

11th International Junior Science Olympiad

Mendoza, Argentina

Experimental Test

December 8th, 2014

Experimental test

EXAMINATION RULES

1. Participants must not enter prohibited items (tools, papers, books, medicine, etc.). Required medicine or personal medical equipment must be previously notified to the monitor.
2. Only examination sheets and the material provided by the monitor can be used throughout the examination.
3. The use of personal safety items: gloves, goggles, and lab coat; are mandatory throughout the whole Experimental Test.
4. Participants must work safely. The equipment and the working station should be kept clean and tidy.
5. Each participant must stay at their designated location and is not allowed to leave the classroom before the Experimental Test time elapsed.
6. It is forbidden to eat or drink throughout the examination. If necessary, the participant can call the monitor and make a request for permission to take a bite out of the examination location.
7. If a participant needs to go to the bathroom, they must be authorized by a monitor.
8. Participants of different teams cannot communicate with each other. Working discussions must be done sparingly and quietly. If the participant needs assistance, they can require it from the monitor.
9. No questions regarding the examination will be answered.
10. A beeping signal will announce the expiration of examination time. From that moment it is forbidden to write anything on the Answer Sheet.
11. After completing the examination, the working material should be organized like at the beginning of the test.
12. The yellow Answer Sheet will be collected by the monitor at the assigned place for each group after the finalization signal.
13. Participants must wait until the monitor collects the Answer Sheet before leaving the room.

INSTRUCTIONS

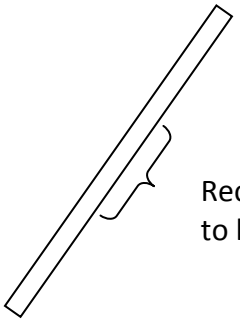
The Experimental Test consists of two parts, which are independent from each other. Therefore, they can be made consecutively or simultaneously. The team should decide how to best optimize working time.

1. Each participant must make sure to have all the working material provided by the monitor (pencil, eraser, ruler and calculator).
2. Each participant must check the papers given, which consists of two sets: experimental instructions and questionnaire, and the yellow Answer Sheet (for submit) and the white Answer Sheet (for draft). **Only the yellow Answer Sheet will be marked.**
3. Each participant must check the personal protective equipment provided (gloves, goggles and lab coat).
4. Each group must make sure to have all the necessary material and reagents for each experiment according to the detailed lists.
5. Each team must indicate on the first page of the Answer Sheet: name of each member of the group, seat numbers, country and signatures. In addition, the team must write the group code, and the participant codes on the top of each page.
6. Results must be written in ink on the yellow Answer Sheet in the assigned space for this purpose. Those results written out of the assigned location, will not be considered. **The results in the white Answer Sheet will not be considered.**
7. The time available to perform the experimental work and record the results on the Answer Sheet is 4 hours. Thirty minutes before and five minutes before expiration time, there will be an announcement indicating the end of the examination approaching. Upon exam time finalization, the group must stop working immediately and wait until the Answer Sheet is collected by a Monitor.

LABORATORY EQUIPMENT SUGESTIONS

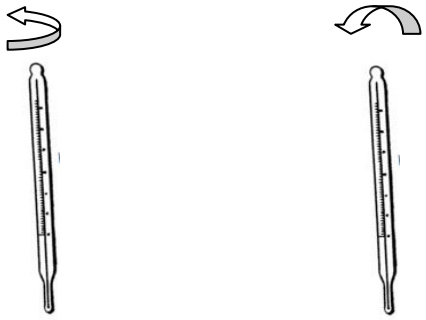
Recommendation for glass rod use:

In order to prevent the glass rod from breaking, it is recommended to hold it from its middle section as indicated in the following drawing.



Recommended place
to hold the glass rod

Recommendation for thermometer use:
For temperature measurements hold the thermometer with the scale pointing towards you.
A thin line of mercury should be seen.
If the mercury line is not evident, move the thermometer slightly (either rotating it or tilting it) until you see the end of the mercury line.



General consideration
If there is any doubt about the use of laboratory equipment, raise your hand to be assisted by the monitor.

Calculator instructions

How to calculate $e^{-2.5}$:

1. Press 2.5
2. Press +/- key
3. Press 2ndf key
4. Press e^x key

How to calculate ln(4):

1. Press 4
2. Press ln key

SECTION 1: ALCOHOLIC FERMENTATION OF GLUCOSE [25.0 Marks]

INTRODUCTION:

Alcoholic Fermentation refers to the decomposition of organic matter with fast and vigorous gas release. It is a biological process in which sugars such as glucose, fructose and sucrose are transformed by microorganisms to obtain energy. The metabolic process can be performed by yeasts, such as Saccharomyces cerevisiae, under anaerobic conditions (absence of air, in particular oxygen). Yeasts are eukaryotic microorganisms classified in the Fungi kingdom. They are unicellular, and have no flagellum. They are round or ovoid cells with a 5 to 10 µm diameter. In particular, alcoholic fermentation (called as such due to the metabolic product ethanol), is the basis for various biotechnological applications including manufacture of alcoholic beverages such as wine, beer and cider drinks; as well as manufacture of bread products. Today it is also used for the large-scale synthesis of ethanol, which is then used as biofuel.

*The yeast Saccharomyces cerevisiae (**Figure 1**) will be used in this experiment. The metabolic process is represented by **equation 1.** In this process the cells get energy and generate ethanol (CH_3-CH_2-OH) and carbon dioxide (CO_2) as waste products. By measuring the volume of released gas during the metabolic process, glucose consumption and ethanol production can be estimated. The identity of the produced gas can also be confirmed.*

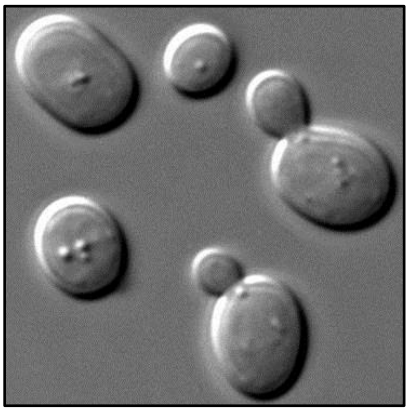


Figure 1: *Saccharomices cerevisiae*
(Image achieved by differential interference contrast microscopy)

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} \underset{\text{Energy}}{238.5\ \text{kJ}} + \underset{\text{Ethanol}}{\mathbf{2}CH_3CH_2OH} + \underset{\text{Carbondioxide}}{\mathbf{2}CO_2(g)}$$

Equation 1

EXPERIMENTATION

Reagents and materials

ID #	Safety material	Quantity
A	Latex gloves	8
B	Goggles	3
C	Absorbent paper roll	1
D	Wastebucket	2

ID #	Material for experimental assay	Quantity
1	Rectangular container	1
2	1000 ml graduated cylinder	1
3	250 ml beaker	2
4	Universal support	1
5	Three-finger clamp	1
6	15 x 15 cm rubber pad	1
7	1000 ml beaker	1
8	Glass rod	1
9	Thermometer, -10 °C to 150 °C	1
10	1000 ml conical/Buchner flask (Kitasato) with rubber stopper and latex tube (reaction flask)	1
11	Stopwatch	1

ID #	Reagent for experimental assay	Quantity
I	Water, 10 L	1
II	Bromothymol blue solution, 25 ml	1
III	Hot water thermos flask, app. 75 °C, 1 L	1
IV	Glucose sample, 4 g	4
V	Yeast sample, 50 g	2
VI	Saturated $Ca(OH)_2$ solution, 25 ml	1

PROCEDURE:

A. ***Instructions for assembling the gas measurement equipment, according to*** **Figure 2.**

This equipment will be used to monitor one of the produced metabolites (gas) by fermentation.

A.1. Pour water into the rectangular container (ID# 1) up to half full. Use water provided in the 10 L container (ID# I) (Figure 2a).

A.2. Fill the graduated cylinder (ID# 2) with the water poured in step A.1 by using a 250 ml beaker (ID# 3) (Figure 2 b).

A.3. Position the universal support (ID# 4) with the three-finger clamp (ID# 5) close to the rectangular container (Figure 2c).

A.4. Cover the mouth of the graduated cylinder with the rubber pad (ID# 6) and hold it with the palm of your hand (Figure 2d).

A.5. Turn the graduated cylinder upside down while keeping it closed with the rubber pad.

A.6. Immerse the graduated cylinder into the rectangular container and take out the rubber pad as shown in Figure 2e.

Important: The graduated cylinder must remain as full of water as possible. Some air could remain trapped but it should be kept at a minimum after taking out the rubber pad.

A.7. Fix the graduated cylinder to the universal support leaving approximately a 2 cm separation between the bottom of the rectangular container and the graduated cylinder, as shown in Figure 2e.

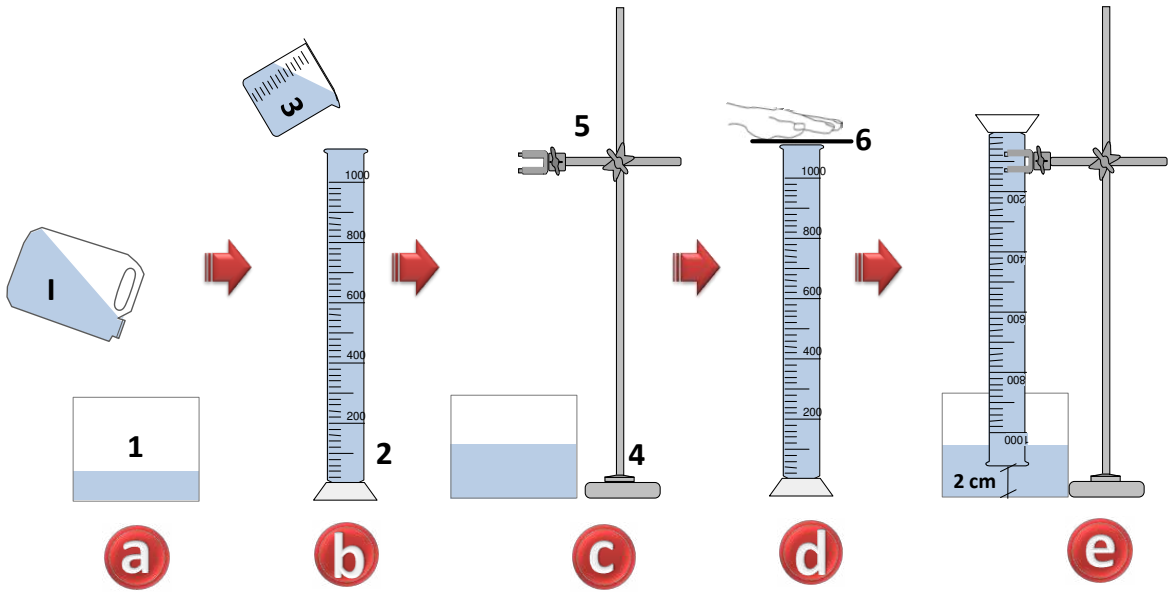


Figure 2

B. ***Instructions for assembling the fermentation reaction flask according to*** **Figure 3.**

B.1. Prepare approximately 750 ml of water within the range of 35-40 °C in the 1000 ml beaker (ID# 7). To this end, use the hot water (ca. 75 °C) provided in the 1 L thermos flask (ID# III) and the water provided in the 10 L container (ID# I). Measure the temperature with the thermometer (ID# 9) and record your reading on the Answer Sheet.

B.2. Label the 250 ml beakers (ID# 3) as "**A**" and "**B"** respectively.

B.3. Prepare **Suspension A** and **Solution B** using the warm water obtained in step B.1, as follows:

> **Suspension A:** Suspend in the 250 ml beaker labelled "A" (ID# 3) all 50 g of yeast supplied to you (ID# V) with 150 ml of warm water (35-40°C). Mix with the glass rod (ID# 8). Bring to a final volume of 250 ml with warm water (Figure 3a).
>
> **Solution B:** Dissolve in the 250 ml beaker labelled "B" (ID# 3) 4 g of glucose (ID# IV) with 150 ml of warm water (35-40°C). Mix with the glass stick (ID# 8). Bring to a final volume of 250 ml with warm water (Figure 3a).

B.4. Pour into the reaction flask (ID# 10) Suspension **A**. In order to ensure a quantitative transference of the yeast, rinse beaker A with a sample of Solution B. Do not dispose of the rinsing residues; pour all of them into the reaction flask. Gently swirl the solution in a circular

motion to homogenize. Place the reaction flask on the rubber pad (ID# 6), which is the same previously used (Figure 3b).

Important: The whole Suspension A and Solution B must be poured into the reaction flask. Do not use additional water for rinsing the beakers.

B.5. Measure the temperature of the resulting suspension and record it on **Table 1.1.** on the Answer Sheet (Figure 3c).

B.6. Cover the reaction flask with the rubber stopper firmly (Figure 3d).

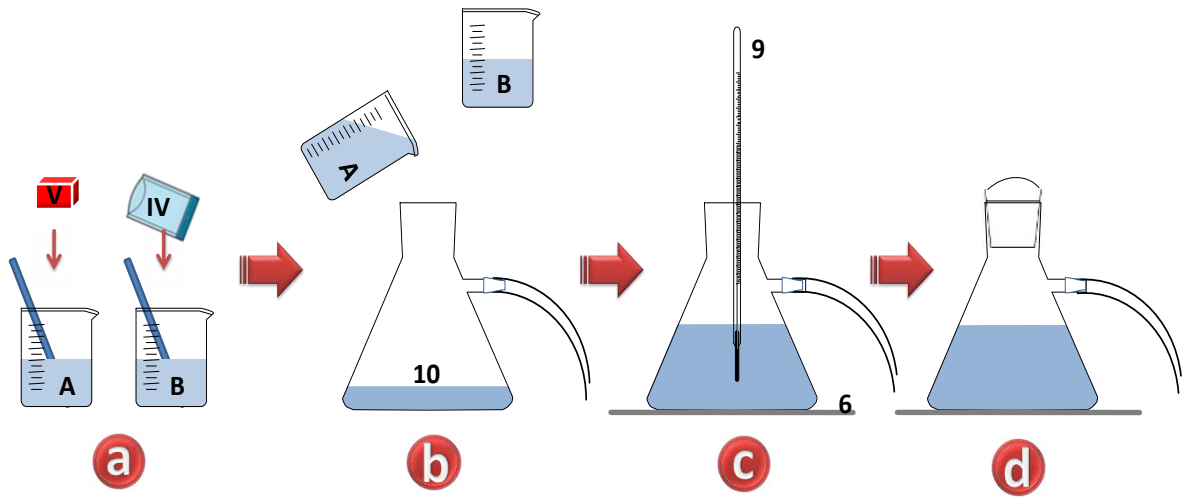


Figure 3

C. *Instructions for assembling the fermentation reaction flask and the gas measurement equipment according to* Figure 4.

C.1. Take the free end of the latex tube and insert it into the inverted graduated cylinder, as shown in (Figure 4). The free end of the latex tube should be at the top of the graduated cylinder after assembling.

C.2. Gently swirl the reaction flask in a circular motion in order to release air possibly trapped in the latex tube. Record the water level in the cylinder on the Answer Sheet. The resulting water level must be considered as the zero-point of the scale, which will be used for measuring the volume of released CO_2 (g).

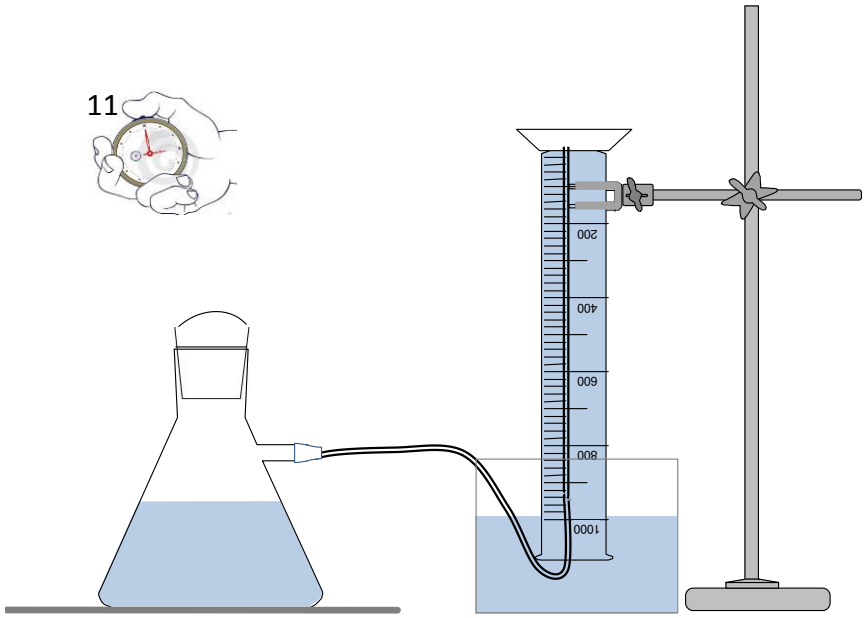


Figure 4

D. ***Instructions for recording the experimental data of the fermentation.***

D.1. As soon as the fermentation reaction flask and gas measurement equipment are assembled (Figure 4) start the stopwatch (ID# 11).

D.2. The reaction flask must be gently swirled in a circular motion, every 1 min for 5 seconds for the duration of the whole experiment.

Important: be careful when swirling the reaction flask because the latex tube should not slip out of the graduated cylinder.

As fermentation occurs in the reaction flask, the gaseous product is released; and most of it is transferred through the latex tube to the water column of the graduated cylinder. The transferred gaseous product will be trapped on the upper part of the graduated cylinder, where the total volume generated can be measured. The gaseous product accumulation will be evidenced by the decrease in the water column of the graduated cylinder. The remaining fraction of the gaseous product dissolves into the reaction flask solution.

D.3. The volume of accumulated gas must be measured every 2 minutes and recorded on **Table 1.1**. For 40 min, record data on the Answer Sheet.

Important: to carry out the reactivation process, do not stop the stopwatch.

Table 1.1: Recording fermentation data [8.5 Marks=7.0 (Record data) + 1.5 (Flow calculation)]

Initial temperature of warm water for preparing suspension A and solution B:		
Initial Temperature of suspension A+B (inside reaction flask):		
Time [min]	**Accumulated gas volume $V_a(t)$; [ml]**	**Flow F(t), [ml/min]**
0		
2		
4		
6		
8		
10		
12		
14		
16		
18		
20		
22		
24		
26		
28		
30		
32		
34		
36		
38		
40		

DO NOT FILL IN THIS TABLE

Instructions for recording experimental data of fermentation reactivation.

E.1. After 40 min of fermentation occuring, firmly hold the reaction flask and close off the latex tube by bending it tightly, so that gas does not pass through it.

E.2. Remove the rubber stopper from the reaction flask.

Important: be careful in order to avoid the latex tube from slipping out of the graduated cylinder.

E.3. Add the second sample of solid glucose (ID# IV) directly in the reaction flask and cover the reaction flask with the rubber stopper, making sure it is tightly closed.

E.4. Release the latex tube ensuring gas movement through it.

E.5. Gently swirl the reaction flask in a circular motion for 30 seconds in order to dissolve the new sample of glucose.

Important: if the graduated cylinder empties out completely it can be filled up again by following the procedure described in Section A. Or else do not disturb the setup of the graduated cylinder.

E.6. The reaction flask must be gently swirled in a circular motion, every 1 min for 5 seconds to record data.

E.7. The volume of the accumulated gaseous product must be measured every 2 minutes for 10 min and recorded on **Table 1.2** on the Answer Sheet.

TABLE 1.2.: Data of fermentation reactivation [2.5 Marks=2.0 (Record data) +0.5 (Flow calculation)]

Time [min]	Accumulated gas volume $V_a(t)$; [ml]	Flow F(t), [ml/min]
42		
44		
46		
48		
50		

DO NOT FILL IN THIS TABLE

E. *Identification of the gaseous product:*

F.1. Reaction with $Ca(OH)_2$

F.1.1. After 10 min of time elapsing of the reactivation stage, take out the latex tube from the graduated cylinder and immerse it into the saturated $Ca(OH)_2$ solution (ID # VI) as shown in **Figure 5**.

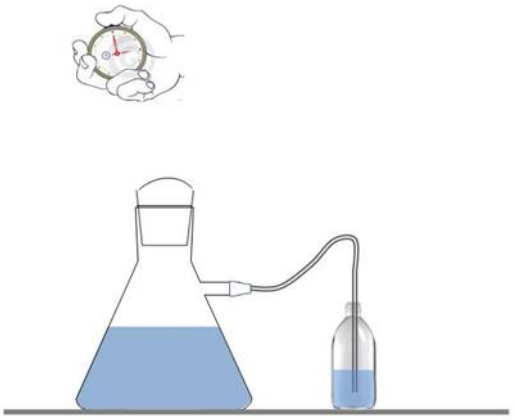


Figure 5

F.1.2. Swirl the reaction flask gently in a circular motion for 60 seconds for the gaseous product to bubble in $Ca(OH)_2$ solution.

F.1.3. Observe whether there is any change in the identification solution ($Ca(OH)_2$).

Choose the correct answer by ticking the appropriate box on the Answer Sheet. [0.5 Marks]

- White precipitate is observed
- Black precipitate is observed
- Precipitate is not observed

DO NOT FILL IN THIS TABLE

F.1.4. Formulate the stoichiometric equation of the reaction on the Answer Sheet. [0.5 Marks]

F.2. Reaction with bromothymol blue indicator.

F.2.1. Take out the latex tube from the $Ca(OH)_2$ solution and immerse it in bromothymol blue solution (ID# II) as shown in **Figure 6**.

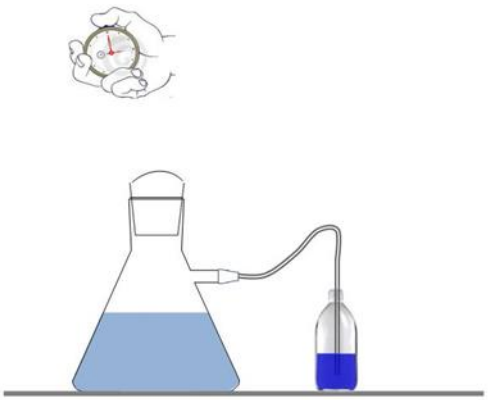


Figure 6

F.2.2. Gently swirl the reaction flask in a circular motion for 60 seconds for the gaseous product to bubble into the bromothymol blue solution.

F.2.2.1. Observe whether there is any change in the bromothymol blue solution.

Choose the correct answer by ticking the appropriate box on the Answer Sheet. [0.2 Marks]

• Yellow color is observed	
• Blue color is observed	DO NOT FILL IN THIS TABLE
• Red color is observed	
• White color is observed	

F.2.2.2. What can you deduce from the observation of the bromothymol blue solution? (Mark the correct answer). [0.2 Marks]

• An increase in pH	
• A decrease in pH	DO NOT FILL IN THIS TABLE
• No change in pH	

F.2.2.3. Formulate the dissociation equation which explains the pH change in the indicator solution. [0.6 Marks]

G. *Data processing and analysis:*

Calculate and complete data in **Table 1.1** and **Table 1.2 (on the Answer Sheet)**.

G.1. Calculate the gaseous product flow (F) for each time interval (Δt=2 min) according to **Equation 2**:

$$F = \frac{V_a(t) - V_a(t - \Delta t)}{\Delta t}$$

Equation 2

Being:

- F: gaseous product flow [ml/min]
- $V_a(t)$: Accumulated volume at time t [ml].

G.2. Plot the data of **Table 1.1** and **Table 1.2** together on the supplied grid **on the Answer Sheet** by using Cartesian coordinate system as follows: [2.5 Marks]

G.2.1**. GRAPH A**: Accumulated gas volume ($V_a(t)$; [ml]) vs. Fermentation time [min].

G.2.2**. GRAPH B**: Gaseous product flow (F(t); [ml/min]) vs. Fermentation time [min]. [1.8 Marks=1.5 Marks (plotting data) + 0.3 identify fermentation stages]

G.3. Draw a cross (X) on the curve of **GRAPH A** at t = 40 min of fermentation.

The initial sample of glucose was partially metabolized after 40 min of fermentation. The glucose that is not metabolized remains in the reaction flask solution and is called ¨residual glucose¨. The metabolized glucose was transformed into gaseous product CO_2 and ethanol. Most of gaseous product CO_2 was transferred to the graduated cylinder through the latex tube. The remaining fraction of the gaseous product CO_2 was dissolved in the reaction flask solution. **Figure 7** *summarizes the process.*

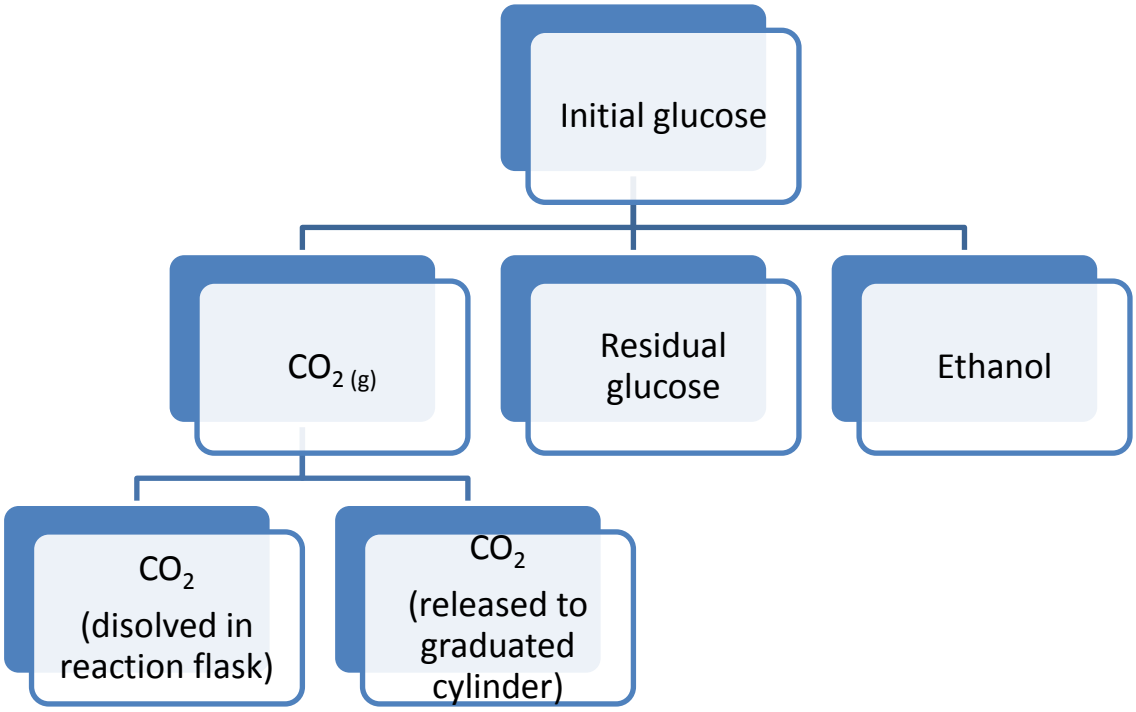


Figure 7

Perform the following calculations on the Answer Sheet.

G.3.1. Calculate the amount (in grams) of metabolized glucose that produced the CO_2 released into the graduated cylinder. To do this, consider the volume of CO_2 accumulated at t=40 min. Use stoichiometric calculations, **Equation 1** and the following data: [1.0 Marks]

Element	Relative Atomic Mass
C	12.010
H	1.008
O	16.000

CO_2 Molar volume at reaction conditions	22.4 L/mol

G.3.2. The amount of **residual glucose** can be estimated according to the following procedure:

G.3.2.1. Identify the time intervals of the fermentation stages (a, b and c) **up to t=40 min** on **GRAPH B**.

a. **An increase in the rate of gas production.**
b. **A decrease** in the rate of gas production.
c. **Constant** rate of gas production.

For such purpose, draw marks or bars in ink on **GRAPH B on the Answer Sheet** to indicate the different fermentation stages and write the corresponding letter.

G.3.2.2. Calculate the natural logarithm (ln) of measured gaseous product flow (F) during the fermentation stage ' A decrease in the rate of gas production' (Stage b, **GRAPH B**), and complete **Table 2.1**. with the calculated values on the Answer Sheet.

Table 2.1.: Calculate the natural logarithm (ln) of data of the fermentation stage corresponding to "A Decrease in the rate of gas production" [0.5 Marks]

Time [min]	F [ml/min]	ln(F)

DO NOT FILL IN THIS TABLE

G.3.2.3. Plot the data of **Table 2.1** on the supplied grid by using a Cartesian coordinate system as follows: ln F vs. time [min] (**GRAPH C**). [1.0 Marks]

G.3.2.4. Draw a straight line that best fits the data points of **GRAPH C**. [1.0 Marks]

G.3.2.5. Calculate the slope (A) and obtain the y-intercept (B) of the fitted line on the **GRAPH C**. Record the calculated values on **Table 2.2** on the Answer Sheet. [0.7 Marks]

Table 2.2: Linear equation of calibration curve

Linear equation	
Slope(A)	DO NOT FILL IN THIS TABLE
y-intercept(B)	

G.3.2.6. There is a hypothetical CO_2 volume (V_h) associated to the residual glucose. This is the volume of CO_2 that could be released into the graduated cylinder if the residual glucose were metabolized. This CO_2 volume is calculated through Equation 3. Calculate and record the value of V_h on the Answer Sheet. [0.2 Marks]

$$\mathbf{V_h = -\frac{e^{[A \times t_h + B]}}{A}}$$

Equation 3

Being:

- $t_h = 40$ min
- V_h: Hypothetical CO_2 volume [ml]
- A: slope of the fitting line obtained in Section G.3.2.5
- B: intercept of the fitting line obtained in Section G.3.2.5

G.3.2.7. Calculate stoichiometrically the mass of glucose that corresponds to the volume V_h of CO_2. That amount of glucose is the residual glucose in the reaction flask. [0.4 Marks]

G.3.2.8. Calculate the **total mass of glucose** that was fermented during the 40 min fermentation. Calculate and record the value on the Answer Sheet. [0.2 Marks]

G.3.2.9. Calculate the **total mass of CO_2 produced** by the glucose consumed calculated in section G.3.2.8. Record the value on the Answer Sheet. [0.4 Marks]

G.3.2.10. Calculate the **mass of CO_2 released** to the graduated cylinder during the 40 min fermentation. Use the V_a (40 min) for calculation. Record the value on the Answer Sheet. [0.4 Marks]

G.3.2.11. Calculate the **mass of CO_2 dissolved** in the reaction flask. Record the value on the Answer Sheet. Assume that no CO_2 is dissolved in the water outside the reaction flask during the whole process. [0.2 Marks]

G.3.2.12. Calculate the **solubility of CO_2** (in g/L) in the reaction flask. Record the value on the Answer Sheet. [0.3 marks]

G.3.3. Calculate the **amount of ethanol** (**in moles**) produced at t=40 min by using stoichiometric calculations and **Equation 1**. Resolve the calculation and record the answers on the Answer Sheet. [0.4 Marks]

G.3.4. Calculate the resulting **concentration of ethanol** (in g/100 ml) in the reaction flaskat t=40 min by using the amount of ethanol previously calculated in G.3.3. Resolve the calculation and record the answers on the Answer Sheet. [0.5 Marks]

G.3.5. Gas production rate slows down at stage ¨b¨ (**GRAPH B**) for some of the reasons detailed below. Consider alcohol concentration calculated previously (G.3.4), and toxic concentration threshold for yeast, which is 14 g/100 ml. [0.5 Marks]

Choose the primary reason by ticking the appropriate box on the Answer Sheet.

a. Yeast death

b. Inhibition of yeast due to the concentration of alcohol.

c. Shortage of fermentable substrate

DO NOT FILL IN THIS TABLE

Time : 4 hrs
Marks : 40

Experimental test

SECTION **2: REFRACTOMETRIC DETERMINATION OF SUCROSE CONCENTRATION** [15.0 Marks]

INTRODUCTION:

Refractometry is a physical testing method of measuring the refractive index of substances. Since the refractive index is a constant for a particular liquid at a given temperature, it can be used to help identify substances, check for purity, and measure concentrations. In fact, the refractive index for many binary mixtures changes linearly with concentration over a wide range of concentrations.

Food and beverage industries use the refractive index to find the concentration of sugar in different samples. The instrument used for this purpose is called ***refractometer.*** *The refractive index is a measure of the speed of light in a medium and therefore, when light passes from one medium to another with different refractive indexes, the path of light is deviated from the original path. This phenomenon is the fundament of the refractometer.*

The refractive index of juices or drinks is influenced by the concentration of soluble solids, which are mainly constituted by sucrose. In addition to sucrose, other soluble solids such as organic acids and salts, can affect the measurement. Consequently, it is considered that the measurement in these samples corresponds to sucrose content, with an accuracy of about 0.5%. The total sucrose concentration is expressed in Brix degrees (° Bx), whose units are grams of sucrose per 100 g of solution [% w/w]. For example: A honey of 25 ° Bx contains 25 g of sucrose per 100 g of honey.

In this experiment the sucrose concentration unit will be g/100 ml.

EXPERIMENTATION

Reagents and materials

ID #	Material for experimental assay	Quantity
12	Home-made refractometer	1
13	Laser source	1
14	Funnel	1
15	50 ml volumetric flask	1
16	125 ml plastic bottle	5
17	25 ml pipette	1
18	Pipette filler	1
19	Marker pen	1

ID #	Reagent for experimental assay	Quantity
VII	Initial solution, 62.5 g/100 ml sucrose, 100 ml	1
VIII	Water squeeze bottle	1
IX	Plastic Pasteur pipette	1
X	Sucrose solution, unknown concentration, 50 ml	1
XI	Honey solution, 50 ml	1
XII	Beverage sample, 50 ml	1

PROCEDURE:

CAUTION:
Do not point the laser beam to the eyes. Turn it on only for the calibration and measuring processes.
Laser specifications: <1 mW, red.

A. ***Instructions for assembling the refractometer according to*** **Figure 8*.***

This equipment will be used to construct a calibration curve and to determine the sucrose concentration of the Samples. The home-made refractometer is almost completely assembled (ID# 13). Only the laser beam alignment is required. For such purpose:

A.1. Turn on the laser source (ID# 13) and move it side to side on the main wooden base until the beam falls on the center of the millimetric scale, as show in **Figure 8**.

A.2. Mark the correct position of the laser source on the main wooden base using the marker provided.

A.3. Take the laser source and peel off the adhesive tape cover on the bottom. Place the laser source in the previously marked position on the main wooden base (A.2) and exert gentle pressure to ensure proper adhesion. Ensure that you have placed the laser source in the correct position before fixing it on the wooden base.

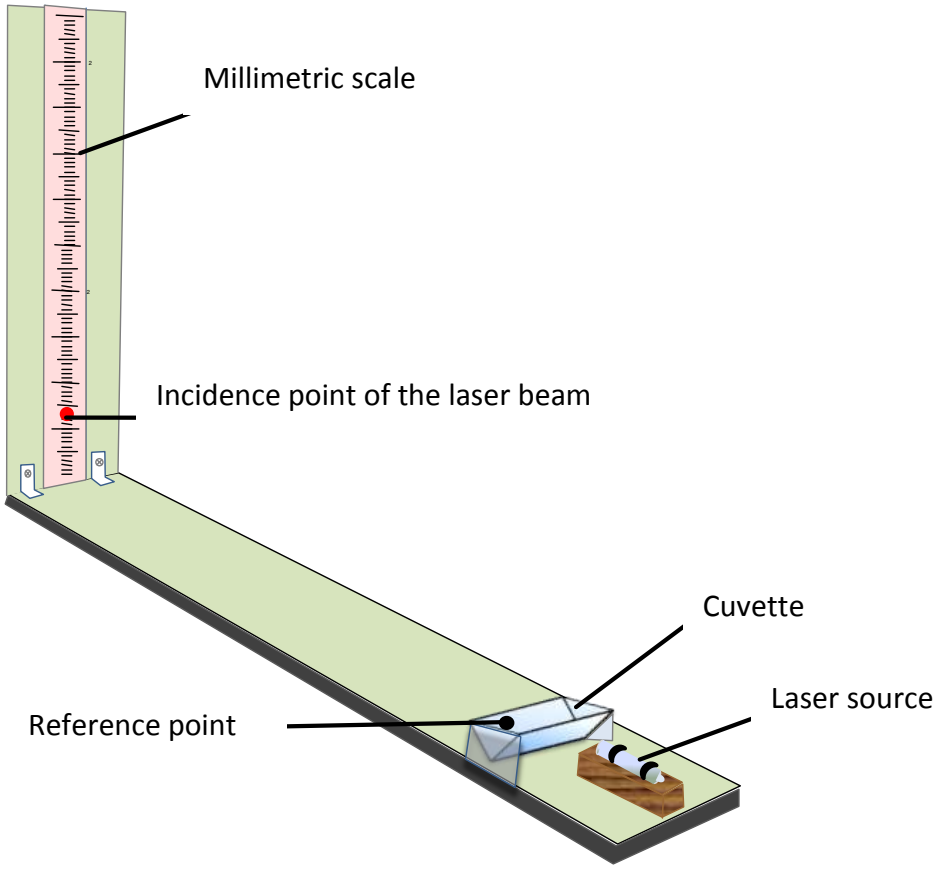


Figure 8

B. ***General instructions for the preparation of the calibration curve:***

B.1. Preparation of calibration solutions

B.1.1. From the initial solution of the sucrose (ID# VII), 5 other solutions of sucrose need to be prepared at 5, 10, 15, 20 and 25 g/100 ml concentration. To do this , some volumes of the initial solution are diluted to the final volume of 50 ml. Calculate the volumes of the stock solution needed to prepare 5, 10, 15, 20 and 25 g/100 ml sucrose solutions (considering that the sucrose initial solution (ID# VII) has an initial concentration of 62.5 g/100 ml and the final volumes of each stock solution will be 50 ml) Record the calculated values on **Table 3.1** on the Answer Sheet. [1.2 Marks]

Table 3.1: Summary of calibration solutions data

Solution identification	Initial Concentration [g/100 ml]	Initial Volume [ml]	Final Concentration [g/100 ml]	Final Volume [ml]
A	62.5		5	50
B	62.5	DO NOT	10	50
C	62.5	FILL IN	15	50
D	62.5	THIS TABLE	20	50
E	62.5		25	50

B.1.2. Prepare sucrose dilutions calculated in the previous section (B.1.1) as follows.

B.1.2.1. Label the plastic bottles (ID# 16) with identification letters A, B, C, D and E, which correspond to different concentration levels of the standard solutions [A=5 g/100 ml, B=10 g/100 ml, C=15 g/100 ml, D=20g/100 ml, E=25g/100 ml]. Different solutions are to be prepared using the pipette (ID# 17), the pipette filler (ID# 18), and the 50 ml volumetric flask (ID# 15). Prepare the solutions in order of increasing concentration.
B.1.2.2. Take the calculated volume of 62.5 g/100 ml sucrose solution (ID# VII) using the pipette and pipette filler.
B.1.2.3. Transfer the taken volume to the 50 ml volumetric flask.
B.1.2.4. Fill the volumetric flask with the water squeeze bottle (ID# VIII) up to a volume close to the calibration mark. Gently shake to homogenize.
B.1.2.5. Complete the volume up to the calibration mark using the plastic Pasteur pipette (ID# IX).
B.1.2.6. Transfer the solutions to the corresponding labelled plastic bottle (ID# 16).

B.2. Data acquisition for the calibration curve using the home-made refractometer (ID# 12).
Special considerations for measurement:

- The cuvette must be fully filled for measurement.
- The cuvette must be placed at the same position for all the measurements. For such purpose, a reference point is drawn on one of the cuvette´s side (**Figure 8**).
- Dry the outside of the cuvette with absorbent paper (ID# C) before each measurement.

General procedure for measurements:

B.2.1. Remove the cuvette from the refractometer, empty it, dry the inside of it, put it back in the original position, fill it with the respective solution, and dry it on the outside..
B.2.2. Turn on the laser source (ID# 13). The laser beam stays on while holding down the switch
B.2.3. Use the marker to draw the point of incidence of the light beam on the millimetric scale.

General procedure to build the concentration scale
The scale should be built from low to high sucrose concentration after measuring the zero of the scale.

B.2.4. Determination of the zero point of the scale: follow the procedure described above (B.2.1-B.2.3) using water (ID# VIII) for determining the reference point (zero) of the scale.
B.2.5. Determination of scale points:
B.2.5.1. Repeat the procedure described above (B.2.1-B.2.3) with sucrose solution ¨**A**¨.
B.2.5.2. On the millimeter scale, measure the linear distance between marks of zero and concentration of sucrose solution ¨**A**¨.
B.2.5.3. Record the obtained data on **Table 3.2** on the Answer Sheet.
B.2.5.4. Repeat the procedure described above (B.2.5.1-B.2.5.3) with the remaining sucrose solutions (**B, C, D** and **E**).

Table 3.2: Calibration curve data [4.0 Marks]

Solution Name	Sucrose Concentration [g/100 ml]	Laser beam deviation from the zero point [mm]
Water	**0**	0
A	**5**	
B	**10**	DO NOT
C	**15**	FILL IN
D	**20**	THIS TABLE
E	**25**	

B.3. Graphic of the calibration curve (**GRAPH D**): [3.0 Marks]

B.3.1. Plot the data recorded on **Table 3.2** (Laser beam deviation from the zero point[mm] vs. Sucrose concentration [g/100 ml]) by using a Cartesian coordinate system.
B.3.2. Draw a straight line that best fits the data points of **GRAPH D**.

B.3.3. Calculate the slope (A) and the y-intercept (B) of the fitted line of **GRAPH D**. Record the calculated values on **Table 3.3** on the Answer Sheet. [0.9 Marks]

Table 3.3: Linear equation of calibration curve

Linear Equation	
Slope	DO NOT FILL IN THIS TABLE
y-intercept	

C. *Instructions for sample analysis (ID# X,ID# XIand ID#XII):*

C.1. Data acquisition of samples with unknown sucrose concentration by using a home-made refractometer (ID# 12).

Procedure for measurements:

C.1.1. Rinse the cuvette with water (ID# VIII). Dry the inside of it with the paper towel provided.
C.1.2. Fill up the cuvette with the sample of unknown sucrose concentration (ID# X).
C.1.3. Dry the outside of the cuvette and place it at the original position for measurement.
C.1.4. Turn on the laser beam.
C.1.5. With a marker, draw the point of incidence of the light beam on the millimeter scale.
C.1.6. Measure the distance between the marks of zero and the sample on the millimeter scale.
C.1.7. Record the obtained data on **Table 3.4** on the Answer Sheet.
C.1.8. Repeat the described procedure (C.1.1-C.1.7) with the remaining samples of unknown sucrose concentration (ID# XI and ID# 0XII).

Table 3.4: Data of analyzed samples [2.1 Marks]

Samples	Millimeters deviation [mm]
ID # X	DO NOT FILL IN THIS TABLE
ID # XI	
ID # XII	

C.2. Determine the sucrose concentration of the samples: [2.3 Marks]

C.2.1. Graphic determination:
C.2.1.1. SampleUse GRAPH D to determine the concentrations of samples X, XI, XII.

C.2.1.2. Record the data obtained in **Table 3.5** on the Answer Sheet.

C.2.2. Analytical determination:
C.2.2.1. Calculate sucrose concentration for the samples using the linear equation recorded in **Table 3.3** and data recorded in **Table 3.4**. Record the calculations on the Answer Sheet.
C.2.2.2. Record the results on **Table 3.5** on the Answer Sheet.

C.2.3. Calculate the percentage concentration difference [D(%)] between graphic and analytical determination by using **Equation 4**. Record the data obtained on **Table 3.5** on the Answer Sheet.

$$D(\%) = \frac{C_G - C_A}{C_A} \cdot 100$$

Equation 4

Being:

C_G: Sucrose concentration graphically obtained
C_A: Sucrose concentration analytically obtained

Table 3.5. Graphical and analytical determination of sucrose concentration in analyzed samples

Sample	Sucrose concentration graphically obtained [g/100 ml]	Sucrose concentration analytically obtained [g/100 ml]	Percentage ConcentrationDifference D[%]
ID # X			
ID # XI	DO NOT FILL IN THIS TABLE		
ID # XII			

D. Additional calculations and data analysis of samples XI and XII:

D.1. Calculate the original sucrose concentration (in °Brix) of the honey using the corresponding sucrose concentration analytically obtained for the honey sample (ID# XI), and considering that for its analysis the honey sample (ID# XI) was prepared as follows: 16 g of honey were diluted in water reaching a final volume of 100 ml. Record the data obtained on **Table 3.6** on the Answer Sheet. [0.3 Marks]

Table 3.6.: Sucrose concentration in original honey sample

Samples	Sucrose original concentration [°Brix]
ID # XI	DO NOT FILL IN THIS TABLE

Honey must have more than 65 °Brix for safe conservation.

D.2. Indicate whether the original honey sample meets the sucrose concentration requirement for a secure conservation. Mark with a cross the correct answer on **Table 3.7** on the Answer Sheet.

Table 3.7.: Safe preservation of honey [0.2 marks]

SAFE	NOT SAFE
DO NOT FILL IN THIS TABLE	

D.3. Calculate the theoretical deviation of the laser beam [mm] for a sucrose solution of 8.5g/100 ml concentration using the two procedures: the graphic representation and the analytical calculation (B.3). Record the calculations on the Answer Sheet. Record the results on **Table 3.8** on the Answer Sheet. [0.5 Marks]

Table 3.8.: Theoretical deviation of the laser beam [mm] for a solution 8.5g/100 ml sucrose concentration

Calculation	Theoretical deviation of the laser beam [mm]
Analytical	DO NOT FILL IN THIS
Graphical	TABLE

The label on the package of the analyzed beverage states that it contains a sucrose concentration of 15 ± 2 g *per serving of 200 ml.*

D.4. Considering the sucrose concentration found in the analyzed beverage, indicate whether it matches the sucrose concentration stated on the package label. Record the calculations on the Answer Sheet. Mark with a cross (X) the correct answer on **Table 3.9** on the Answer Sheet. [0.5 marks]

Table 3.9.: Analysed beverage specification according to the statement on the package label

Yes	DO NOT FILL IN THIS TABLE
No	