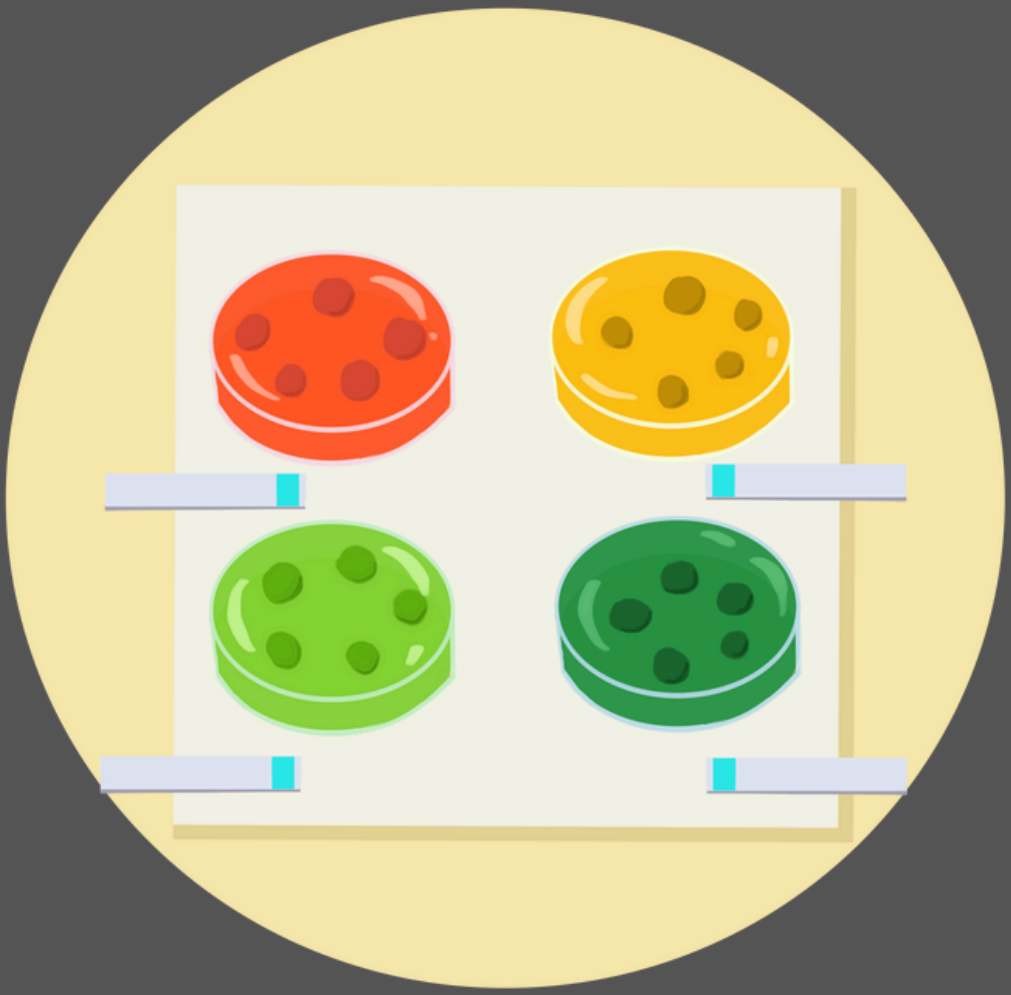
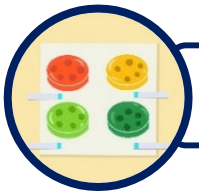


Yeast Bead Invertase Investigation



Yeast Bead Invertase Investigation

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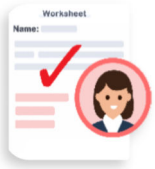
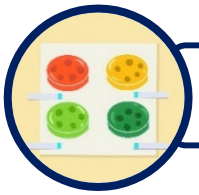


Notes for teachers

- 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 Bead Invertase Investigation

Overview

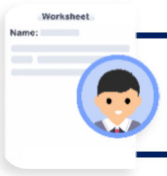
- The *Yeast Bead Invertase Investigation* is about the industrial application of immobilised yeasts for the production of invert syrup.
- Immobilised yeasts, known as yeast beads, contain invertase (Bryer, 2016).
- Students investigate the effect of pH on the activity of yeast bead invertase.
- Students collect semi-quantitative data (i.e. the colour intensity of glucose test strips) to determine the invertase activity.
- Students are given the opportunity to design and carry out an experiment and assess the accuracy of the measurement tool, and the reliability of the data.

Teaching Plan

Prerequisite knowledge (scientific ideas)

- Properties, actions, and roles of enzymes
- Factors that affect the actions of enzymes

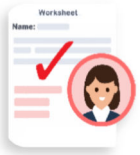
Lesson	Lesson sequence	Duration (mins)	Resources
Stage ❶ Preparing for the investigation • It is situated in an authentic context related to the industrial application of invertase in chocolate making (**Contextualisation**). • Students read information to familiarise themselves with the background of the investigation (*Reading Materials*).			
Before Lesson 1	• The teacher distributes *Worksheet 1* for students to complete at home so that they can be familiar with the background of the investigation.		*Worksheet 1*
1	• The teacher discusses the investigation context with students. • The teacher provides feedback on students' responses in *Worksheet 1*.	40	Student Samples 1
Stage ❷ Designing the investigation • Students interact with a virtual laboratory to familiarise themselves with the materials and apparatuses they use in the investigation (*Virtual Laboratory*). • Students use a template to design their own experimental set-ups (*Investigation Planning Template*). • Students have the chance to evaluate their own and their peers' experimental set-ups (*Self & Peer Evaluation*).			
2	• The teacher presents the main investigation context and discusses with students questions related to their experimental designs. • The teacher provides students with the laboratory manual for preparation at home.	40	Teacher Notes 1
Stage ❸ Carrying out the investigation • Students collect data using a template (*Data Collection Sheet*).			
3	• 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 assess the limitations of the data collected in answering the investigation question.			
Before Lesson 4	• Students complete data reporting and analysis at home. • The teacher collects and marks student responses.		Teacher Notes 2
4	• The teacher provides feedback on students' performance related to data reporting and analysis.	40	Teacher Notes 2



Instructional Materials

Stage ❶ Preparing for the investigation

Student Worksheet 1



Notes for teachers

- Teachers can distribute *Worksheet 1* and ask students to read the background information related to the investigation and design their experimental set-ups at home.
- Teachers can collect students' drawing using a *Google Form.*
- Scan the QR code to get a copy of the *Google Form*.

Task 1

- Read the scenario and answer the questions that follow:

Scenario

Invertase is an enzyme that catalyses the breakdown of sucrose into fructose and glucose. It is widely used in the food industry to produce creams, jams, and artificial honey.

Yeast (*Saccharomyces cerevisiae*) is a rich source of invertase. Yeast cells are immobilised to form yeast beads, which can be easily removed from the sucrose solution and reused.

In this investigation, you will study yeast bead invertase. Read the information in the *Data File* to familiarise yourself with the background of this investigation. You will use your biological knowledge of enzymes and the design of valid and reliable experiments to complete this investigation.

Question

1. You have found that you can control the time for making invert sugar syrup by changing the temperature during the making of invert sugar syrup. Therefore, you want to investigate the effects of temperature on the activity of yeast bead invertase.

The virtual laboratory provides students with opportunities to get familiar with materials used in the investigation.

- You have been provided with the following materials:

20% Sucrose solution	Water bath	Plastic dropper
Yeast beads	Glucose test strips	Thermometer
Timer	Vials	Forceps
Measuring cylinder	Spotting plate	Colour chart
White tile	Spoon	

- You may also make use of other common apparatuses in the laboratory.

Scan the QR code to see the materials

(a) Briefly describe how you would use the above materials to plan an experiment to achieve the aim of the investigation.

- You can draw your experimental design.
- Write down any important experimental design decisions
- The *Investigation Planning Chart* (scan the QR code) can help you with this.

Data File

Your biology teacher asks you to read the following source materials to prepare for planning a scientific investigation on yeast bead invertase:

Source 1: Industrial application of invertase

Invertase is an enzyme that catalyses the breakdown of sucrose into fructose and glucose.

$$\text{sucrose} \xrightarrow{\text{invertase}} \text{fructose} + \text{glucose}$$

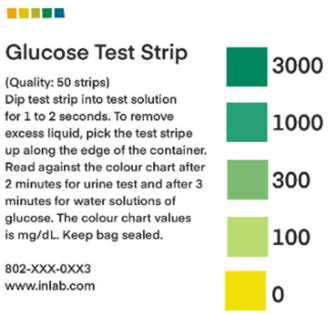
Invertase is commonly used in the food industry to produce 'invert syrup' from sucrose solutions. Invert syrup contains a mixture of fructose and glucose from sucrose treated with invertase. The products formed in the enzyme reaction (i.e., fructose and glucose) have a higher solubility than the substrate (i.e., sucrose).

A well-known use of invertase is in the production of chocolates with a soft centre, such as *Lindt Lindor* milk chocolate truffles. One way to make this type of chocolate is to add a small amount of invertase to the solid sugar filling, which consists of table sugar (sucrose). The mixture of fructose and glucose from the sucrose treated with invertase becomes liquid in the chocolate shell.

Scan the QR code to learn more about how invertase is used in the food industry.

Source 2: Testing for glucose concentration in urine samples

Glucose is found in urine samples from patients with diabetes. A convenient way to detect the presence of glucose in urine is to use a glucose paper test strip.



A glucose test strip contains *glucose oxidase*. This enzyme catalyses the conversion of oxygen into hydrogen peroxide. The hydrogen peroxide produced reacts with the substrate on the test strip to form a coloured substance. The simplified reactions are as follows:

$$\text{glucose} + \text{oxygen} \xrightarrow{\text{glucose oxidase}} \text{intermediate X} + \text{hydrogen peroxide}$$

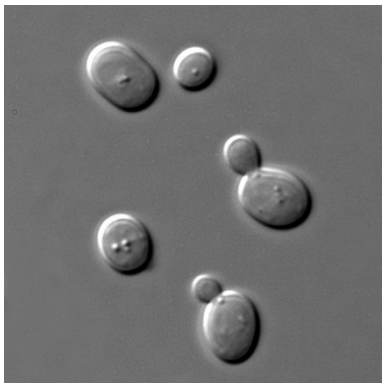
$$\text{substrate} + \text{hydrogen peroxide} \longrightarrow \text{water} + \text{coloured substance}$$

After dipping in the urine sample, the paper test strips change colour if glucose is present. The resulting colour indicates the concentration of glucose semi-quantitatively.

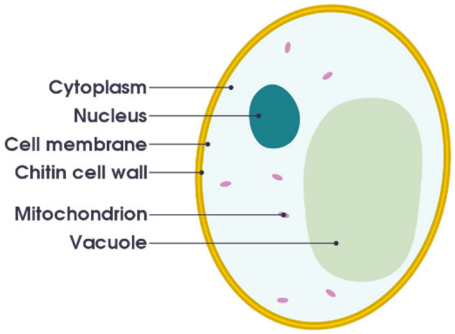
Scan the QR code to learn how to use a glucose test strip.

Source 3: What are yeast and yeast beads?

Yeast (*Saccharomyces cerevisiae*) is a eukaryotic organism. A eukaryotic cell has a true nucleus and membrane-bound organelle. Although yeast cells have cell walls, the chemical composition of their cell walls is different from that of plant cells. The following diagrams show yeast cells under a light microscope and a drawing of a yeast cell, respectively.



Yeast cells under a microscope

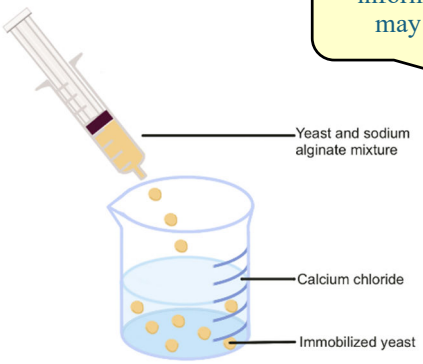


Drawing of a yeast cell

Yeast cells contain invertase, which catalyses the conversion of sucrose to fructose and glucose.

Scientists use yeast for many industrial applications, including brewing beer and making bread. In some applications, scientists immobilise whole yeast cells to form yeast beads using sodium alginate and calcium chloride.

The reading materials provide information about yeast beads, which may be unfamiliar to the students.



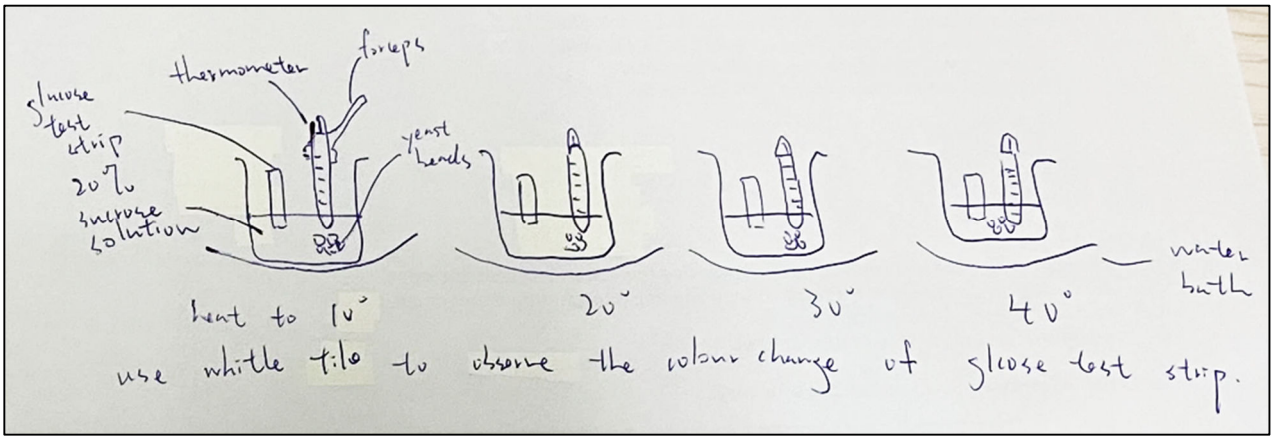
Yeast in alginate solution + Calcium chloride ⟶ Yeast beads

(Yeast immobilised in insoluble calcium alginate)

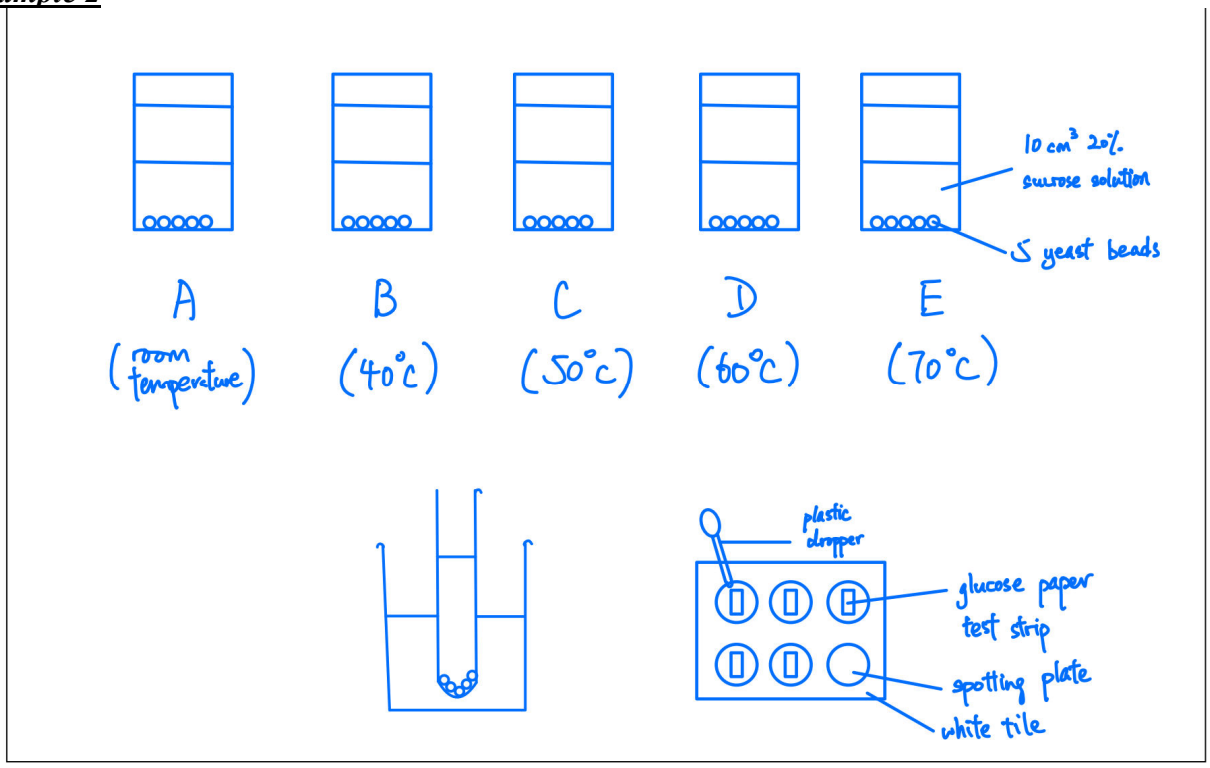
Immobilised yeasts are also active. The yeast beads can be collected and reused after the reaction.

Scan the QR code to learn how to make yeast beads.

Sample 1



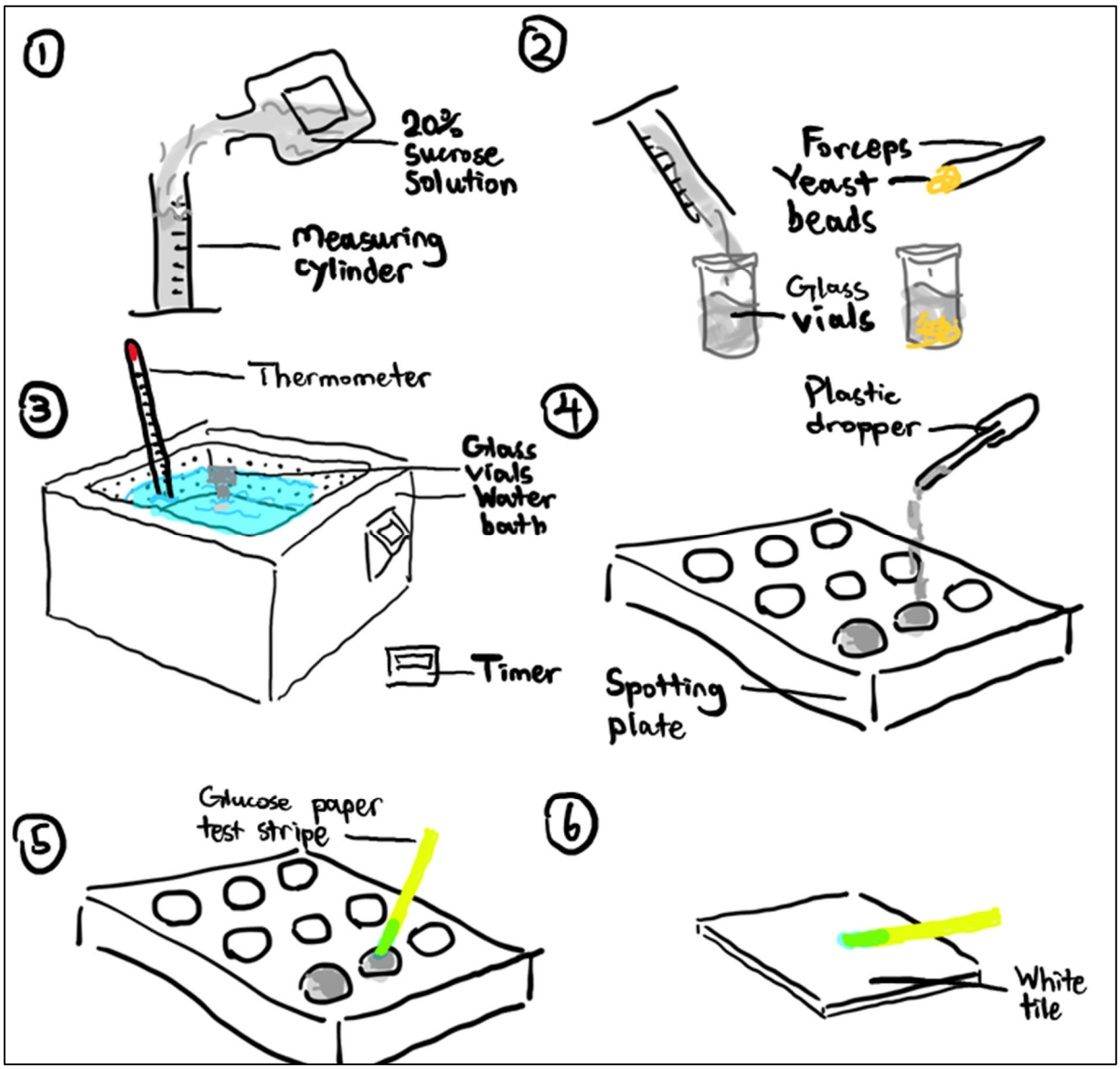
Sample 2

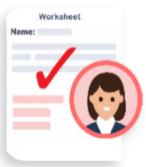


Brief explanation of my design:

Firstly, make some yeast beads by using a plastic dropper. Next, measure 10 cm3 of 20% of sucrose solution by using a measuring cylinder, then transfer the solution into a glass vial and repeat this step for four times. After that, use a water bath and put a thermometer to seek the respective wanted temperatures of the solution, also set a timer for 12 mins. Then, transfer five yeast beads into each glass vial and start the timer once the beads are ur into the vial by a spoon. Afterwards, place a spotting plate on a white tile and add five glucose paper test strips on the plate by a forcep while waiting the experiment to complete. After 12 minutes, use a plastic dropper to add two drops of each of the mixture to the plate. At last, observe the colour change of the test paper to deduce how much sucrose has been converted into glucose.

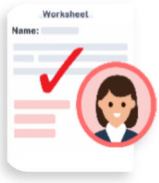
Sample 3





Notes for teachers

- Teachers can select student drawings (anonymised) for discussion.
- Teachers can discuss the following scientific inquiry skills: (1) range and interval of independent variable; (2) measurement of dependent variable; (3) significant assumptions; (4) important precautionary steps; (5) number of yeast beads used.



Notes for teachers

- The following shows the main investigation context that requires students to design an investigation with another independent variable.
- There are some questions that teachers may use 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.

Task 2

Scenario

Invertase is an enzyme that catalyses the breakdown of sucrose into fructose and glucose. Invertase is often used in the food industry for the production of invert syrup from sucrose.

Yeast (*Saccharomyces cerevisiae*) is a rich source of invertase. Yeast cells are immobilised and form yeast beads that can be easily removed from the sucrose solution and reused.

Since sucrose solutions with different food additives have different pH values, the efficiency of the yeast beads in converting sucrose into fructose and glucose may be different. In this study, you would like to investigate the effect of pH on the invertase activity of the yeast beads.

Design of the investigation

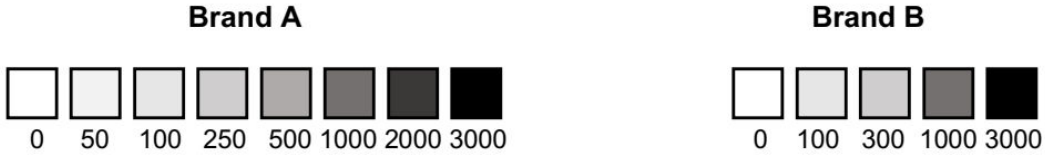
Your teacher has given you the following materials:

5% Sucrose solution at pH 3, 5, 7, 9	Distilled water	Timer
Buffer solution* (pH = 3, 5, 7, 9)	Glucose test strip	Forceps
Yeast bead	Petri dish	Spoon
Plastic vial	Measuring cylinder	White tile
Spotting plate	Plastic dropper	Colour chart

* Buffer solutions are used to maintain the pH of the solution mixture.

Possible questions

1. You have found two different brands of glucose paper test strips in the laboratory. Below you can see the colour charts of the two brands, which you can use to determine the concentration of glucose.



Which brand, A or B, will you use in this investigation? Why?

2. State *one* significant assumption in this investigation.
(An assumption is something we think it is true, though we cannot be sure. A significant assumption is the one that the experiment cannot make any conclusion without assuming it to be true).

3. Your teacher has also given you the following reminders:

Terms are defined using student-friendly language.

Reminders

- *Place the yeast beads in the petri dish containing the buffer solution for at least 5 minutes before mixing with the sucrose solution.*
- *Gently shake the plastic vials with the yeast beads and the sucrose solution from time to time.*

Suppose you have overlooked your teacher's reminders and

(a) forgot to put the yeast beads into the buffer solution before adding them to the sucrose solution.
(b) shook the plastic vials too vigorously and spilled half of the sucrose solution out of the plastic vials.

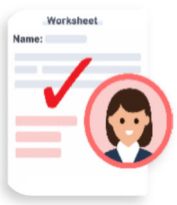
Explain how each of these mistakes would impact the experimental results.

Hints: Be sure to include the following parts in your answers:

- the effect on the data being collected
- explanation for the effect

The checklist serves to scaffold students' responses.

	Impact on experimental results
(a)	
(b)	



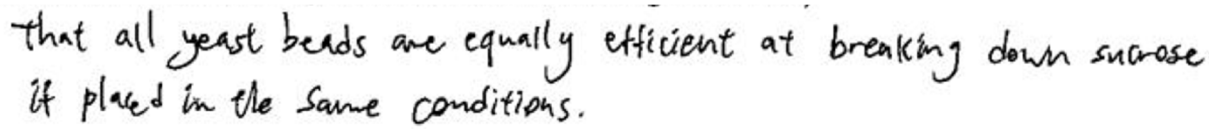
Notes for teachers:

- Q.1 assesses students' ability to reduce measurement errors by choosing the glucose test strip brand that is more sensitive.
- Q.2 assesses students' ability to identify the significant assumption.
- Q.3 assesses students' ability to explain how specific steps can impact on the validity of the data.

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

Sample 1

Sample 2



About the samples

- Neither sample mentioned the significant assumption concerning the relationship between the measurement and the dependent variable.

The following examples demonstrate varying levels of sophistication in quality:

Unattained

- Environmental conditions are the same.
- Yeast beads have the same size and shape.

Basic

- All yeast beads work the same.

Good

- Amount of invertase in each yeast bead is the same.

Excellent

- Glucose is only contributed by the activity of invertase in the yeast beads.

The following are some examples of students' responses to Q.3(a):

Sample 1

	Impact on experimental results
(1)	The pH value of sucrose solution will be affected, because if yeast beads didn't put into buffer solution, the pH value of yeast beads and sucrose solution will be diffrent. So the result won't be the pH you want.

Sample 2

	Impact on experimental results
(1)	The amount of glucose may be higher initially for the set-ups with lower pH. The buffer solution ensures that the yeast beads are already at the pH of the sucrose solution. In this case, the yeast beads will only change its pH once in contact with the sucrose solution, meaning that it will not be denatured beforehand and more of the enzymes will be able to break down sucrose into glucose.

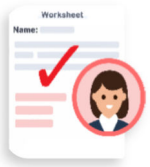
Sample 3

	Impact on experimental results
(1)	Since ~~it is not~~ at yeast beads were not added into their respective buffer solutions, pH of yeast beads are not the same ~~as~~ with sucrose solutions, that the pH of solution obtained after the expiriment is different from the initial pH of the sucrose solution, resulting in either higher or lower glucose concentration ~~as~~ in the solution obtained.

About the samples

- Sample 1 did not clearly explain how omitting the precautionary step would impact the yeast beads' ability to reach the desired pH at the start of the experiment.
- Both Samples 2 and 3 described the effect of this missing step. Sample 3 specifically stated that the effect could result in either a higher or lower glucose concentration, depending on the pH profile of the invertase enzyme. In contrast, Sample 2 assumed a lower pH would lead to higher invertase activity, despite lacking experimental evidence to support that claim.

Laboratory Manual



Notes for teachers

- Teachers can distribute the manual for students to read and prepare before the investigation.
- Teachers can ask questions to check if students fully understand the procedures (e.g., how many glucose test strips do you need for this experiment? Why?).
- The *Supplementary Resource* section contains the list of materials.
- Teachers can print the *Data Collection Sheet* and laminate the printouts for use in class.
- Scan the QR code to view the process of the experiment.

Task 3:

- Read the following procedures to carry out the investigation.

Procedure

Preparation of yeast beads

1. Add 10 mL of 10% yeast (in a vial) to 10 mL of 2% sodium alginate solution in a 50 mL-tube.
2. Mix the solution well by inverting the 50 mL-tube to make a yeast–sodium alginate solution.
3. Hold the plastic dropper (without cap) with a stand and clamp.
4. Pour 50 mL 2% $CaCl_2$ (calcium chloride) into a plastic cup/100 mL-beaker.
5. Assemble the set up shown in *Figure 1*.
6. Add the yeast sodium alginate solution to the plastic dropper (a bead should form when the drop comes into contact with the $CaCl_2$ solution and falls to the bottom of the beaker).
7. Wait 5 minutes until the beads have hardened.
8. Discard any floating yeast beads with a plastic spoon.
9. Collect the beads with a sieve.
10. Wash the beads several times with distilled water from a wash bottle over a plastic cup.

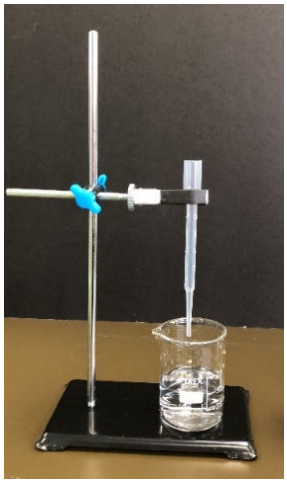


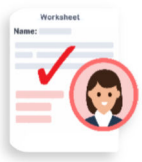
Figure 1

Incubation of the yeast beads in buffer solution

1. Add 5 mL buffer solution (pH = 3.0, 5.0, 7.0, 9.0) to 4 different petri dishes.
2. Use a spoon to gently move at least 15 yeast beads into each buffer solution (pH = 3.0, 5.0, 7.0, 9.0).
3. Wait at least 5 minutes.

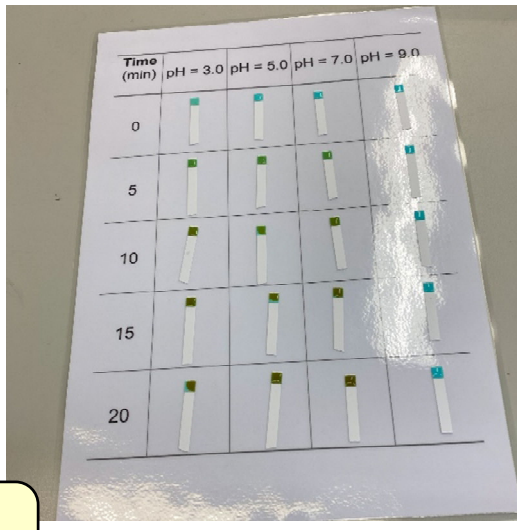
Testing the invertase activity

1. Transfer 5 mL of the sucrose solution with a pH of 3.0 into a plastic vial.
2. Repeat *Step 1* with the sucrose solution with different pH values (5.0, 7.0 and 9.0).
3. Transfer 15 yeast beads into each plastic vial using a spoon and a pair of forceps.
4. At time = 0 minute, remove a small drop of sample from each vial with a plastic dropper and place it on the laminated spotting plate sheet.
5. Close the plastic vial and swirl it gently from time to time.
6. Repeat *Step 4* at time points 5, 10, 15 and 20 minutes.
7. When you have collected all the samples, dip the glucose paper test strip into each sample.
8. Observe and record the colour change, if any, after 1 minute.
9. Determine the glucose concentration from the colour chart.



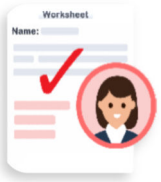
Notes for teachers

- Remind the technician to adjust the relative concentration of sucrose solution as different brands of glucose test strips have different sensitivity.
- Laminated data collection sheets are commonly used in microscale activities.
- Scan the QR code for copy of the *Data Collection Sheet*.



The *Data Collection Sheet* provides guidance for students to collect data.

Teacher Notes 2



Notes for teachers

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

Task 4

1. Based on the data collected, describe and explain the effect of pH on yeast bead invertase activity.

2. Tom performed the same experiment. He found that all the glucose test strips of the samples at pH 7 and 9 at 0, 5, 10, and 20 minutes gave a negative result.

   You found that your group's results are similar to the general trend observed in the class data. You suspected that Tom's results are anomalous data and had some errors.

   (a) By comparing your results with those of Tom, identify the possible errors in Tom's results.

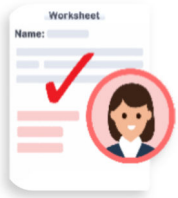
   (b) How would you further confirm that the results by Tom are anomalous?

   (c) Explain the possible causes for the errors.

3. Tom would like to determine the optimum pH of yeast bead invertase accurately.

   (a) Based on the data, explain the limitations of the experimental design in finding the optimum pH of yeast bead invertase.

   (b) Describe how you would modify this experiment to obtain a more accurate estimate of the optimum pH of yeast bead invertase.

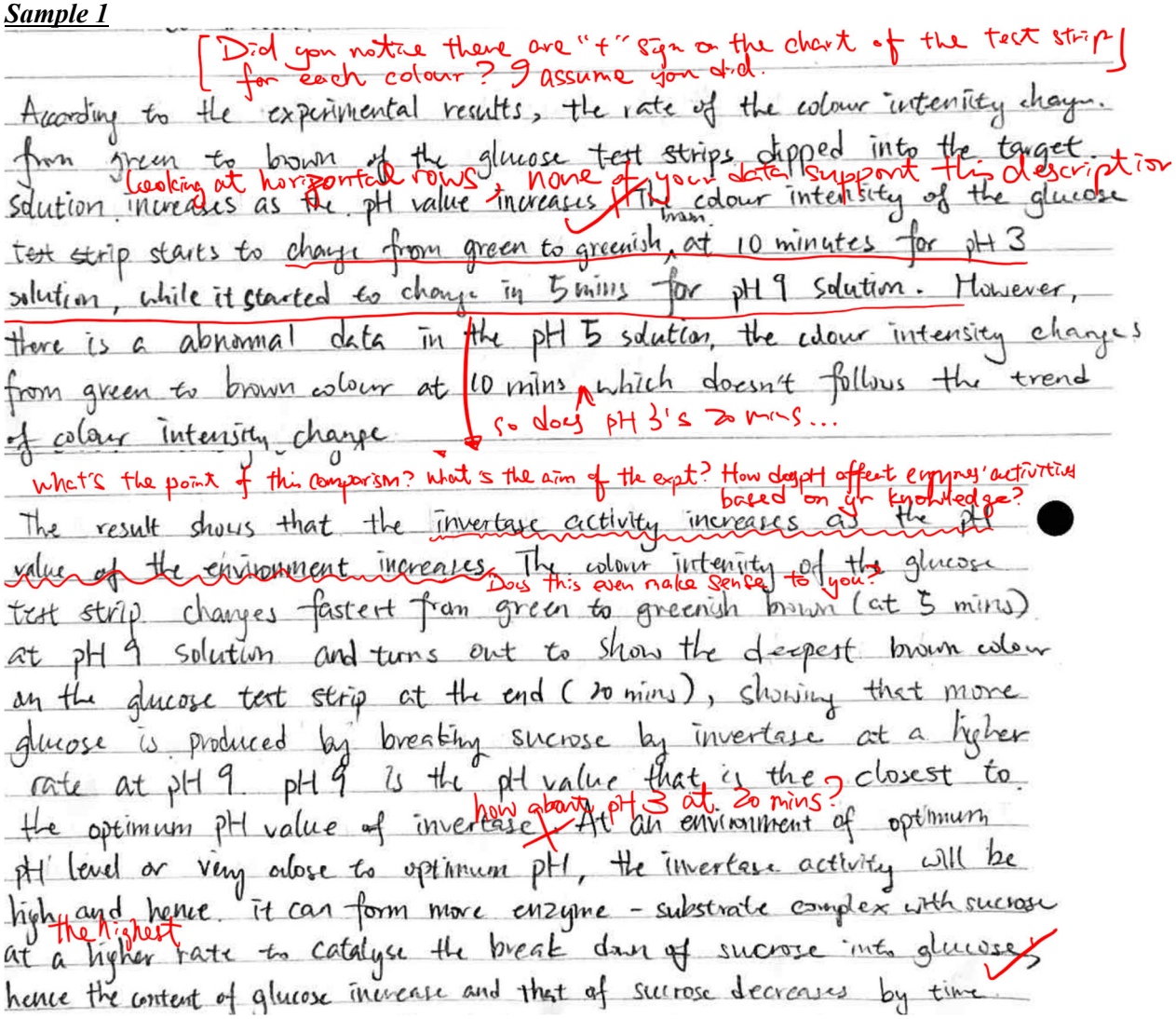


Notes for teachers

- Q.1 assesses students' ability to describe and explain data in simple data sets.
- Q.2 assesses students' ability to identify data inconsistent with the general trend or patterns observed within the class data and suggest ways to confirm if the data are anomalous as well as explanations for the occurrence.
- Q.3 assesses students' ability to assess the adequacy of the selection of the range and interval of the independent variable in determining the optimum pH of the yeast bead invertase, and suggest possible modifications to improve the accuracy of the designs to achieve this aim.

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

Sample 1


Did you notice there are "+" sign on the chart of the test strip for each colour? I assume you did.

According to the experimental results, the rate of the colour intensity chaym. from green to brown of the glucose test strips dipped into the target solution increases as the pH value increases. The colour intensity of the glucose test strip starts to change from green to greenish, at 10 minutes for pH 3 solution, while it started to change in 5 mins for pH 9 solution. However, there is a abnormal data in the pH 5 solution, the colour intensity changes from green to brown colour at 10 mins which doesn't follows the trend of colour intensity change

looking at horizontal rows, none of your data support this description

so does pH 3's 20 mins...

what's the point of this comparism? what's the aim of the expt? How does pH affect enzyme activities based on yr knowledge?

The result shows that the invertase activity increases as the pH value of the environment increases. The colour intensity of the glucose test strip changes fastert from green to greenish brown (at 5 mins) at pH 9 solution and turns out to show the deepest brown colour on the glucose test strip at the end (20 mins), showing that more glucose is produced by breaking sucrose by invertase at a higher rate at pH 9. pH 9 is the pH value that is the closest to the optimum pH value of invertase. At an environment of optimum pH level or very close to optimum pH, the invertase activity will be high and hence it can form more enzyme - substrate complex with sucrose at a higher rate to catalyse the break down of sucrose into glucose, hence the content of glucose increase and that of sucrose decreases by time.

Does this even make sense to you?

how about pH 3 at 20 mins?

the highest

Sample 2

As the pH of sucrose solution increased from 3 to 5, the color intensity of glucose test strip remained the same for the first 15 minutes. However, at the 20th minute, the color intensity of glucose test strip of pH 5 was higher than that of pH 3.

As the pH of sucrose solution increased from 5 to 9, most of the color intensity of glucose test strip at their respective time frame decreased, while only some remained unchanged.

??

As time went on, the color intensity of glucose test strips increased. not for pH 9.

As the optimum pH of invertase is at pH 4.5, when pH of sucrose solution increased from 3 to 5, the pH was closer to the optimum pH. Hence as pH of sucrose solution increased from pH 3 to 5, a smaller proportion of invertase would be denatured, so the chance of formation of enzyme-substrate complex increases, more sucrose can be broken down to fructose and glucose, hence the invertase activity increases.

However, as pH of sucrose solution increased from 5 to 9, the pH became further away from the optimum pH. Hence as pH of sucrose solution increased from pH 5 to 9, a larger proportion of invertase would be denatured, so the chance of formation of enzyme-substrate complex decreases, less sucrose can be broken down to fructose and glucose, hence the invertase activity decreases.

Sample 3

Among all pH, pH7 took the shortest time to produce the largest amount of glucose. Also, after calculating the invertase activity by glucose amount/time, it is shown that the invertase activity increased from pH3 to pH7, but decreased from pH7 to pH9, the invertase activity was the highest in pH7. This indicates that pH7 is the optimum pH.

At optimum pH, which is pH7, invertase activity is at its maximum. While at unsuitable pH, invertase activity decreases because unsuitable pH causes denaturation of enzymes. Substrates can no longer fit into the active site of enzymes to form enzyme-substrate complexes as the shape of active sites are changed, this causes invertase to slowly lose its catalytic ability permenantly and results in above results.

About the samples

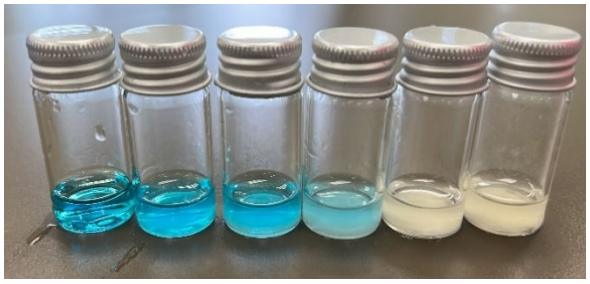
- Sample 1 did not describe the trends and patterns observed in the data obtained and did not provide explanation for the trends and patterns observed.
- Sample 2 described the trends and patterns observed in the data obtained, but the description lacked clarity. In the explanation, it was wrongly stated that the optimum pH was 4.5, and the explanation did not explain why extreme pH would denature the enzyme.
- Sample 3 described the pH profile of the yeast bead invertase by relating it to the data obtained. It also explained the effect of extreme pH on the enzyme activity.

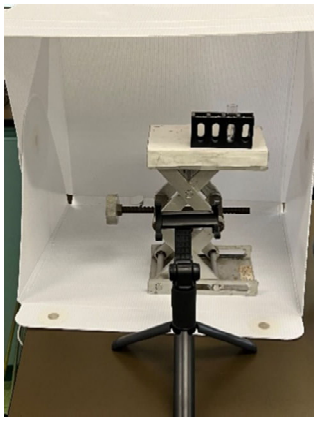
Supplementary Resources

Possible Modifications

1. Investigating the rate of yeast bead invertase quantitatively

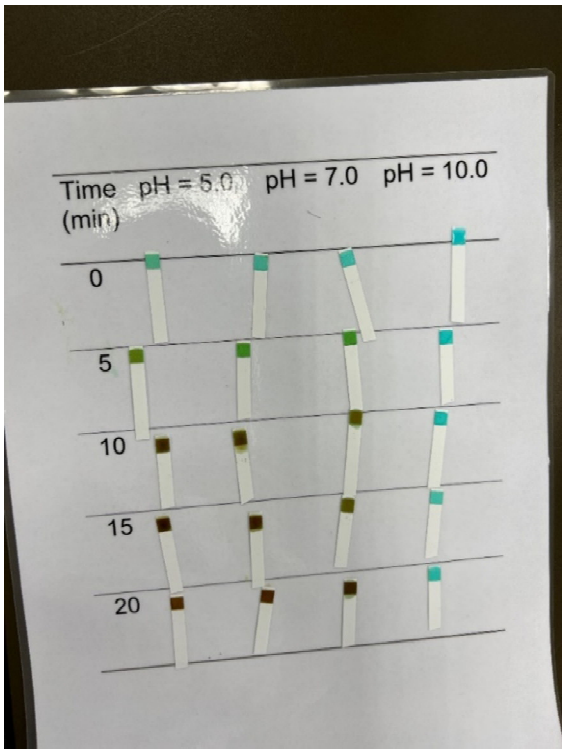
- The reducing sugars produced by the invertase can be quantified using quantitative Benedict's test.
- A colorimeter can be used to determine the amount of reducing sugar produced.
- Details can be found in Hale (2023).

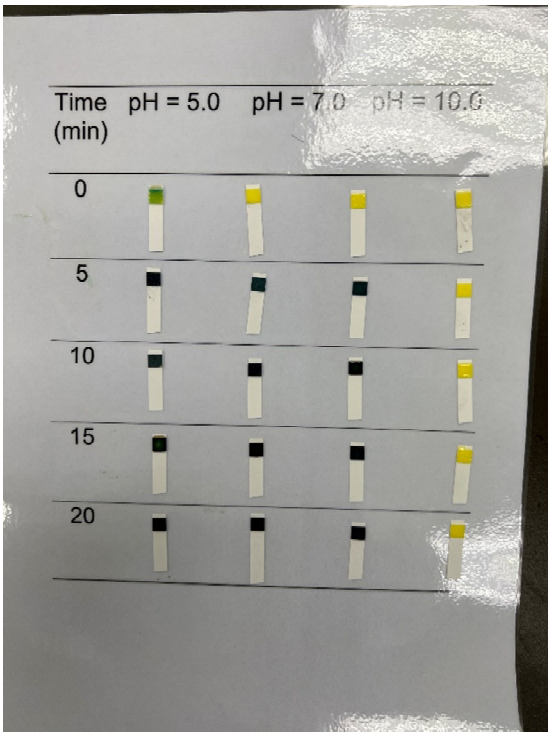




2. Comparing the sensitivity of two brands of glucose test strips

- The sensitivity of different brands of glucose test strips can be compared.





Technician Notes

Materials for Task 3

Chemicals to be prepared

- 10% yeast extract (1 mL in 1.5 mL tube) (to be prepared on that day) (Add 15 g yeast in 150 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.)
- 2% sodium alginate (food grade). Add 200 mL of distilled or deionised water and a stir bar. Heat the solution. Slowly add some sodium alginate to dissolve it. Add more powder slowly (5 g in total). Make up volume to 250 mL. Store the solution at 4°C.

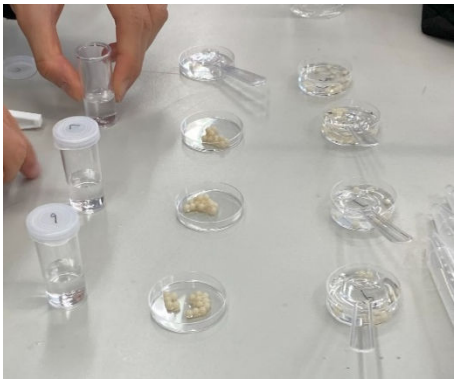
Scan the QR code to watch a video on how to prepare 2% sodium alginate.

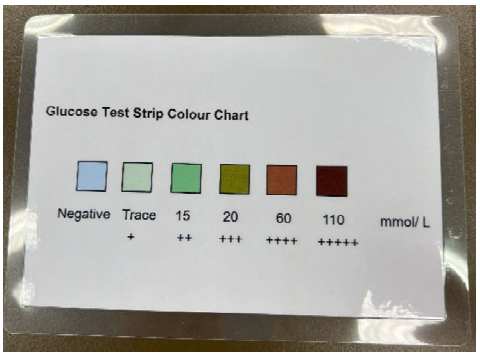
Materials for each group

• 10 mL 2% Sodium alginate in 50 mL tube	• Plastic forceps X 4	• Glucose test strips X 20
• 10 mL 10% Yeast in a plastic vial	• 3 mL Plastic disposable pipette (with part of the head cut)	• Rubbish bin
• 100 mL Beaker X2	• Mini petri dish X 12	• 50 mL 2% calcium chloride
• Spoon (for removing floating yeast bead)	• Stand and clamp	• Timer
• Sieve	• Wash bottle (with distilled water)	

Notes:

- Use 5% to 20% sucrose solution depending on the brand of glucose test strips.
- Do *not* use sodium phosphate buffer as alginate beads can react with the buffer.
- The time for *Step 8* (i.e., testing the invertase activity) depends on the brand of glucose test strips.





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Bryer, P. J. (2016). Exploring catalase and invertase activity using sodium alginate–encapsulated yeast (yeast spheres). *Journal of Microbiology & Biology Education*, *17*(3), 490–491.

Hale, J. (2023) Using immobilised yeast synoptically at A-level. *School Science Review*, *104*(387), 13–17.

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