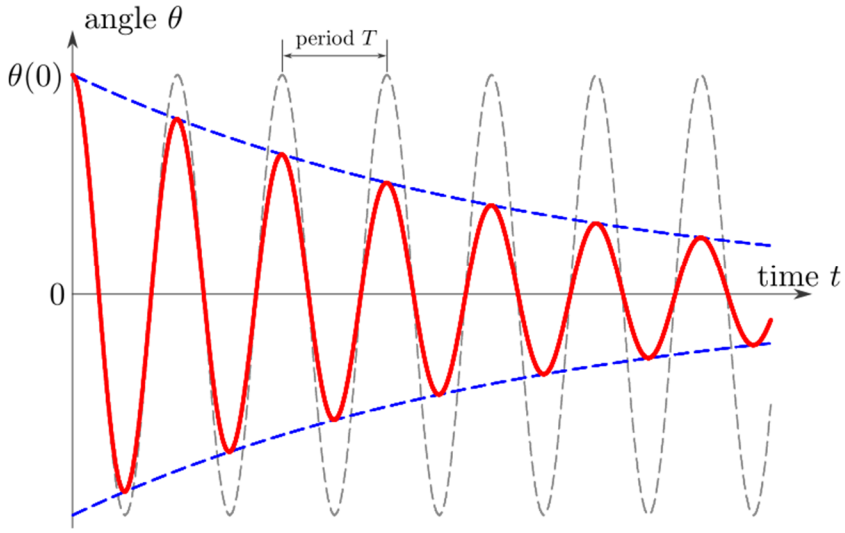

Figure 2.1 Time dependence of the pendulum displacement θ without drag (gray dashed line), and with drag (solid red line). The blue dashed line shows the exponential decrease of the maxima of the pendulum with drag. $\theta = 0$ corresponds to pendulum equilibrium position. Period T is time between two maxima, either without or with drag. (Here we present the simplified case valid for small damping constant γ).

Your task is to measure the damping constants γ for cylindrical bodies of different dimensions L, by measuring the time dependence of the falloff of the maximal displacement at prescribed points. Furthermore, you will check whether the damping constant is proportional to the dimensions L.

2.2 Experimental Setup

The experimental setup we will use consists of a pendulum – a cylindrical body suspended from a string (see Figure 2.2 a)). The mass of the body is 30.0 g, and its center of mass is 18.0 mm below its upper edge (Figure 2.2 b)). The position of the center of mass is denoted with a thin groove. The string is assumed to be massless and unstretchable. Attach the string to the pivot point, as shown on Figure 2.2 c).

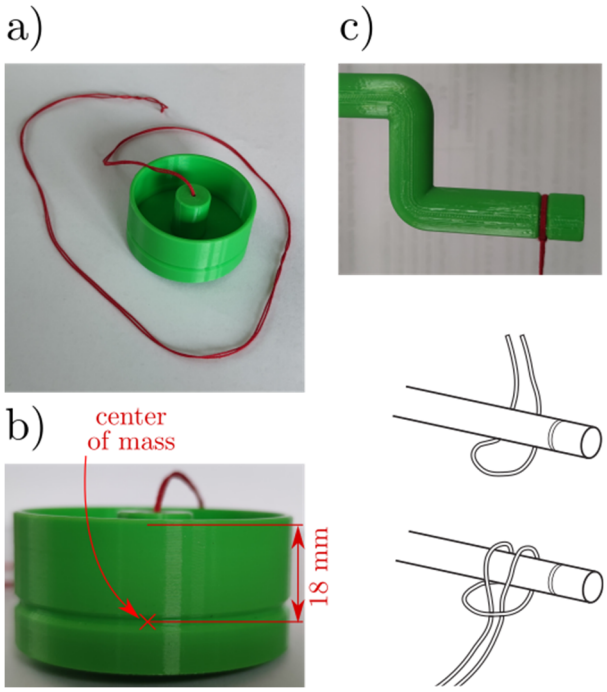

Figure 2.2

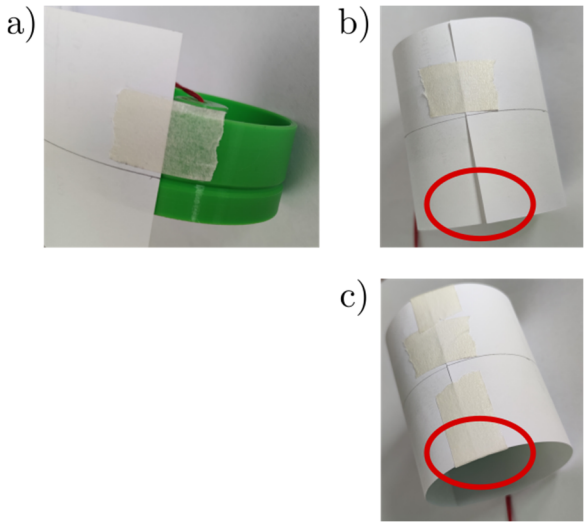

Figure 2.3

Cylindrical bodies of different heights will be made by securing papers of different dimensions L = 3.5 cm, 5.0 cm, 7.0 cm, 14 cm, and 21 cm with a Scotch tape (see Figure 2.3.a)). Align the middle of the paper with the groove. Wind the paper several turns and secure it with additional Scotch tape (see Figure 2.3.b)). In order to eliminate unwanted air friction effects, use Scotch tape to straighten and secure any loose paper ends or edges, as seen on Fig. 2.3.c). The mass of paper is not negligible and can be calculated from using paper's surface density of 80 g/m^2. (Mass of the tape can be ignored.)

The angle of the pendulum's displacement from equilibrium can be measured using the paper with printed angle markings, which you will pin to the polystyrene board (Figure 2.4 a)). Attach the plastic board holder to the other side (Figure 2.4 b)).

Assemble everything on a clamp stand as shown in Figure 2.5 a) and b). Adjust the two upper right angle clamps so that the zero degree marking corresponds to the position of the pendulum in equilibrium. Ensure that the pendulum's pivot is aligned with the origin marked on paper. Adjust the polystyrene board to be as vertical as possible by rotating the third right angle clamp.

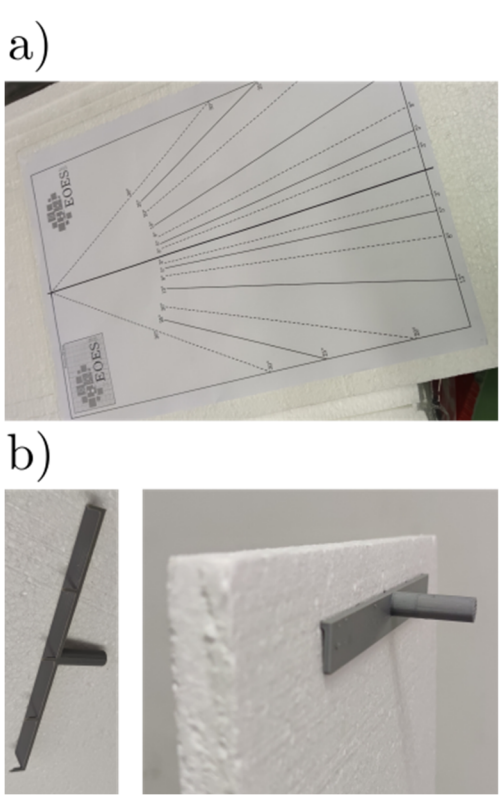

Figure 2.4

Figure 2.5

The measurement process consists of measuring the time elapsed from the initial maximum angle (20 deg) to several other marked angles. It is easiest to keep these angles fixed, so the experimental setup is set up according to the following table:

angle θ	**comment**
20 deg	The maximum deflection that we consider as the initial maximum deflection $\theta(0)$
13 deg	The values of the maximum deflection angles for which you will measure the elapsed time
8 deg	
5 deg	
3 deg	

Table 2.1 Fixed angles used for measurements.

To measure time use the included stopwatch.

2.3. Measurements

2.1. Using the Scotch tape attach the paper with dimension of L = 3.5 cm to a pendulum, forming a cylindrical-like body. Make sure that the middle line of the paper (thin line) is aligned with the center-of-mass groove. Start the pendulum by releasing it from rest so that its initial deflection is at least 25 deg. Then observe when the maximum deflection coincides with 20 deg and start timing with the stopwatch. Measure the time it takes for the maximum deflection to reach the values in Table 2.1. Record your measurements in Table 2.2 marked 'L = 3.5 cm' on the Answer Sheet. Repeat the described series of measurements once more. (3.2 p)

2.2. Determine the period of oscillations more accurately, by measuring the time required for at least 20 consecutive periods. (For period measurements, ensure that the deflection is not larger than approximately 8 deg.) Write the value in Table 2.2 marked 'L = 3.5 cm' on the Answer Sheet. (0.4 p)

The expression for the period T of a pendulum of length R, when drag is neglected, is

$$T = 2\pi\sqrt{\frac{R}{g}} \tag{2.2}$$

where R is the distance between the pivot and the center of mass of the pendulum (Figure 2.2 a)), and g is the acceleration due to gravity (g = 9.81 m/s^2).

2.3. Calculate the period of oscillation using equation (2.2). Determine the relative error between the measured and the calculated period values (relative to calculated value) and enter them on the Answer Sheet. Show your calculations. (3 p)

2.4. Repeat the measurements described in 2.1 and 2.2, but with attached paper of the dimensions L = 5.0 cm, 7.0 cm, 14.0 cm, and 21.0 cm Enter all measurements in the appropriate Table(s) 2.2 on the Answer Sheet. (14.4 p)

2.5. Since we expect an exponential decay of the maximum deflections, we need the values of the natural logarithms of the predefined angles. For this purpose, fill in the lower right table in Table 2.2 on the Answer Sheet. (1 p)

We say that the influence of drag on the period of the pendulum is negligible (non-negligible) if the measured values differ by less (more than or equal to) 5%.

2.6. Given the measured values of the oscillation period T, is the drag caused by the paper cylinder negligible*, or* non-negligible*? Write the answer on the Answer Sheet. Show your calculations. Does the period T of the pendulum decrease or increase as the height of the cylinder increases?* (1 p)

Taking the natural logarithm of both sides of Eq. (2.1), we get:

$$\ln\theta(t) = \ln\theta(0) - \gamma\, t, \tag{2.3}$$

which means that the time dependence of the logarithm of the maximum deflections on time has a slope '–γ'.

2.7 Using the values from Table 2.2, plot time dependence of calculated values of the logarithm of the angle ($\ln\theta$), on graphing paper (Plot 2.1) on the Answer Sheet. Plot ALL data, without averaging. (6.5 p)

2.8. For each dimensions L, draw a straight line that best follows the points, and from the slope of that line determine the damping factor γ to three significant figures. Fill in the first two columns of Table 2.3. on the Answer Sheet with the obtained values. (7.5 p)

From theoretical considerations it can be shown that the constant γ is proportional to the length of the cylinder (L) and inversely proportional to the total mass m of the pendulum:

$$\gamma = C\frac{L}{m}, \tag{2.4}$$

where C is some constant. Since we increased the mass suspended from the pendulum by adding paper (but did not change the distance of the center of mass from the suspension point), the measured values depend not only on L, but also on the total mass m of the pendulum and the paper. The dependence on m, however, can be 'absorbed' in the dimension L, so we *renormalize* it (m_0 is the mass of the cylinder without paper):

$$L_i \longrightarrow L_i\frac{m_0}{m_i} = L_i\frac{m_0}{m_0 + m_{i,\mathrm{paper}}} = L_{\mathrm{ren},i} \tag{2.5}$$

and expression (2.4) can be written as

$$\gamma = \left(\frac{C}{m_0}\right)\left(L_i\frac{m_0}{m_0 + m_{i,\mathrm{paper}}}\right) = \left(\frac{C}{m_0}\right)L_{\mathrm{ren},i} \tag{2.6}$$

2.9. For each value of the cylinder dimension L_i, determine the corresponding renormalized value $L_{\mathrm{ren},\,i}$, and enter the value in the last column of Table 2.3 on the Answer Sheet. (2.5 p)

2.10. On the graphing paper (Plot 2.2) on the Answer Sheet, plot values of γ as a function of the renormalized *dimension $L_{\mathrm{ren},\,i}$.* (2 p)

2.11. Using a ruler, draw the line of best fit on the graphing paper (Plot 2.2), and from its slope determine the value of constant C and enter it on the Answer Sheet. Show your calculations. (2 p)

Problem 3. Heat it up! Speed it up! Step it up!

Materials and equipment

- Pre-weighed dry yeast (labelled)
- Pre-weighed glucose powder (labelled)
- 500 mL laboratory beaker with demineralized water – 1
- 300 mL Erlenmeyer flask – 1
- 100 mL graduated cylinder – 1
- Glass rod – 1
- Glass tubes – 10
- Rubber stopper designed for glass tubes - 10
- Sterile medical needles – 10
- Plastic syringe (5 mL volume) – 10
- Thermostat/incubator – in the laboratory, set at 35 °C
- Tube rack
- Indoor air thermometer and clock located in the laboratory
- Light microscope
- Microscopic glass slides – clean (x 3)
- Microscopic glass slides – marked (x 3)
- Pencil, marker pen
- Bunsen burner – 1; in the laboratory, operated by lab assistant
- Laboratory tweezers – 1
- Sterile swabs – 3
- Disposable gloves
- Paper towel (for protecting the table and drying stained samples)
- Staining dish, plastic – 1
- Crystal violet stain in a dropper
- Wash bottle with demineralized water
- Wash bottle with ethanol
- Immersion oil for microscopy in a dropper

Introduction

Note: This task is divided into several parts, so be sure to read the entire assignment thoroughly before starting. Take a moment to review the answer sheet to ensure you are fully prepared—this will help you manage your time effectively. While waiting for the results of Step 3.1. (which takes approximately one hour), you should begin working on Step 3.5. Remember: Only answers recorded on the Answer Sheet will earn you points.

In this task, we will continue exploring kinetics, focusing on how metabolic rate influences microbial metabolism. You will investigate this relationship and its real-life implications. Additionally, you'll discover another interesting use of purple dye (Crystal violet) —a key element in the chemistry task your colleagues are working on!

The growth and metabolism of microorganisms, such as bacteria and yeasts, are highly temperature-dependent. A widely accepted metric, the Q_{10} temperature coefficient, quantifies how the metabolic rate of biological systems increases with a 10 °C rise in temperature. For both bacteria and yeasts, this coefficient is typically around 2, meaning their metabolism doubles for every 10 °C increase.

Simplifying further, we can assume that under ideal conditions, the rate of microbial division also doubles with each 10 °C rise. For example, if a cell takes 10 hours to divide at 5 °C, it will take only 75 minutes at 35 °C. Microbes thrive in warmth!

STEP 3.1. Do an experiment to determine the Q_{10} coefficient for baker's yeast.

In this assignment, you will use baker's yeast *Saccharomyces cerevisiae* as a model organism, providing a safer alternative to bacteria, even though there are billions of bacteria actively living in your mouth as you read this!

Experimental setup:

1. Prepare a 1 % w/v solution in an Erlenmeyer flask by adding all of the previously weighed glucose powder from the labelled package in 300 mL of demineralized water.
2. Dissolve the pre-weighed dry yeast in the glucose solution to create your working suspension, using a laboratory glass rod to stir and assist the process.
3. Prepare 10 glass tubes for the experiment.
4. Distribute the yeast suspension into the tubes, **filling each tube all the way to the top**. Place a paper towel under the rack to catch any spillage, but don't worry - you'll clean it up later. ***Note:*** *As you distribute the suspension, be sure to gently stir the suspension continuously to ensure even distribution in each tube. If left unstirred, the yeast will quickly settle at the bottom of the flask.* **Work quickly and efficiently to avoid inaccurate results.**
5. After filling the tubes with the yeast suspension, securely cap each tube with a rubber stopper designed for glass tubes. The stoppers will not go all the way, but this will be fine once you proceed with the next step.
6. Insert a sterile medical needle into each tube by carefully puncturing the rubber stopper (See **Figure 1.1.**). Now you can press the stoppers all the way. ***Note: Be extremely cautious with the needles to avoid injuring yourself or puncturing your finger!***

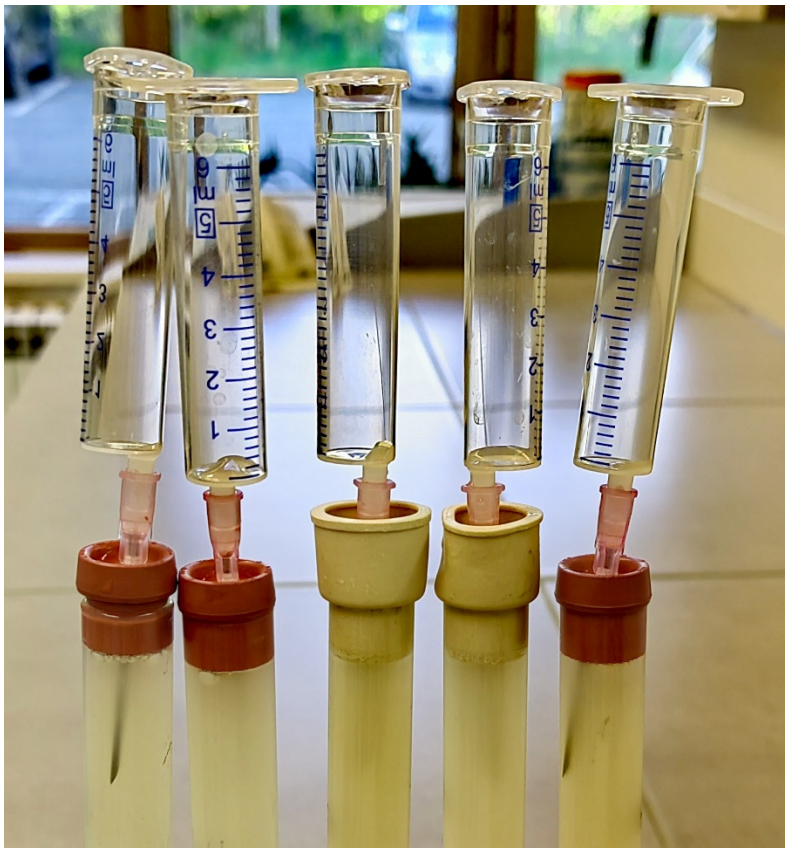

Figure 1.1. *Experimental setup: Inserting a medical needle into a glass tube by puncturing the rubber stopper and then attaching the syringe.*

7. Now, attach a 5 mL plastic syringe to each needle. Ensure the syringe is without the plunger. ***Note:*** *If you have trouble removing the plunger from the syringe, simply pull with a bit more force.* **To prevent false results, first insert the needles into all the tubes, and only attach the plastic syringes just before placing the samples in the incubator.**
8. Incubate 5 glass tubes at room temperature (you can leave them on the desk) and 5 glass tubes at 35 °C (in the pre-set thermostat) for 60 minutes. Be sure to label the tubes with marker pen; indicate your team name and treatment temperature, so that you can recognize your rack after 1 hour. Measure the room temperature by checking the indoor air thermometer located in the laboratory (ask the lab assistant for its location). **Write down the treatment temperatures in Table 3.1.1. on your Answer Sheet.**
9. As the yeast ferments during the incubation, it will expel water into the syringe, allowing you to measure the volume of expelled suspension. **After 60 min**, **as soon as you get back to your work desk** read the volume of water expelled into the syringe. Then, calculate the mean volume of expelled suspension in all treated tubes for each temperature treatment. **Enter the values in Table 3.1.1. on your Answer Sheet.** (2 p)

STEP 3.2. Calculate the Q_{10} coefficient

To calculate the Q_{10} coefficient, use the widely accepted Arrhenius model, that says:

$$Q_{10} = \left[\frac{k(t_2)}{k(t_1)} \right]^{10°C/(t_2 - t_1)}$$

If we assume that the value of *k* is proportional to the volume of expelled suspension in your experiment, we can define the following:

Q_{10} – the Q_{10} coefficient
$k(t_1)$ – the volume of expelled suspension at the lower temperature of incubation
$k(t_2)$ – the volume of expelled suspension at the higher temperature of incubation
t_1 – the lower temperature reading in degrees Celsius
t_2 – the higher temperature reading in degrees Celsius

3.2.1. *Using the Arrhenius model, calculate the Q_{10} coefficient and* ***write it down on the Answer Sheet.*** *Round it to one decimal place. The Q_{10} is important because you will need it to solve the following steps.* When you calculate the Q_{10}, please call the lab assistant for a check-up. If it is correct, carry on with your assignment. If wrong, the lab assistant will give you the right answer, but you will lose 3 points. (3 p)

STEP 3.3. Bacterial growth at different temperatures

Now, the Q_{10} coefficient also applies to bacteria. Imagine yourself in a hypothetical situation, enjoying a delicious rice bowl, perhaps a "*Risotto frutti di mare*". Since the risotto was properly cooked, it initially contains no bacteria. However, as you eat, you inadvertently transfer bacteria from your mouth to the meal. After having your meal, you decide to save the leftovers for later. But, it is very important where you leave them, as we will see next.

For next task, firstly you will need to determine the generation time of bacteria in your imaginary bowl. The definition of generation time is – *Time that takes the bacterial population to double in numbers*. However, it can be simplified, and considered as the time it takes the bacteria to divide in two. Your task is to calculate how many bacterial cells will be present in your bowl tomorrow (**after 24 hours**) if you store the bowl in the **refrigerator (4 °C)**, leave it outside on your balcony during autumn (**14 °C**), leave it in this laboratory (**24 °C**) or if you leave it **outside** at a constant temperature of **34 °C**, which is a common summer temperature in Zagreb. You started with transferring 500 bacterial cells from your mouth to the bowl during your meal.

To do this, you need to know how much faster the bacterial metabolism is at different temperatures, and the Q_{10} coefficient tells you exactly that. So, for next calculations, you will assume that the Q_{10} obtained from the yeast experiment also applies to bacteria in your bowl.

3.3.1. On the Answer Sheet, write down the generation time of bacteria in YOUR rice bowl by using the Q_{10} coefficient obtained from the yeast experiment. In your case, you have a given generation time at 4 °C which is 8 h. **Round generation time to a single decimal place**. (1.5 p)

EXAMPLE to help you: if the Q_{10} coefficient is, let's say three, and the generation time at 4 °C is 12 hours; that means that for each 10 °C the speed of bacterial metabolism triples, so the generation time at 14°C is 4 hours, etc. This example is written in the ***Table 3.3.1.***

Table 3.3.1. Bacterial generation time

	Example of calculated generation time
Q_{10} coefficient	3.0
Generation time (h) at 4 °C	12.0
Generation time (h) at 14 °C	4.0
Generation time (h) at 24 °C	1.3
Generation time (h) at 34 °C	0.4

3.3.2. Calculate the number of bacteria in your bowl after 24 h, if stored at various temperatures. Write down the data in the Table 3.3.2. on the Answer Sheet. (4 p)

Instructions:

- The number of bacteria at given time point can be calculated according to following equation:

$$N = N_0 \times 2^n$$

where N is the number of bacteria at given time, N_0 is the starting number of the bacteria, and n is the number of divisions.

STEP 3.4. On the Answer Sheet, answer the questions from 3.4.1 to 3.4.8. using experimental observations and your knowledge. (total: 9 p)

STEP 3.5. Prove that there are bacteria in your mouth

While the yeast ferments (STEP 3.1.) your task is to prove that bacteria inhabit your mouth. The most effective method is to visualize them. Your assignment involves using Crystal violet to stain and observe the bacteria from your mouth.

Procedure (by following these steps, you will be able to personally see them, and thus confirm the presence of bacteria in your mouth!):

1. Label the microscope glass slide(s) on a frosted edge with a pencil. You can choose the number of slides to prepare, but remember that duplicates are always a good idea. Write your

initials and a label (e.g., UP) on the upper side of the glass slide, using a pencil. Ensure you write on the frosted edge to avoid interfering with the sample.

2. **Clean the microscope glass slide:** You will now need the Bunsen burner, and help of the lab assistant. Read the following and approach the lab assistant: hold one end of the slide with tweezers and briefly pass both sides through the tip of a blue rushing flame to remove any greasy residues. ***Note:*** *Do not hold the slide in the flame; just pass it quickly through. Place the slide on a work surface and allow it to cool for about two minutes.*
3. **Collect a sample of your mouth epithelial cells:** Using a sterile swab, gather a sample from the inside of your cheek and along the teeth and gum line. Apply firm, rotating movements to ensure an adequate collection of cells from both cheeks. This should take at least 5-10 seconds – be sure to apply pressure so the mucosal cells and bacteria stick to the swab. If you are too gentle, you will not transfer epithelial cells and bacteria from your mouth to the swab. Once you remove the swab from your mouth, immediately spread the sample evenly across the entire surface of the microscope glass slide by rolling the swab. ***Note:*** *work quickly; if you delay, the bacteria may not transfer to the slide. Discard the swab in the waste bin and allow the slide to dry for about two minutes on the work surface.*
4. **Fixate the sample:** Using tweezers, hold one end of the slide and pass the non-sample side (the lower side) through the blue rushing flame cone three times to fixate the sample. Lab assistants will assist with the fixation process. After fixation, place the slide back on the surface and allow it to cool for two minutes.
5. **Stain the sample:** Cover your work area with paper and wear disposable gloves. Place the slide on the holder of the staining dish. Using a plastic dropper, completely cover the sample area with Crystal violet stain solution, ensuring the dropper does not touch the slide. Allow the stain to set for one minute.
6. **Rinse the slide:** Place several paper towel sheets on your work surface under the staining dish. While holding one end of the slide with tweezers, tilt the slide and rinse it using the wash bottle: first rinse with ethanol (96 v/v/ %), and **quickly after** rinse with demineralized water. It's important to be quick, otherwise ethanol will destroy your sample. Keep rinsing with water until the runoff is clear. Now, place the rinsed slide on the prepared paper and blot with paper towels to remove excess water. ***Note:*** *It's important to remove all water to ensure proper viewing under the microscope with the immersion lens.*
7. **Microscope examination:** Finally, examine the stained sample under the microscope using the immersion objective to observe the bacteria. ***Note:*** *if you are unable to find a picture, ask the lab assistant to help you, but in that case you will lose 1.5 points.*
8. **IMPORTANT:** Kindly ask you, when you are done with microscopy, call the lab assistant to clean the immersion objective. Otherwise, the oil can dry out and harm the objective.

3.5.1. Observe and draw stained cells under the microscope, following the instructions on the Answer Sheet. (5.5 p)

STEP 3.6. Take a look at some other bacteria (3 p + 4 p + 5 p)

3.6.1. – 3.6.3. On the desk you have several microscopic slides marked: X1, X2 and X3. On these slides you have previously prepared samples of different microorganisms that are already fixated. So, do the same staining procedure as described above. Simply **repeat the steps from 5 to 7**.

Again, draw representative images of all three samples on the Answer sheet under the 3.6.1, 3.6.2 and 3.6.3. Mark key features, if there are any.

STEP 3.7. Finally, answer questions from the 3.7.1 to 3.7.3. in the Answer sheet, using experimental observations and your knowledge. (1 p + 1 p + 1 p)