Yeast Respirometer Investigation

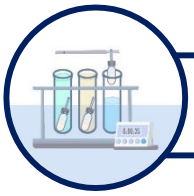

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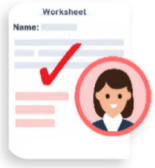
Table of Content

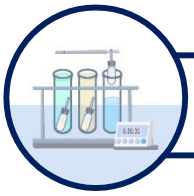
		Page
1	**Introduction**	**1-2**
	• Overview	1
	• Teaching Plan & Key Features	1-2
	• Important Notes	2
2	**Instructional Materials**	**3-15**
	• Student Worksheet 1	3-4
	• Student Samples 1 (Worksheet 1)	4
	• Student Worksheet 2	5
	• Student Worksheet 3	6
	• Teacher Notes 1	7-10
	• Laboratory Manual	11
	• Teacher Notes 2	12-15
3	**Supplementary Resources**	**16-19**
	• Possible Modifications	16-17
	• Technician Notes	18-19
	• References	19



Notes for teachers

- Scan the QR code to get the electronic files.
- Teachers are strongly encouraged to adapt and modify these resources as necessary.

Innovations in Biology Investigations



Yeast Respirometer Investigation

Overview

- The *Yeast Respirometer Investigation* is related to the use of sugar substitutes in breadmaking. Students investigate the effects of different types of sugar substitutes on the rate of yeast fermentation.
- Students are given the opportunity to design and carry out experiments in which they identify significant assumptions, consider limitations in measurement, and evaluate different experimental designs (i.e., within- and between-subject designs).

Teaching Plan

Prerequisite knowledge (scientific ideas)

- Alcoholic fermentation process
- Alcoholic fermentation as an enzyme-catalysed reaction

Lesson	Lesson sequence	Duration (mins)	Resources
Stage ❶ Preparing for the investigation • The investigation is set in a decision-making context (**Decision-making Task**). • The investigation is situated in an authentic daily-life context related to the use of sugar substitutes for breadmaking (**Contextualisation**). • Students have the opportunities to design their own respirometers and trial run their designs (*Trial Run*). • Students evaluate own and other set-ups in terms of their feasibility and accuracy (*Self & Peer Evaluation*). • Students read information to better understand the working principles of different set-ups (*Reading Materials*).			
1	• The teacher introduces the investigation context to students in *Worksheet 1*. • The teacher provides materials for students to design and trial run their set-ups.	40	*Worksheet 1*, Student Samples 1
Before Lesson 2	• The teacher distributes *Worksheet 2* for students to complete at home and be familiar with the working principles of different set-ups.		*Worksheet 2*
Stage ❷ Designing the investigation • The teacher shows students the microscale respirometer.			
2	• The teacher provides feedback on students' responses in *Worksheet 2* in class. • The teacher shows students the microscale respirometer and asks them to explain how the set-up can be used for investigating the effect of sugar substitutes.	40	*Worksheet 3*
3	• The teacher discusses with the students some questions related to the experimental design. • Teacher provides students with laboratory manual for preparation at home.	40	Teacher Notes 1
Stage ❸ Carrying out the investigation • Students watch pre-recorded video that show the procedures of how to set up the respirometers (*Video with Guidance on Procedures*). • The teacher performs demonstration to show how to assemble to microscale set-up (*Teacher Demonstration*). • Students use microscale instrumentation that reduces the time of the experiments (**Microscale Instrumentation**). • Students collect more complex data sets by setting up replicates (**Complex Data Set**). • Students use cameras to record data (*Digital Tool*).			

4	• The teacher performs a demonstration of how to assemble the microscale respirometer. • Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills. • Students carry out the investigation.	40	Laboratory Manual
Stage ❹ Explaining and evaluating data • Students evaluate the validity of using two parameters to measure the fermentation rate of the yeast (i.e. the number of bubbles produced in a fixed period, and the time for the colour of the indicator to change from green to yellow). • Students use data to make informed decisions on whether and how to use sugar substitutes to produce breads with similar textures and appearances.			
Before Lesson 5	• Students complete data reporting and analysis at home. • Teacher collects and marks student responses.		Teacher Notes 2
5	• Teacher provides feedback on students' performance related to data reporting and analysis**.**	40	Teacher Notes 2

Important Notes

- Students are *not* required to explain why yeasts can use sugar substitutes for fermentation. Rather, they are expected to use data to make decisions about which sugar substitute(s) to replace refined sugar.

Instructional Materials

Stage 1 Preparing for the investigation

Student Worksheet 1

Notes for teachers

- Teachers can distribute *Worksheet 1* and instruct students to design experimental set-ups to measure the rate of yeast fermentation.
- Teachers can provide students with concrete materials for their trial runs to see if their set-ups are feasible. See the *Supplementary Resource* section for the list of materials.
- The *Supplementary Resource* section provides examples of possible set-ups.

Task 1

- Read the scenario and complete the questions that follow:

Scenario

Adam is weight conscious. He has recently replaced refined sugars (e.g., sucrose) with sugar substitutes (i.e., calorie-free sweeteners). He wondered if sugar substitutes can be used to replace sucrose in breadmaking. He would like to investigate the effects of different sugar substitutes on the rate of yeast fermentation.

Refined sugar

Sugar substitute 1

Sugar substitute 2

Sugar substitute 3

To perform the investigation, he must first assemble a set-up that allows him to measure the fermentation rate of yeast. The following shows a list of apparatuses and materials that he can find in the science laboratory:

Yeast	Test tube	Syringe	Phenolphthalein
Balloon	Boiling tube	Syringe cap	Straw
String	Rubber tubing	Paraffin oil	Glass bottle
Boiled sugar substitutes 1, 2, 3	Plastic dropper	Dropper bottle	Sodium hydroxide solution
Boiled distilled water	Timer	Ruler	Water
Boiled sucrose solution	Electronic balance	Measuring cylinder	Petri dish

1. Based on the materials and apparatuses given, draw *at least two* set-ups that your group thinks Adam can use to measure the fermentation rate of yeast.
Notes:
- Your group can trial run your set-ups.
- Be prepared to show your peers the set-ups.

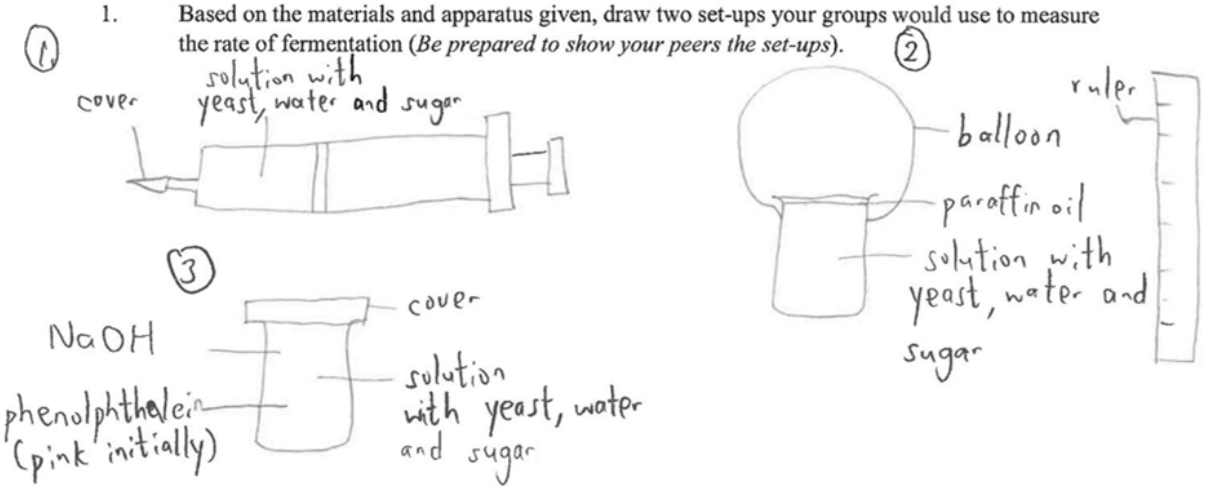
Students had opportunities to try out their designs.

2. Briefly explain how you will use the set-ups to investigate the effect of the three sugar substitutes on the rate of fermentation.

Student Samples 1 (Worksheet 1)

Directions:

1. Based on the materials and apparatus given, draw two set-ups your groups would use to measure the rate of fermentation (*Be prepared to show your peers the set-ups*).

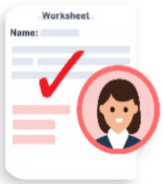


2. Briefly explain how you will use the set-ups to investigate the effect of the three sugar substitutes on the rate of fermentation.

(1) measure the volume of CO_2 produced per unit time

(2) measure the increase in height of balloon per unit time

(3) measure the time for the disappearance of the pink colour (CO_2 neutralize NaOH)



Notes for teachers

- The student sample shows the drawing of some set-ups designed by students.
- Teachers can distribute *Worksheet 2*, which prompts students to analyse the principles of different set-ups after they have explained their set-ups.
- Students' responses in *Worksheet 2* can be collected using a *Google Form*.
- Teachers can read *Appendix 3* from Chan et al. (2021) for the possible set-ups and their working principles.
- Scan the QR code to access *Appendix 3*.

Task 2

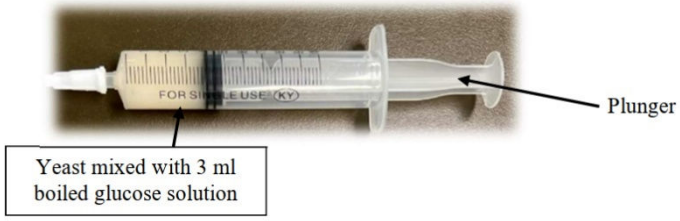
- Adam found the following information.

Source 1: **Experimental set-ups for investigating the rate of yeast fermentation**

Adam found two experimental set-ups that can be used to measure the rate of yeast fermentation:

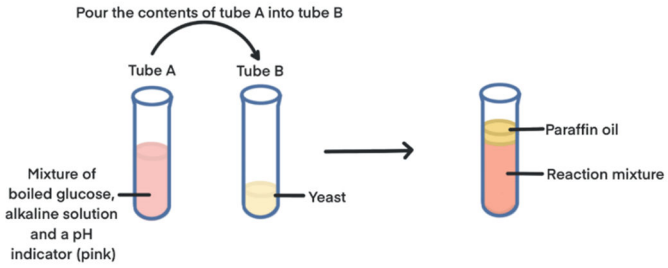
Set-up 1

- This set-up uses an airtight syringe containing yeast mixed with boiled sugar solution. To measure the rate of yeast fermentation, the initial position of the plunger at the beginning of the experiment is recorded. Readings are then taken at specific time intervals.



Set-up 2

- This set-up involves two tubes. Tube A contains a mixture of boiled glucose solution and an alkaline solution containing a pH indicator (pink under alkaline pH values and colourless when the pH decreases to 7 or lower). Tube B contains yeast. The two tubes are mixed, and a layer of paraffin oil is added to the reaction mixture. The time taken for the disappearance of the pink colour in the reaction mixture can be used to indicate the rate of yeast fermentation.



Answer the following questions about the working principles of the two set-ups Adam has given to you:

(a) Write a word equation for yeast fermentation.

(b) Based on your answer in (a),

(1) explain why experimental S*et-up 1* can be used to measure the yeast fermentation rate. (*Hints:* Consider the products formed in yeast fermentation. What will happen to the position of the plunger after the experiment starts? Why? How can the fermentation *rate* be determined?)

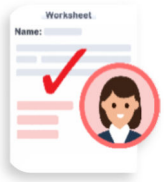
(2) explain why experimental *Set-up 2* can be used to measure the yeast fermentation rate. (*Hints:* Consider the products formed in yeast fermentation and determine if they have an effect on the pH of the reaction mixture. What is the relationship between the time taken for the disappearance of the pink colour in the reaction mixture and the rate of yeast fermentation?)

(c) (1) Adam reminded you that the boiled glucose solution should be cooled before mixing with the yeast when using both set-ups. Explain why this step is necessary.

(2) Suggest another precaution you will need to take when assembling *Set-up 1*.

Scan the QR code to get a copy of the *Google Form*.

Student Worksheet 3



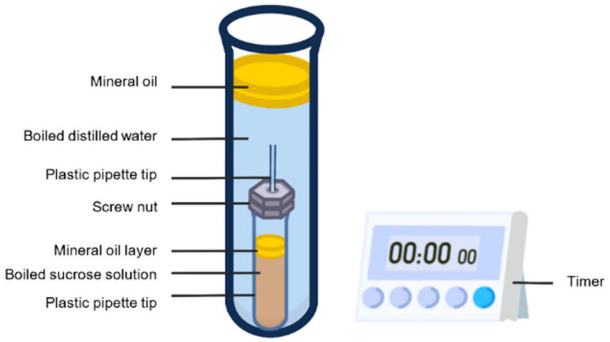
Notes for teachers

- Teachers can provide feedback on student responses in *Worksheet 2*.
- Teachers can show students the microscale respirometer and ask them to think about how the set-up can be used to measure the rate of yeast fermentation.
- See Chan (2016) for more information about the set-up.
- Teachers may show a video to the students. Scan this QR code to access the video.

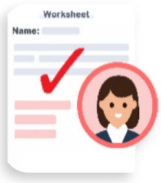
Task 3

Possible questions

1. Andrew suggested using the following experimental set-up to measure the dependent variable. To ensure that the apparatus is functioning properly, he recommends first testing it with the boiled sucrose solution.
   (a) Predict *one* observable change during the experiment.
   (b) Describe how you would use the experimental set-up to compare the yeast fermentation rate among sugar substitutes (1, 2, 3).
   (*Hint:* In your answer, please include [1] how to manipulate the independent variable, [2] how to measure the dependent variable, and [3] the relationship between the dependent variable and the measurement method.)



Teacher Notes 1



Notes for teachers

- After introducing the microscale respirometer, the following shows the main investigation context for students to work on.
- Some questions can be used by teachers to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

Task 3

Scenario

Some people who try to lose weight eat less food containing refined sugar. However, the breadmaking process uses a lot of refined sugar (i.e., sucrose). In recent years, it has become more common to use sugar substitutes (zero-calorie sweeteners) to replace refined sugar to reduce calorie intake.

Can sugar substitutes replace refined sugar in making bread? If so, which type of sugar substitute allows the yeast to ferment the best?

Here are some of the materials and apparatuses in the science laboratory:

Yeast	Boiling tube	Plastic dropper	Paraffin oil
Boiled sucrose solution	Boiled distilled water	Timer	Screw nuts
Boiled sugar substitute 1	Boiled sugar substitute 2	Boiled sugar substitute 3	Universal indicator

Using your biological knowledge of yeast fermentation, design a valid and reliable experiment to carry answer the investigation question.

Possible questions

1. Adam and his friends are discussing the possible significant assumptions of this design.

Amy

A significant assumption is that the fermentation rate is higher for sugar substitute 1.

Is it the assumption that the carbon dioxide produced during fermentation does not dissolve in the sugar substitute solution?

Adam

Carlos

I would say that the rate of carbon dioxide produced by the yeast indicates the rate of fermentation is one of the significant assumptions.

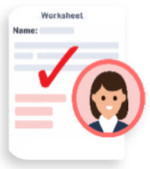
I have one more idea, which is the sugar substitute solution should be boiled before the experiment.

Betty

Which person(s) do you agree with? Put a "✓" into the appropriate box(es) below.
(*Hints:* There can be more than one assumption.)

- ☐ Amy
- ☐ Adam
- ☐ Carlos
- ☐ Betty

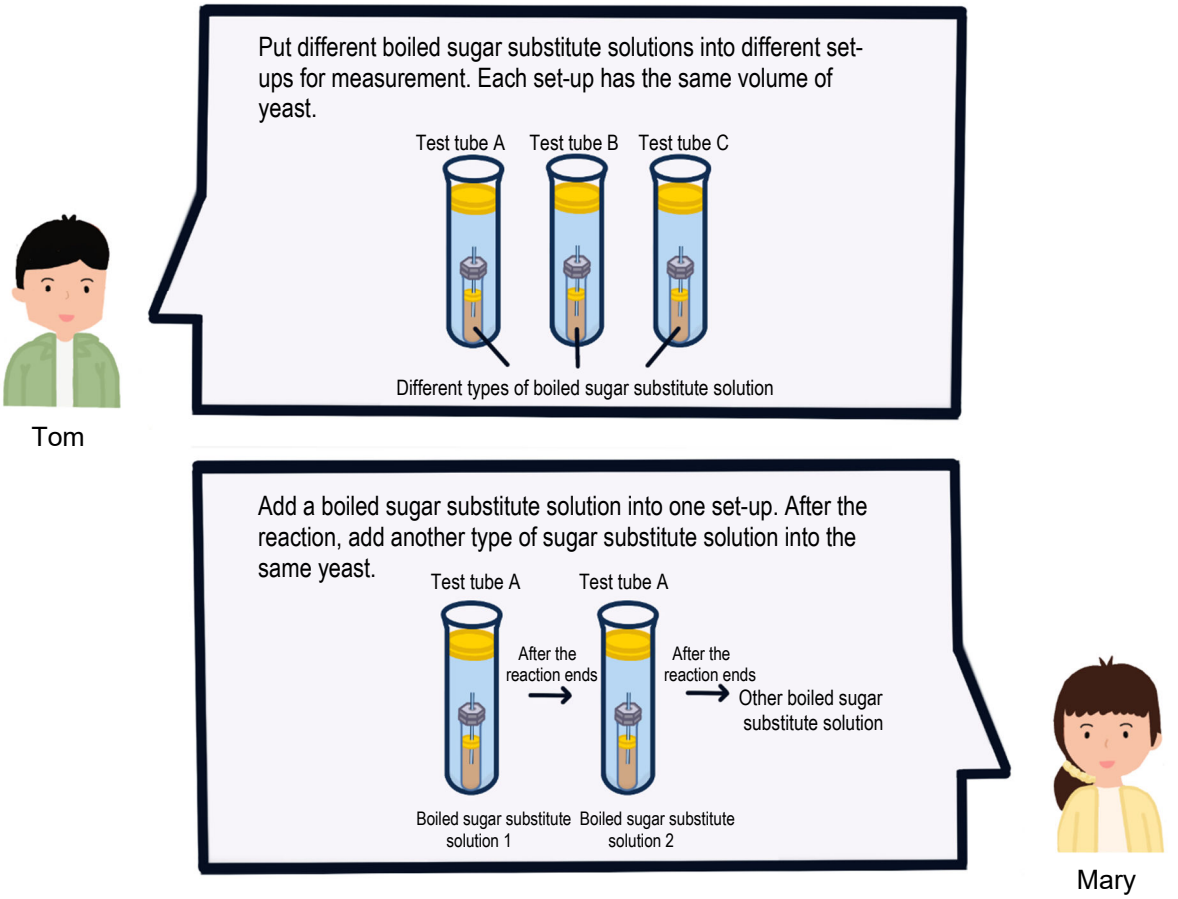
This question format enables the identification of distractors.



Notes for teachers

- Q.1 assesses students' ability to identify significant multiple assumptions from the choices. Distractors include precautionary steps and predicted results.

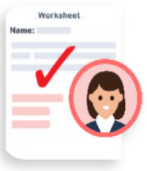
2. Tom and Mary are discussing two experimental designs to achieve the aim of this experiment:



Which design would you choose? Why?
(Please put a '✓' in the box of the experimental design you choose.)

☐ Tom's design
☐ Mary's design

Explanation:

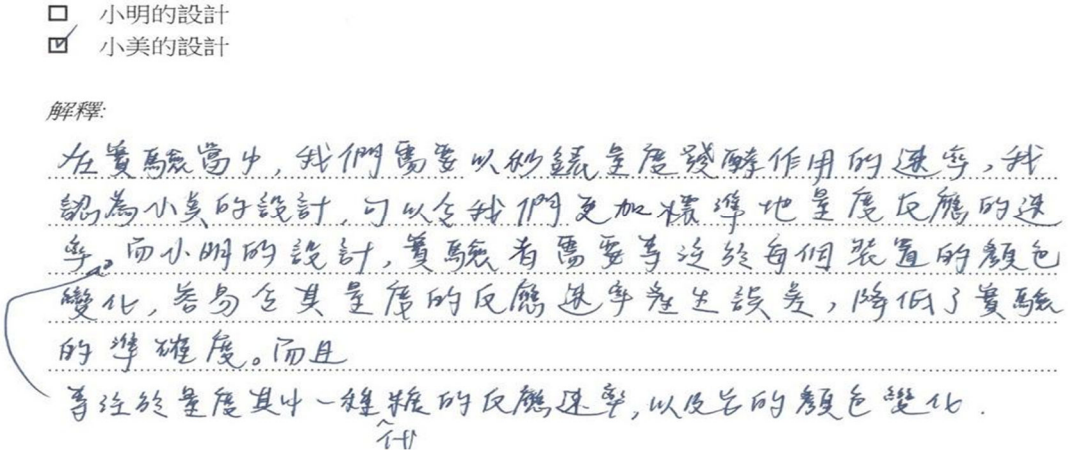


Notes for teachers

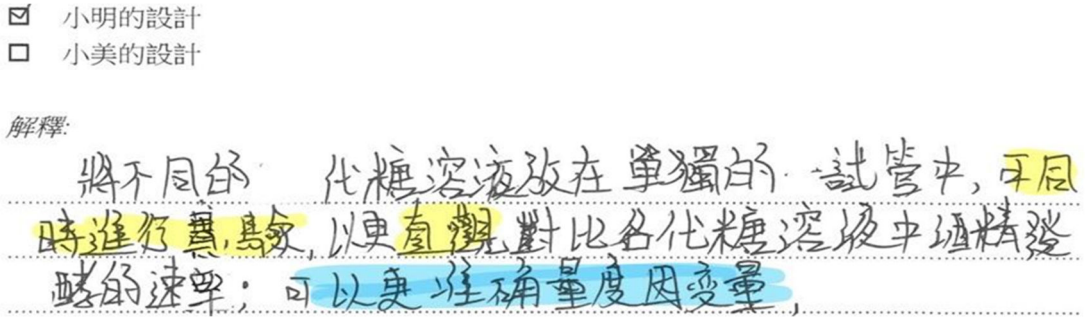
- Q.2 assesses students' ability to analyse alternative designs (i.e. within- and between-subject designs) in terms of generating valid and reliable data.

The following are some examples of students' responses to Q.2:

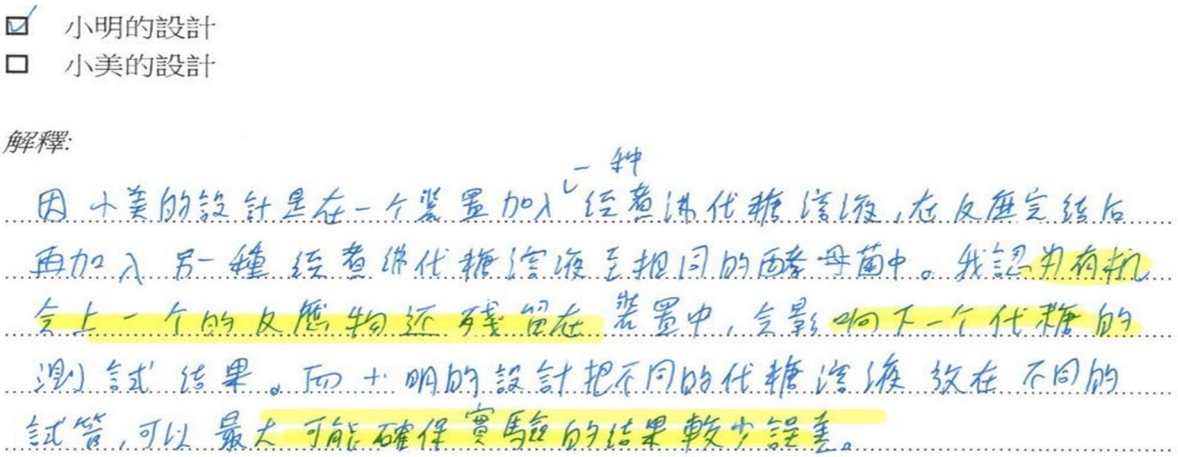
Sample 1



Sample 2



Sample 3

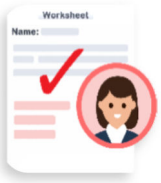


About the samples

- In Sample 1, the better design was not chosen.
- In Sample 2, the better design was chosen but the student did not explain why the design allows for the collection of more valid data. The student provided reasons not related to the validity of the data (i.e. less time).
- In Sample 3, not only was the better set-up chosen but also the carry-over effect was explained. Other effects include the death of the yeast or the change in the pH of the solution as a result of the previous treatment.

Stage ❸ Carrying out the investigation

Laboratory Manual



Notes for teachers

- Teachers can distribute the manual for students to read and prepare before the investigation.
- The pre-recorded video provides the steps for assembling the microscale respirometer.
- Teachers can perform a demonstration to show students how to set up a microscale respirometer and highlight the critical steps.
- Teachers can ask questions to check if students fully understand the procedures (e.g., how many respirometers do we need to set up?).
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

Task 4

- Read the following procedures to carry out the investigation.

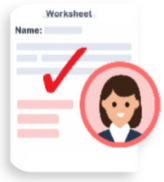
Procedure

1. Measure 15 mL of boiled distilled water containing a universal indicator using a measuring cylinder. Transfer it to a 25-mL boiling tube.
2. Expel 3 mL of air from a 3-mL plastic pipette.
3. Use the 3-mL plastic dropper to suck up 1 mL of yeast extract solution.
4. Invert the dropper to allow the liquid to flow into the bulb portion.
5. Expel the air from the 3-mL plastic dropper containing the yeast extract.
6. Use the 3-mL plastic dropper to suck up 1 mL of the boiled sucrose solution, sugar substitute 1, sugar substitute 2, sugar substitute 3, or distilled water.
7. Invert the dropper to allow the liquid to flow into the bulb portion.
8. Gently squeeze the bulb of the pipette to mix the yeast extract and sugar/sugar substitute/distilled water solution.
9. Expel the air from the 3-mL plastic dropper.
10. Use the 3 mL plastic dropper to suck up 400 µL of paraffin oil.
11. Invert the dropper to allow the liquid to flow into the bulb portion.
12. Secure two screw nuts onto the neck of the pipette.
13. Use forceps to place the entire set-up into the test tube, ensuring that the dropper is fully submerged in the water.
14. Add a layer of paraffin oil.
15. Start a timer and record the time required for the universal indicator to change from green to yellow.
16. Allow the set-up to reach equilibrium for 2 minutes and then record the number of bubbles generated within 15 minutes.
17. Repeat *Steps 1* to *16* one more time.

Scan this QR code to see how to assemble the experimental set-up.

Stage ❹ Explaining and evaluating data

Teacher Notes 2



Notes for teachers

- The following are possible questions that teachers can use to guide students in thinking about or assessing their scientific inquiry skills related to data analysis and interpretation.
- Student work samples are shown below to illustrate possible student thinking to some of the questions.

Task 5

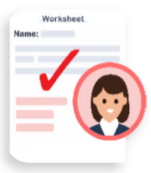
Possible questions

1. According to the data you collected, which of the following parameters can more accurately measure the yeast fermentation rate? Explain your answer.
   (Put a '✓' in the appropriate box.)
   ☐ Time taken for the universal indicator to change from green to yellow
   ☐ Number of bubbles produced within 15 minutes

2. Adam wants to use sugar substitutes instead of sucrose to make bread. He hopes his bread will be as fluffy as when using sucrose. Based on the data you collected, answer the following two questions:
   (a) Which sugar substitute should Adam use? Explain your answer.
   (Put a '✓' into the appropriate box.)
   ☐ Sugar substitute 1
   ☐ Sugar substitute 2
   ☐ Sugar substitute 3

   (b) Propose a method to make the fermentation rate using the selected sugar substitute similar to the rate using sucrose.
   (*Hint:* Consider the factors that affect the yeast fermentation rate.)

3. Adam found that yeast can use one of the sugar substitutes. He would like to determine the optimum temperature at which the yeast ferments the sugar substitute. How would you modify the experimental design of this investigation to achieve this goal?



Notes for teachers

- Q.1 assesses students' ability to assess the appropriateness and accuracy of the methods to determine the dependent variable based on their data.
- Q.2 assesses students' ability to make informed decisions based on their data.
- Q.3 assesses students' ability to modify the experimental designs for answering a new investigation question.

The following are some examples of students' responses to Q.1:

Sample 1

2. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。
（在下列方格 ✓ 以選出你的答案）

☑ 通用指示劑由綠色轉為黃色所需要的時間

☐ 十五分鐘內所產生的氣泡數目

因為試管內的通用指示劑，受酵母所發酵釋出二氧化碳及氣泡，从二氧化碳是酸性物質，使通用指示劑由綠色轉為黃色，若釋放大量二氧化碳在通用指示劑裏，使通用指示劑發揮更清晰的黃色。

Sample 2

2. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。
（在下列方格 ✓ 以選出你的答案）

☐ 通用指示劑由綠色轉為黃色所需要的時間

☑ 十五分鐘內所產生的氣泡數目

酵母十五分鐘內所產生的氣泡數目較為明顯及最快能看到酵母發酵的速率。而綠色轉為黃色的色差中，不能確保是否已完全轉為黃色。

Sample 3

2. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。
（在下列方格 ✓ 以選出你的答案）

☐ 通用指示劑由綠色轉為黃色所需要的時間

☑ 十五分鐘內所產生的氣泡數目

因為數氣泡數目是最客观反映CO_2釋出的量，如果使用指示剂变色时间为指标，不同人着颜色变化的定义不同，例如怎样才算黄色，或者淺黄色算不算等等，根据颜色是主观的，每人对色觉的敏感度不一，因此数气泡数目能最大程度减少CO_2释出需时及数量之误差，更能显示实验的準确度。

About the samples

- In Sample 1, the right measurement parameter was not chosen, and invalid reasons were provided.
- In Samples 2 and 3, the right parameter was chosen. Sample 3 further explained why relying on visual inspection by naked eyes as a measurement method is an important limitation (i.e. subjective judgment of colour change).

The following are some examples of students' responses to Q.2:

Sample 1:

4. 如果以其中一種實驗中使用的代糖取替蔗糖以製造麵包，在相若的發酵時間下，令麵包的鬆軟程度像使用蔗糖那樣。***請根據你所收集的數據***回答以下兩題。

(a) 你可以使用哪一種代糖？***請解釋你的答案***。
（在下列方格 ✓ 以選出你的答案）
☐ 代糖 1 號
☐ 代糖 2 號
☑ 代糖 3 號

因它的反應速率最接近蔗糖，說明在使用3號代替蔗糖製造面包時，鬆軟程度會最接近蔗糖面包。

(b) 提出*一項*方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。
(提示：考慮影響酵母發酵速率的因素)

使用該代糖時，增加酵母菌的份量，調整至與它們的酵母發酵速率接近一致

Sample 2

(a) 你可以使用哪一種代糖？***請解釋你的答案***。
（在下列方格 ✓ 以選出你的答案）
☐ 代糖 1 號
☐ 代糖 2 號
☑ 代糖 3 號

代糖3号的發酵程度最快，它產生的氣泡時間較其他代糖快，缺氧呼吸的速率也快可以最大程度令麵包較鬆软，代糖3号在實驗過程中產生最多的氣泡有29個而代糖2号有16个，代糖1号有7個。蔗糖就有29個，因此代糖3号与蔗糖的發酵速率相若，能与蔗糖一樣令麵包鬆软程度一樣。

(b) 提出*一項*方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。
(提示：考慮影響酵母發酵速率的因素)

根據實驗代糖3的發酵速率比蔗糖的發酵速率快。這有机会是因为温度是代糖3的最適温度，酶的活躍度最勁，發酵速率最快，而也有机会是因份量有差别。可以把温度下降或上升減低酶的活躍度，平衡代糖与蔗糖的發酵速率。

Sample 3

4. 如果以其中一種實驗中使用的代糖取替蔗糖以製造麵包，在相若的發酵時間下，令麵包的鬆軟程度像使用蔗糖那樣。***請根據你所收集的數據***回答以下兩題。

(a) 你可以使用哪一種代糖？***請解釋你的答案***。

（在下列方格 ✓ 以選出你的答案）

☐ 代糖 1 號

☐ 代糖 2 號

☑ 代糖 3 號

因為根據本實驗結果的記錄中，蔗糖溶液的試管中的通用指示劑由綠色轉為黃色所需要的時間為約11分27秒，而代糖3號所需時間為約11分45秒，時間差距相當接近，但其餘的糖溶液所需時間為約13分鐘，及沒有結果。由此可見，代糖3號是除了蔗糖溶液外使酵母發酵效率最好的溶液，因此產生較多的乙醇和二氧化碳，所以代糖3號溶液的氣泡釋能出量和蔗糖溶液差不多為最高，在15分鐘內均產生了約32個氣泡，而氣其他糖溶液的氣泡為3個或4個，因較多氣泡能使麵包更鬆軟，所以代糖3號是最佳之選。

(b) 提出*一項*方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。

(提示：考慮影響酵母發酵速率的因素)

煮沸該代糖溶液，如煮沸該代糖溶液能去除溶液當中的氣、氣体，減少酵母發酵時的阻礙因素，使酵母能在乾淨沒有太多雜質的環境下發酵，增加酵母發酵時的速率，因此達到與使用蔗糖一地相近的水平。

About the samples

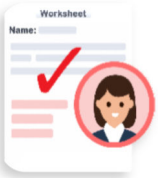
- In all three samples, correct decisions concerning the type of sugar substitute were made based on the data collected. However, Sample 1 did not refer to the data collected to justify their decisions. Sample 3 is detailed in comparing all the data collected for decision-making.
- Sample 3 did not make use of biological principles in terms of the factors that can speed up the yeast fermentation rate to make the necessary modifications. Sample 1 identified the method while Sample 2 showed some issues with the use of data.

Supplementary Resources

Possible Modifications

1. Investigating yeast bead fermentation

- A syringe can be used to set up a yeast respirometer conveniently.
- If yeast beads are used, the reaction mixtures can be collected, and the solution can be used for titration against an alkaline solution containing a pH indicator.



Notes for teachers

- Teachers can use the following procedures.
- The procedures for making yeast beads can be found in *Yeast Bead Invertase/Catalase Investigation*.
- Scan the QR code to view a video that shows the whole experiment.
- Read the *Technician Notes* section for the materials required for this experiment.
- A video showing how to set up a yeast respirometer using a syringe is available via the QR code alongside the procedure.

1. Prepare the yeast beads.

2. Set up the respirometers.

3. Perform a titration to determine the number of drops of solution for colour change of the alkaline solution.

Procedure

Setting up the respirometer

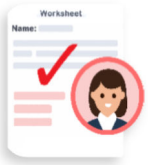
1. Remove the plunger from a 10-mL syringe.
2. Cap the syringe using a plastic syringe cap.
3. Use a pair of forceps to transfer 50 yeast beads to the syringe.
4. Pipette 2.5 mL of distilled water into the syringe.
5. Place the syringe with the distilled water vertically on the rack.
6. Repeat *Steps 1* to *5* with the sucrose solution and sugar substitutes (1, 2).
7. Insert the plunger in the syringe with distilled water.
8. Invert the syringe so that the yeast beads sink to the bottom of the syringe.
9. Remove the plastic cap from the syringe.
10. Gently tap the syringe to remove air bubbles.
11. Gently press the plunger to push the distilled water up to the top of the syringe.
12. Cap the plastic syringe to ensure that it is airtight.
13. Repeat *Steps 7–12* with the sucrose solution and sugar substitutes (1, 2).
14. Start the timer when all the respirometers are set.
15. Record the initial position of all plungers at $t = 0$ minute.
16. After 20 minutes, the final positions of all plungers are recorded.

A video showing some mistakes in experimental procedures can sensitise students to avoid similar mistakes in their own experiments.

Scan this QR code to see how to assemble the experimental set-up.

Determining the volume of sugar solutions for neutralisation of the alkaline solution

1. Pipette 1 mL of alkaline solution with a pH indicator into eight 5-mL conical flasks.
2. Use a dropper to withdraw all the solutions from each syringe to a 10-mL beaker.
3. Add each solution dropwise to the conical flask containing alkaline solution on a white tile. Gently shake the conical flask after adding each drop. Keep adding sugar solution until the blue colour changes to green.
4. Record the exact number of drops of sugar solution that was added. Write >20 if the colour has not changed after adding 20 drops.
5. Repeat *Step 3* and *Step 4* one more time and calculate the average result.



Notes for teachers

- Teachers can ask the technician to perform a trial run to adjust the alkalinity of the alkaline solution. This will be done by adjusting the volume of the sodium carbonate solution added. The goal is to find the alkaline solution composition where the positive control (i.e., sucrose solution) requires less than 10 drops to change the colour of the alkaline solution.

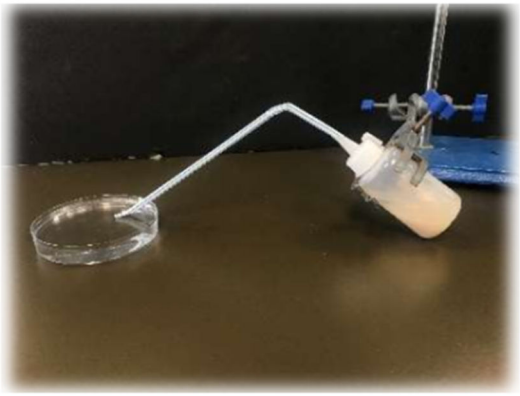
Technician Notes

Materials for Task 1

15% Yeast (activated)	Test tube	Syringe	Phenolphthalein
Balloon	Boiling tube	Syringe cap	Straw
String	Rubber tubing	Paraffin oil	Glass bottle
10% Sucrose solution	Plastic dropper	Dropper bottle	0.1 M Sodium hydroxide solution
Boiled distilled water	Timer	Ruler	Water
Straw	Electronic balance	Measuring cylinder	Petri dish

* Containers of varying sizes can be provided to students.

Possible set-ups



Materials for Task 4

Chemicals to be prepared

- Boiled water (containing universal indicator) (Boil tap water and add appropriate volume of universal indicator [i.e., when the solution is sufficiently green]).
- 10% Sugar solution (boiled)/Distilled water (boiled) (Dissolve 10 g in 100 mL sugar solution. Boil the sugar solution/distilled water. Adjust pH to 7.4.)
- 15% Yeast extract (1 mL in 1.5 mL tube) (to be prepared on that day) (Add 15 g yeast in 100 mL distilled water. Add a spoonful of sugar. Stir on a magnetic stirrer. Wait for 30 minutes to activate the yeast. Keep stirring. Aliquot just before the experiment.)

Materials for each group

• 25 mL Measuring cylinder	• *>150 mL Boiled distilled water (containing universal indicator)	• 1 mL Boiled 10% sucrose solution/sugar substitute 1/sugar substitute 2/sugar substitute 3/distilled water in 1.5-mL tube X 2
• Boiling tubes X 10	• 1 mL 15% Yeast extract in 1.5-mL tube X 10	• 400 μL Paraffin oil X 10
• Boiling tubes rack	• 3 mL Plastic dropper (tip = 3.5 cm) X 10	• Paraffin oil in dropper bottle
• Timer	• Screw nut X 20	• Camera (supplied by students)

* Volume depends on the size of the boiling tubes.

Materials for Yeast Bead Syringe

Materials for each group

• 10 mL Syringe X 5	• Syringe cap X 5	• Autopipette (P-1000)
• 5 mL Conical flask X 10	• Forceps X 5	• Autopipette tip (P-1000)
• >8 mL *Alkaline solution with a pH indicator	• >2.5 mL Boiled sugar substitute solution 1, 2	• >2.5 mL Boiled distilled water
• Plastic dropper X5	• >2.5 mL Boiled sucrose solution	• Timer
• 25 mL Beaker X 5		

*40 mL 0.04% Bromothymol blue + 10 mL 2% Na_2CO_3 + 150 mL distilled water.
Materials for making yeast beads can be found in *Yeast Bead Invertase/Catalase Investigation*.

References

Chan, K. K. H. (2016). A simple micro-scale set-up for investigating yeast respiration in high school biology classrooms. *The American Biology Teacher*, *78*(9), 669–675.

Chan, K. K. H., West-Pratt, J., Ng, R. C. K. (2021). Using yeast fermentation as a context for meaningful learning of procedural understanding. *The American Biology Teacher*, *83*(1), 26–32.

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