Cambridge Assessment International Education

Teaching Pack

Investigating the rate of aerobic respiration in yeast

Cambridge International AS & A Level Biology 9700

Version 1.0

Cambridge Pathway

Contents

Icons used in this pack:

Briefing lesson

Planning lesson

Lab lesson

Debriefing lesson

Introduction

This pack will help you to develop your learners' experimental skills as defined by assessment objective 3 (AO3 Experimental skills and investigations) in the course syllabus.

Important note

Our *Teaching Packs* have been written by **classroom teachers** to help you deliver topics and skills that can be challenging. Use these materials to supplement your teaching and engage your learners. You can also use them to help you create lesson plans for other experiments.

This content is designed to give you and your learners the chance to explore practical skills. It is not intended as specific practice for Paper 3 (Advanced Practical Skills) or Paper 5 (Planning, Analysis and Evaluation).

This is one of a range of *Teaching Packs* and each pack is based on one experiment. The packs can be used in any order to suit your teaching sequence.

The structure is as follows:

Briefing lesson (1 hour*)

This lesson reinforces the key concepts, relevant skills, knowledge and understanding linked to the experiment.

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Planning lesson (1 hour*)

This lesson focuses on planning an experiment.
It ends with a demonstration video of the experiment.

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Lab lesson (1 hour*)

This lesson focuses on carrying out the experiment including the collection and recording of observations, measurements and estimates.

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Debriefing lesson (1 hour*)

This lesson focuses on the analysis and interpretation of data.
This includes making conclusions, evaluating methods and the quality of data, and how improvements could be made.

** the timings are a guide only; you may need to adapt the lessons to suit your circumstances.*

In this pack you will find lesson plans, worksheets and teacher resource sheets.

Experiment: Investigating the rate of aerobic respiration in yeast

This *Teaching Pack* focuses on a respiration experiment.

Yeast is a unicellular organism able to respire in both aerobic and anaerobic conditions. In this experiment, your learners will use a redox indicator to measure the rate of aerobic respiration in yeast at different temperatures.

This experiment has links to the following syllabus content (see syllabus for detail):

- 12.2 Respiration

The experiment covers the following experimental skills, as listed in **AO3: Experimental skills and investigations:**

- plan experiments and investigations
- collect, record and present observations, measurements and estimates
- analyse and interpret data to reach conclusions
- evaluate methods and quality of data and suggest improvements.

The following techniques are used:

- measuring a dependent variable by recording the time to a colour change
- calculating a mean
- finding the rate of reaction using 1/time
- drawing a line graph with a smooth curve.

Prior knowledge

Knowledge from the following syllabus topics is useful for this experiment.

- 12.1 Energy
- 13.2 Investigation of limiting factors
- 18.1 Classification

Briefing lesson: Measuring respiration rate in yeast

Resources	• Worksheets A, B, C and D
Learning objectives	By the end of the lesson: • ***all*** learners should be able to suggest several different ways to measure the rate of respiration in yeast • ***most*** learners should understand what a redox indicator is used for • ***some*** learners will be able to explain why it can be difficult to distinguish between aerobic and anaerobic respiration in yeast.

Timings	Activity
5 min	**Starter/Introduction** Ask learners to review and compare the raw materials and products of aerobic and anaerobic respiration in a ‘quick-fire’ callout. You could ask learners in turn to contribute an element of the two simple equations and build them up on the board. Ensure learners understand that oxygen is absent from the anaerobic equation and the products are carbon dioxide and either lactic acid or ethanol, rather than carbon dioxide and water. Make sure learners understand that aerobic respiration involves a number of dehydrogenation reactions (remind them of what type of reaction this is if necessary). You could show the image on Worksheet A and ask learners to highlight with a marker pen where dehydrogenation reactions occur.
25 min	**Main lesson** Organise learners into groups of 2–4, and give each learner Worksheet B. Ask them to use the information on the fact sheet to help them think of methods to measure the rate of respiration of a yeast culture in the laboratory. Circulate the groups to prompt discussion. If they have already used respirometers, learners might suggest measuring the rate of oxygen uptake. Other suggestions could include measuring the rate of carbon dioxide production (volume of gas; bubbling rate; or rate of foam formation), rate of glucose uptake (measured as decreasing concentration of glucose) or the rate of ethanol production. Carbon dioxide dissolves to form carbonic acid in solution, so they might suggest measuring the rate of change in pH decrease; if no one mentions this, remind them about the carbonic acid and see if anyone suggests measuring pH. Ask learners to consider the apparatus required and the practicality of each of their suggestions. Circulate the groups and challenge their suggestions. Have a class discussion to summarise. Highlight the problems associated with each of their approaches. A list of possible methods and their drawbacks for using with yeast cultures are included in the answers to Worksheet B; you can use these to help the discussion. Elicit that the usual approaches are to measure the rate of carbon dioxide formation or the rate of pH change; make sure learners understand the reasons why. Discuss the difficulty with distinguishing between aerobic and anaerobic respiration in a yeast culture given that both can occur simultaneously; this a commonly misunderstood point in practicals that involve measuring respiration in yeast cells. For example, when measuring anaerobic respiration, even if the container is made air-tight, sufficient oxygen will initially remain dissolved in the yeast suspension for some

Timings	Activity
	of the respiration to be aerobic. When measuring aerobic respiration, parts of the yeast culture further from the surface may become anaerobic and if yeast cells have access to plenty of sugar, such as in laboratory cultures, they switch to anaerobic respiration even if there is oxygen present. Unless specific steps are taken to avoid these situations, measuring the rate of carbon dioxide formation or the rate of pH change will measure both forms of respiration at once.
25 min	Split the class into two halves: give each learner in one half of the class Worksheet C and the other half Worksheet D. Explain that one will discuss measuring anaerobic respiration and the other aerobic respiration. Ask learners to work in pairs to read the information on their worksheet and give them time to answer the questions together. Then, ask a volunteer from each half of the classroom to very briefly outline the method they have been given, without explaining why it only measures one type of respiration. Ask the other side of room to suggest why it measures only that type. Give out the alternative worksheet to each learner, so that all learners now have both worksheets. Then have a class discussion about each question on both worksheets, asking learners from each half of the room for suggestions (suggestions can come from either side of the room). Answers for both worksheets are given in the answer section. Ensure that you cover any points that learners found difficult. In particular, ensure that learners understand that redox indicators highlight aerobic respiration because aerobic respiration involves far more dehydrogenation reactions compared to anaerobic respiration, so is much more effective at decolourising redox indicators. You will need to monitor this activity closely to make sure that learners all write down the correct answers to each worksheet. Ensure that all learners have annotated Worksheet D with (or written in their notebooks) the advantages and disadvantages of each redox indicator.
5 min	**Plenary** Emphasise the difference between the direct measurement of rate (volume of gas produced per minute; time taken for colour change of redox indicator) in these experiments as opposed to the measurement of an aspect related to the rate (such as distance moved by a bubble in a certain time in a respirometer). Your learners should recognise this distinction when they are planning an experiment.

Planning lesson: Measuring aerobic respiration

Resources	• Annotated Worksheet D and Worksheet E • *Investigating the rate of aerobic respiration in yeast* video
Learning objectives	By the end of the lesson: • ***all*** learners should be able to describe a basic plan involving a redox indicator and a range of temperatures • ***most*** learners should plan to time how long decolourisation takes at a range of temperatures, repeating readings to find mean times • ***some*** learners will be able to describe a detailed plan that standardises all necessary variables and take steps to minimise the error involved in determining the end-point.

Timings	Activity
10 min	**Starter/Introduction** Explain to your learners that they are going to plan an experiment to investigate the effect of changing the temperature on the rate of aerobic respiration in yeast. Ask them how they can ensure they're only measuring aerobic respiration; they should recall from the previous lesson that they need to use a redox indicator. Ask for suggestions of how they could use the decolourisation of the redox indicator to measure the rate of respiration. Elicit that they need to add the redox indicator to the yeast and then record the time it takes for the colour change to occur. They can use the time to calculate a rate. In groups of 2–3, ask learners to discuss how they might ensure a comparable end-point each time when watching for a redox indicator to decolourise. Ideas might include viewing the tube against the same background each time; having the same person decide; and using a standard decolourised tube against which to compare others. List all the ideas on the board for the whole class to see. Make sure the list includes the importance of not agitating the mixture while timing, to avoid reoxidising the redox indicator which would lead to inaccurate measurements.
35 min	**Main lesson** Explain that they are now going to plan the experiment. Tell them that they should use 2 cm^3 of yeast suspension and 6 cm^3 of redox indicator for each temperature. You might need to prompt learners during the writing of their plans. In particular, ensure that they justify their choice of redox indicator and explain clearly how they will identify the end-point at which they stop timing. Remind learners to describe the steps they will take in sufficient detail that another person could follow their plan. Worksheet E provides some prompts for planning an experiment.

Timings	Activity
15 min	**Plenary** Learners watch the '*Investigating the rate of aerobic respiration in yeast*' video as an example of how to carry out the experiment. Ask them to identify any key points they have missed out of their own procedure. For example, it is likely that some learners will not have recognised the need for temperature equilibration of the yeast suspension and/or the redox indicator solution before mixing. Some may not have recognised that the yeast samples must be timed sequentially rather than attempting to observe them all at once. Give learners some time to make suitable adjustments to their plan. **Circulate to make sure each learner's method is appropriate as they will use this in the *Lab lesson* (or collect them in to check in detail before the next lesson).** For example, you might need to adjust the temperatures used.

Lab lesson: Measuring aerobic respiration

Resources	• *Teacher walkthrough* video • All equipment as listed in the *Teacher notes*

Learning objectives	By the end of the lesson: • ***all*** learners should be able to draw a results table and collect some data • ***most*** learners should be able to draw a results table with correct column headings and units and collect a full set of data • ***some*** learners will include a column for mean time taken and recognise and repeat anomalous readings.

Timings	Activity
5 min	**Starter/Introduction** Explain to your learners that they will be carrying out the experiment using methylene blue (reassure them that DCPIP is also a suitable alternative, but given that oxidised DCPIP is pink in more acidic conditions, which could be the case for a rapidly respiring yeast suspension, methylene blue is likely to better). Depending on the time available, you might also need to explain that they will not be able to take replicate measurements but, instead, will pool class results. Learners should create a suitable results table for recording their results before starting their experiment.
50 min	**Main lesson** Learners should collect and set up their apparatus. Make sure you draw their attention to the safety precautions they should take, for example, the care needed when using the water baths at higher temperatures and the need for test-tube holders. (They should **not** remove boiling tubes from water baths using their bare hands as shown in the video!). Learners carry out their method for finding the time taken for decolourisation at different temperatures. During the lesson, you should check that learners have drawn appropriate tables and have included the correct units in the column headings. **Safety** Circulate the classroom at all times during the experiment so that you can make sure that your learners are safe and that the data they are collecting is accurate. Ask learners to make a note of any problems they have with any of the steps in their method, and if they can think of any improvements now that they're actually carrying out each step.
5 min	**Plenary** Learners calculate mean times or submit their results to the pooled class data.

Teacher notes

Watch the *Teacher walkthrough* video and read these notes.

Each group will require:

- 20 cm^3 yeast–glucose suspension
- 40 cm^3 0.005% methylene blue solution
- 12 boiling tubes (plus one for a reference yeast suspension if required)
- boiling tube rack (or 500 cm^3 beakers for each water bath)
- 2 ×10 cm^3 syringes or graduated pipettes
- test-tube holder
- timer
- access to 6 water baths, set to 5°C (ice), 25°C, 40°C, 50°C, 60°C and 80°C.

Safety

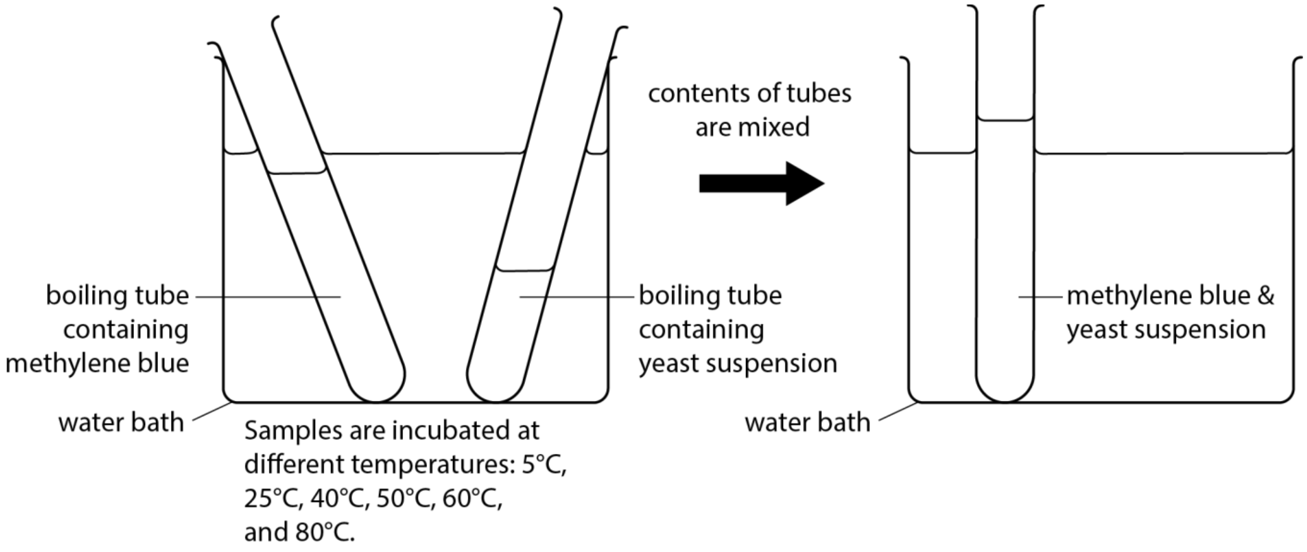
The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners. The information is **not** exhaustive and does not include storage or handling instructions.

Learners should always wear gloves, eye protection and lab coats. There should not be any eating or drinking in the lab. Hands should be washed thoroughly at the end of the experiment.

It is your responsibility to carry out an appropriate risk assessment for this experiment.

Substance	Hazard	First aid
Hot water baths	MEDIUM HAZARD	Flood burnt area with water for at least 10 minutes. For serious injuries see a doctor.
Methylene blue (dilute) aqueous solution	LOW HAZARD	**In the eye:** Flood the eye with gently running tap water for at least 10 min. See a doctor. **Swallowed:** Do no more than wash out the mouth with water. Do not induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor. **Dust breathed in:** Remove the casualty to fresh air. See a doctor if breathing is difficult. **Spilt on the skin or clothing:** Remove contaminated clothing. Wash off the skin with soap and plenty of water. Rinse contaminated clothing. **Spilt on the floor, bench, etc.:** Scoop up solids (take care not to raise dust). Wipe up solution spills or any traces of solid with a damp cloth and rinse it well.
Yeast	ALLERGENS	Gloves should be worn if learners have allergies. If discomfort persists, see a doctor.

Experiment set-up



You should make up solutions as per the manufacturer's instructions. However, here is an example:

Material/equipment	When	How
Yeast–glucose suspension	At least 16 hours in advance.	1. Add 20 g dried yeast to 100 cm^3 of 1% glucose solution. 2. Add 0.1 g sodium phosphate. 3. Add 0.2 g sodium bicarbonate. 4. Mix 5. Insert aquatic aerator and magnetic stirrer. 6. Leave at 20–25°C for at least 16 hours. A rate of 5 bubbles per second is probably sufficient, but this isn't overly important – as long as the suspension is agitated (mixed) and aerated overnight, to promote growth. It might take some trial and error.

Teacher method

This is your version of the method that accompanies the *Teacher walkthrough* video.

Before you begin

Think about:
- the number of groups you will need (group size 2–4 learners)
- the amount of equipment/chemicals required
- how much time you will need for the water baths to reach temperature before the lesson
- the volume of yeast–glucose suspension needed for the number of groups you will have.

Experiment

Walk around the learners during the experiment in case they encounter any difficulties.

Steps	Notes
1. Learners should collect the equipment they require from the front of the class.	
2. They should label six boiling tubes with the six different temperatures to be used.	*Labels should be at the top of the tubes, near the lip.*
3. A syringe should be used to add 6 cm^3 of methylene blue solution to each tube.	
4. Learners should label a further 6 boiling tubes with the six different temperatures to be used.	*Labels should be at the top of the tubes, near the lip.*
5. They should use a syringe to add 2 cm^3 of yeast suspension to each tube.	*It is important that learners gently stir the yeast suspension before measuring it into boiling tubes. It is important that learners gently stir the yeast-glucose suspension before measuring it into boiling tubes. The yeast cells will slowly settle to the bottom of the beaker, so without stirring, samples taken from the top of the beaker would varying vastly in numbers of yeast cells. Stirring before each measurement will ensure that this variable is standardised.*
6. A pair of boiling tubes, each with the same matching temperature label, should be placed into each water bath.	

Steps	Notes
7. The pairs of tubes are left to equilibrate in each water bath for at least 10 minutes.	
8. For one of the pairs of tubes, learners pour the methylene blue into the tube with the yeast–glucose suspension, invert the tube to mix the contents and immediately start the timer. 9. The boiling tube containing the mixture is placed back into the water bath as carefully as possible without shaking the contents.	*Inversion ensures effective mixing. It is important that the timer is started* ***immediately****. Only one pair of boiling tubes should be mixed at any one time, per group.* *It is important that the tube is not disturbed during the incubation to avoid reoxidising the redox indicator, which would invalidate the results. However, the boiling tubes can be very gently lifted from the beaker to check the colour more closely, but must be replaced immediately to ensure that the incubation continues at the correct temperature.*
10. Learners watch the tube for the blue colour to disappear.	*Using another boiling tube containing yeast–glucose suspension as a reference for comparison will help learners to judge the end-point. A thin blue layer at the surface of the yeast suspension can be ignored because this layer is not likely to contain yeast cells, as they gradually sink and form sediment.*
11. Learners stop the clock at the end-point and record the time taken (in seconds) for decolorisation.	*Decolourisation of the methylene blue should occur within 5 minutes when the experiment is conducted at 40 °C. If it is taking longer than this, the methylene blue solution can be diluted further and then the time multiplied up by the appropriate factor.*
12. Learners repeat steps 8–11 for the remaining pairs of boiling tubes and record the time taken (in seconds) for decolorisation.	*Pairs of tubes must be processed sequentially because learners cannot monitor more than one tube at once for decolorisation.*
13. If time allows, learners repeat the entire sequence for each temperature, to gain further readings in order to calculate a mean time for each temperature.	

Clean-up

After the experiment learners should:

- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up
- return all equipment and any unused chemicals to you.

Alternative methods

If you do not have access to the required equipment or the suggested method would not work for your class, here are some possible alternatives that you could use.

If thermostatic water baths are not available:
Learners could set up and maintain their own water baths using Bunsen burners. They can add 300 cm^3 of boiling water to a beaker, then add cold/hot water until the desired temperature is reached. A thermometer should be used to monitor the temperature every 5 minutes and cold/hot water is added as required. The ice water bath can be made by filling a 500 cm^3 beaker with 150 cm^3 of water and then adding 10–15 ice cubes. A thermometer is used to monitor the temperature; it should reach 5 °C after 10–15 minutes.

Alternative to repeats:
Since the pairs of boiling tubes must be processed sequentially, time may not allow for repeat readings at each temperature. Instead, class data could be pooled in order to calculate mean times for each temperature.

Debriefing lesson: Interpreting rate measurements

Resources	• Worksheet F • Data tables from the *Lab lesson*

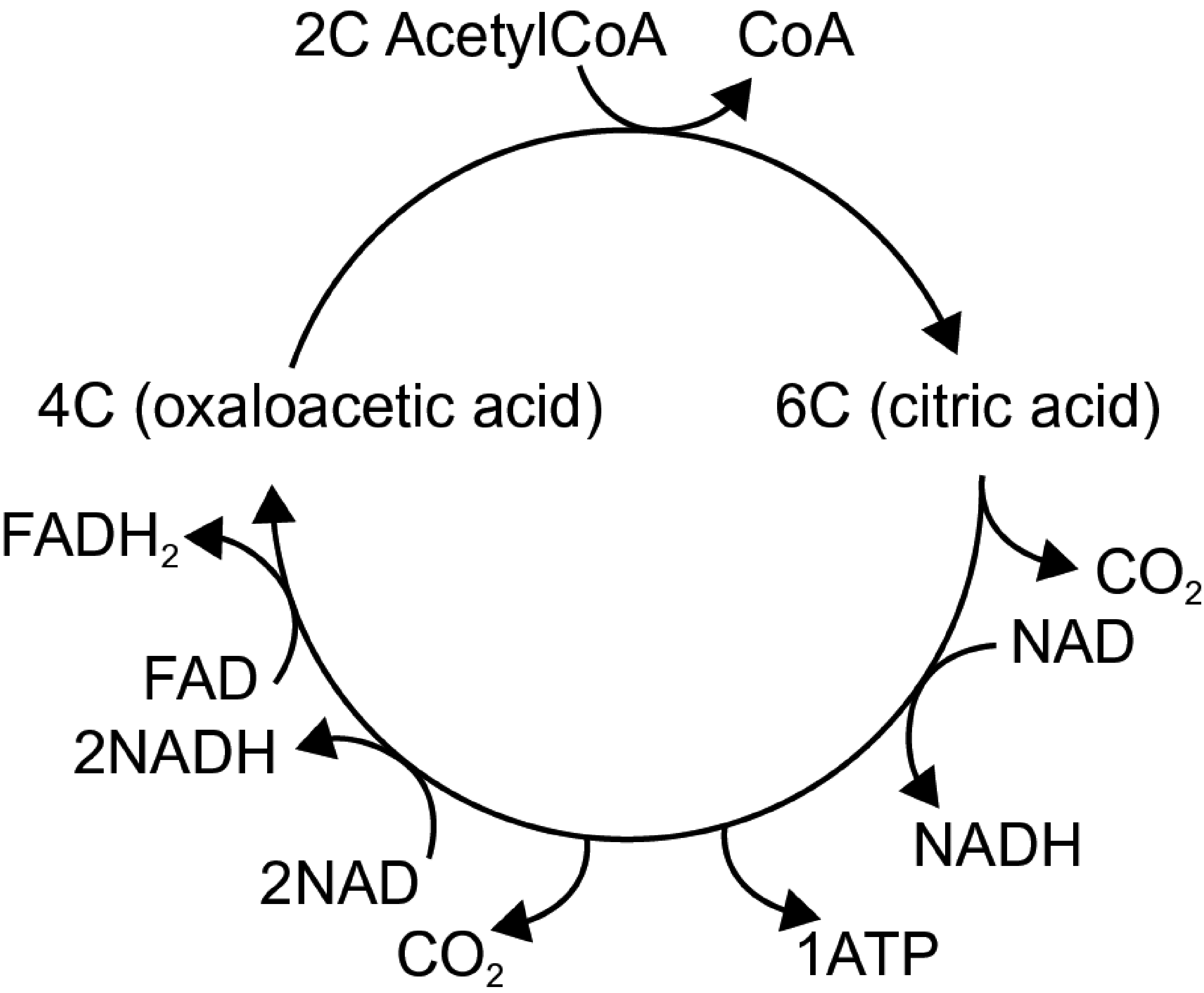
Learning objectives	By the end of the lesson: • ***all*** learners should understand the use of 1/t as a measure of rate • ***most*** learners should be able to process their data and plot a suitable graph • ***some*** learners will understand that, because of the uncertainty of the end-points, they can have confidence in the overall trend but less confidence in the accuracy of individual mean times.

Timings	Activity
15 min	**Starter/Introduction** Give your learners Worksheet F. Ask them to consider the data in groups of 3–4 and discuss how the experiment was carried out. Elicit that it should be clear from the table headings what was done. Ask them to identify the independent and dependent variables, which columns are raw data and which columns show processed data. Ask the groups to discuss how the data was processed; then they should complete the table by filling in the missing values. Check that they use the appropriate number of significant figures; make sure learners understand that the degree of accuracy should be same as used for the raw data, and why this is the case. Ask them to consider if the table presents the final results clearly enough and if not, how this could be improved.
10 min	**Main lesson** Ask learners to identify the independent and dependent variables in the experiment they carried out during the *Lab lesson*. Ask them if there was a control in the experiment and if not, to suggest a suitable control (using boiled yeast at room temperature). Ask them to describe some sources of possible errors in the investigation and how these could have been avoided. Possible errors include inconsistent judgement of the end-point and disturbance of the tubes causing reoxidation of the methylene blue. Point out that any disturbance would cause a random error, whilst if the end-point judgement was inaccurate but consistent, it would cause a systematic error.
5 min	With these errors in mind, ask them to explain how confident they are in the accuracy of their time values. Ask them what this means about their data. Elicit that if they avoided any random error, they can have confidence in the overall trend but less confidence in the accuracy of individual mean times. Provided that the judgement of the end-point was consistent, any error is systematic so the shape of the curve will be reliable as opposed to its position relative to the axes.
20 min	Ask your learners to suggest how their values for mean time taken for decolourisation could be processed to provide a measure of the rate of aerobic respiration in the yeast. Ensure they understand that it is not possible to quantify decolourisation per unit of time in the same way that, for example, mass loss can be

Timings	Activity
	quantified in g/minute. Introduce or remind learners of the concept of using the reciprocal (1/t) as a measure of rate. Now ask your learners if they have designed their results tables to include processed data and if not, how they will present it. Learners process and present their data by calculating the reciprocals of their mean times. To generate larger values that are easier to plot on a graph, you can suggest they multiply the reciprocal by 100 (100/t) provided they indicate this on the axis label by '× 100'. Worksheet F provides some guidance for learners when tabulating processed data by illustrating how to best present processed data, when to include with raw results, or when to put in a separate table. Ask learners what type of graph they should draw to present their processed data, and why. Once they have established it's a line graph, ask them if they should draw a line of best fit, curve of best fit, smooth curve or a set of ruled straight lines to join the points. Make sure they know the appropriate choice (a smooth curve) and can explain why (the graph follows the expected trend for enzyme-catalysed reactions). Learners then plot a line graph of rate of decolourisation against temperature. If required, a set of example results and an example graph is provided on Worksheet G.
	**Plenary** Learners interpret their graph. You might need to prompt them to recall the effect of temperature on the rate of enzyme-catalysed reactions and the concept of an optimum temperature. However, it is important they understand that it is not the yeast that has an optimum temperature, but rather the yeast enzymes involved in respiration, of which there are many. Ask them to use their graph to identify the temperature at which yeast enzymes are most active. Ask them to recall the natural habitats of yeast from Worksheet B and whether this value makes sense. Ask them to suggest why the value is higher than they might expect. With prompting, they might be able to suggest the type of yeast used in the experiment (baker's or brewer's yeast, rather than a wild strain seen on Worksheet B) and suggest that it may have been selected for its ability to carry out fermentation at higher temperatures. Ask learners to suggest how this investigation could be modified to more **accurately** determine the optimum temperature for yeast respiration. With prompting, they should be able to suggest repeating the experiment at closer temperature intervals around the peak of their curve.

Worksheets and answers

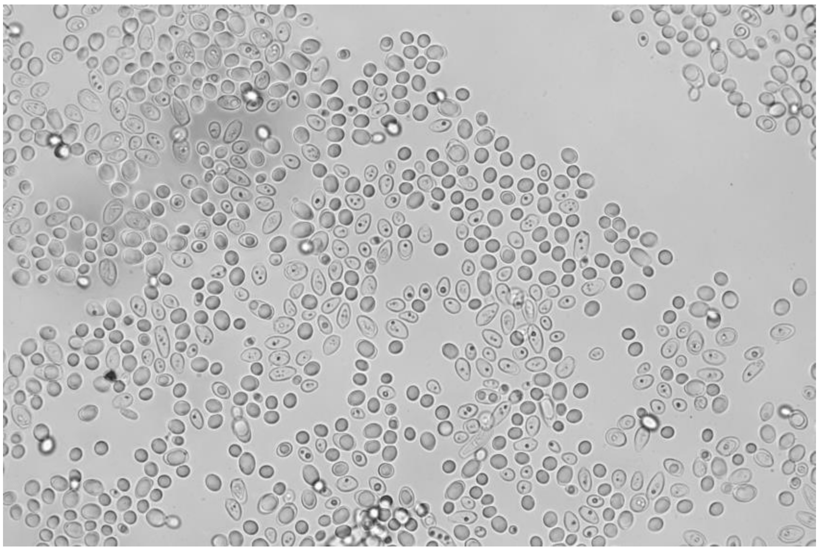
Worksheet A: Outline of Krebs cycle



Worksheet B: Yeast fact sheet

Introduction

Yeast is a microscopic unicellular fungus. Yeast cells are roughly spherical and about 4 µm in diameter. This photo shows what they look like under a light microscope.

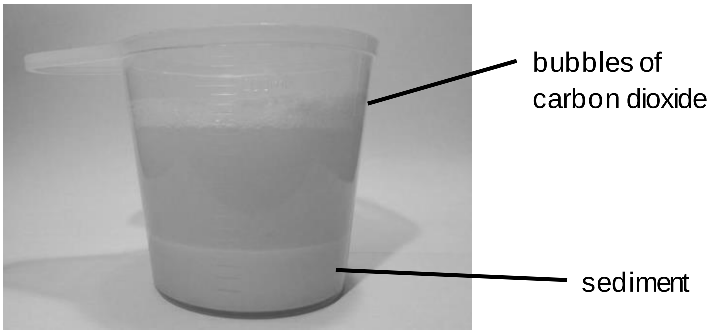


Yeast cells are found growing on the surfaces of fruits, leaves and in flowers where they feed on sugars from the plant. Yeast is able to respire in both aerobic and anaerobic conditions. Both conditions often occur at the same time in a yeast culture.

In the laboratory

To grow yeast cells in a laboratory, the cells are suspended in an aqueous solution of a suitable respiratory substrate such as glucose. The cultures are cloudy because of the cells suspended in the liquid. Yeast cells are non-motile so the culture has to be stirred continuously to prevent the cells slowly sinking to the bottom of the container to form a layer of sediment.

Near the top of the container, the yeast cells are exposed to oxygen from the air that dissolves at the surface. Lower down the container, the oxygen might be used up, creating anaerobic conditions.



Yeast cells respiring aerobically (near the top of the container) obtain dissolved oxygen from the solution and release carbon dioxide and water into the solution. When they respire anaerobically (those near the bottom of the container), they release carbon dioxide and ethanol into the solution.

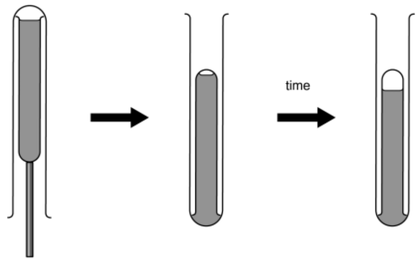
Carbon dioxide produced by the yeast during respiration dissolves in the solution to form carbonic acid. However, when yeast is respiring rapidly, bubbles of carbon dioxide are forced out of solution and rise to produce foam at the surface.

Worksheet C: Measuring anaerobic respiration

One way to be sure that an experiment is measuring the rate of just anaerobic respiration in yeast cells is to create completely anaerobic conditions in the yeast culture. This can be achieved with a very small volume of yeast culture in a closed environment, because the yeast cells will rapidly use all the oxygen and no further oxygen can diffuse into the culture from the air.

Method:

1. Fill a small test-tube with a small volume of the yeast culture.
2. Use a glass rod to push the small test-tube to the top of an inverted test-tube of standard size.
3. Turn the set-up over quickly so that the yeast culture remains trapped in the small test-tube.



There is very little of the liquid's surface in contact with the air, so the small volume of yeast culture rapidly becomes anaerobic. As the yeast respires anaerobically, carbon dioxide is forced out of solution and collects as a gas bubble at the top of the small test-tube. The volume of gas collected in a fixed period of time can be measured.

The experiment was carried out for a variety of substrates. The volume of carbon dioxide was measured after 20 minutes.

Substrate	Fructose	Sucrose	Glucose	Maltose	Lactose
Volume of CO_2 / mm^3	390	930	1260	30	20
Rate of anaerobic respiration /					

1. Decide on a suitable measure of the rate of anaerobic respiration and add units to the heading in the appropriate cell. Calculate the rate for each substrate.

2. How should these results be presented graphically? Why?

3. Suggest how the volume of carbon dioxide might have been measured.

4. Suggest an improvement to the experiment set-up that would increase the certainty that only anaerobic respiration is taking place.

5. Identify two variables that would need to be standardised for the results to be accurate and justify your choice.

6. Why do the results not give the total volume of carbon dioxide produced by the yeast cells in 20 minutes?

7. Why would this method not be appropriate for finding the effect of temperature on the rate of anaerobic respiration?

Worksheet D: Measuring aerobic respiration

One way to be more certain that an experiment is measuring the rate of just aerobic respiration in yeast cells is to use a redox indicator.

Remember that during the Krebs cycle there are a series of reactions, some of which are dehydrogenation reactions. In dehydrogenation reactions, dehydrogenase enzymes remove hydrogen atoms from respiratory substrates. These hydrogen atoms are taken up by hydrogen acceptors (NAD and FAD).

The dehydrogenation reactions are not usually visible but can be observed as a colour change if a redox indicator is used. Redox indicators are artificial hydrogen acceptors that will accept some of the hydrogen atoms during the Krebs cycle that are normally accepted by NAD and FAD. The redox indicators are one colour when they are oxidised (the normal colour in air where oxygen is present) but they change colour when they are reduced by accepting hydrogen atoms. This change in colour can provide a visual check on the rate of aerobic respiration.

Three redox indicators are shown in the table.

Redox indicator	Colour when oxidised	Colour when reduced
Methylene blue	Blue	Colourless
Triphenyltetrazolium chloride (TTC)	Colourless	Pink
Dichlorophenol indophenol (DCPIP)	Blue (pink in acidic conditions)	Colourless

A redox indicator can be added to a yeast culture to measure the rate of aerobic respiration.

It is important that redox indicators:

- are not toxic to cells or organelles
- have an intense colour visible at very low concentration (this helps to avoid poisoning the cells or organelles)
- show a distinct colour change that is not obscured by the natural colour of the liquid they are added to, such as a cloudy yeast culture.

1. Suggest why redox indicators highlight aerobic respiration.
2. Use the information above to write down the advantages and disadvantages of each redox indicator if used with a yeast culture. Explain your judgements.
3. Which redox indicator(s) would be most suitable for use with a yeast culture? Justify your decision.

Worksheet E: Planning prompts

A good plan should focus on 'what' will be investigated and 'how' this will be achieved. Ask yourself the following questions when planning an experiment.

What …	How …
is the investigation about?	does your understanding predict what might happen?
is expected to happen (hypothesis)?	will I test my hypothesis?
are the variables (independent and dependent)?	will I change or measure each variable? will I standardise any other appropriate variables?
data will I collect?	will I collect the data accurately?
apparatus should I use?	should the apparatus be used/arranged?
techniques should I use?	will the techniques be used?
sequence of steps should I take?	will I order each step so the plan is easy to follow?
risks are there?	will I make it safe?

Things to consider:

- How you will know your yeast suspension is at the right temperature
- Which redox indicator you will use and why
- How you will time the decolourisation
- How many different temperatures you will use, and what will be the intervals between them
- When you will add the redox indicator to the yeast suspension
- What you will standardise whilst varying the temperature
- What range of temperatures you will use
- How you will heat the yeast suspension to different temperatures
- How you will measure the volume of yeast suspension and redox indicator
- How you will decide when it has decolourised
- How many times you will measure the time for decolourisation at each temperature.

Remember that you will have 2 cm^3 of yeast suspension and 6 cm^3 of redox indicator for each temperature.

Worksheet F: Presenting processed data

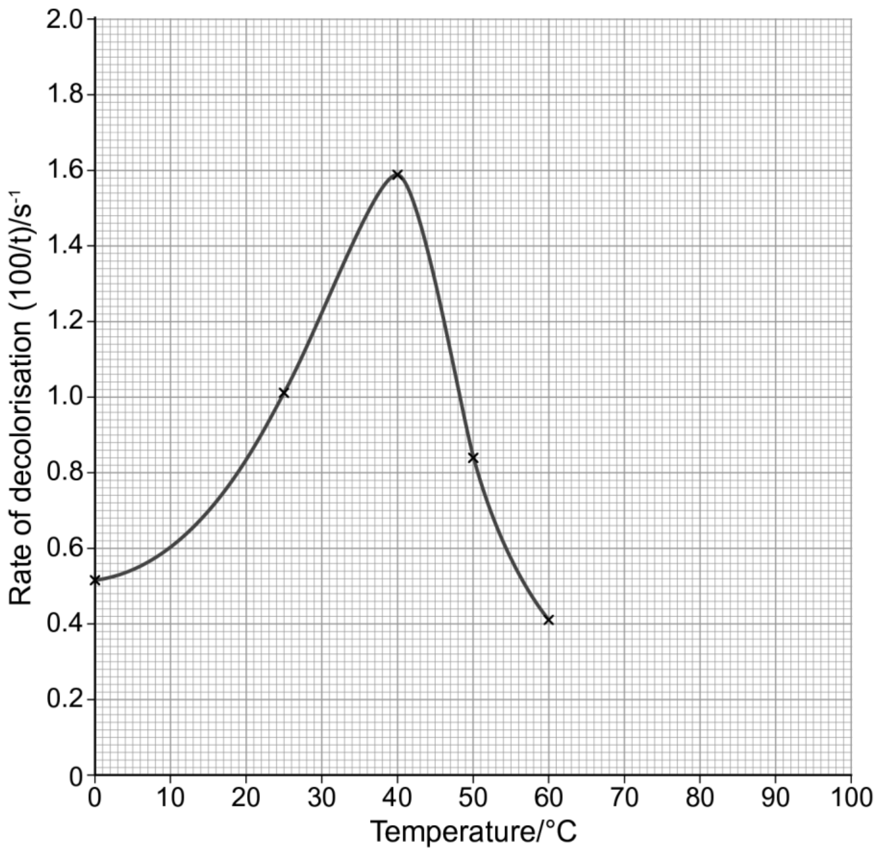
The table below shows data from an experiment on transpiration.

Leaves were hung in warm air for 30 minutes.

leaf	greased surface	initial mass of leaf / g	final mass of leaf / g	change in mass / g	percentage mass loss / %
A1	neither surface	9.1	5.9	-3.2	35.2
A2	neither surface	9.3	6.2	-3.1	33.3
B1	upper surface	13.5	11.3	-2.2	16.3
B2	upper surface	13.6	11.2		
C1	lower surface	13.8	12.6	-1.2	8.7
C2	lower surface	13.8	12.7		
D1	both surfaces	14.4	14.2	-0.2	1.4
D2	both surfaces	14.2	14.1	-0.1	0.7

Worksheet G: Example results

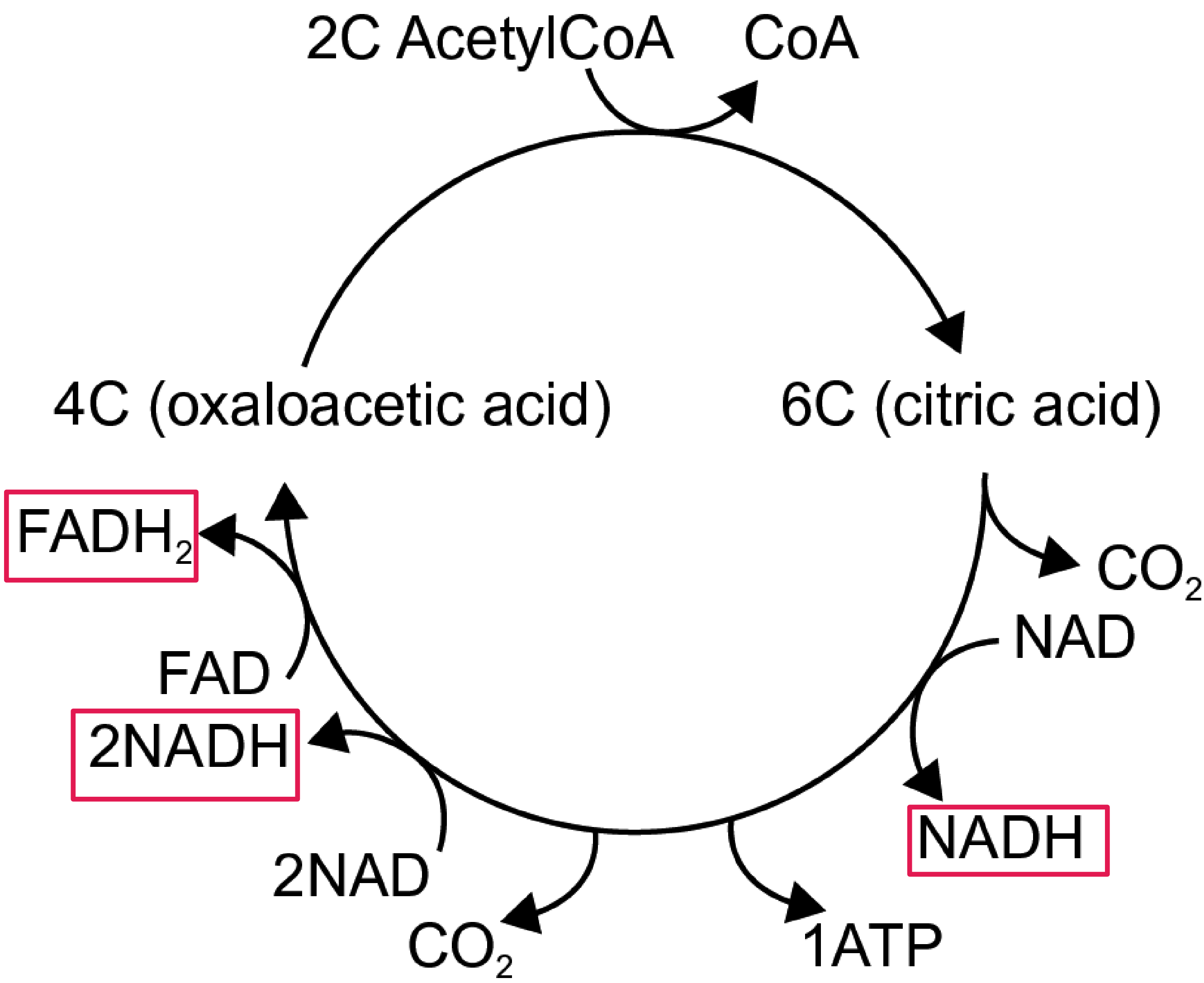
Temperature / °C	Time taken for methylene blue to decolourise / seconds				Rate of decolourisation / (100/ time)/ s^{-1}
	Sample A	Sample B	Sample C	Mean	
0	168	180	178	175 (3 sf)	0.571
25	94	101	96	97	1.03
40	66	63	60	63	1.59
50	115	118	124	119	0.840
60	246	230	254	243 (3 sf)	0.412
80	No reaction	No reaction	No reaction	N/A	N/A



The results of this investigation can be explained by what we know about the effect of temperature on the rate of enzyme-catalysed reactions. As the temperature increases, the rate of respiration increases. This is because the kinetic energy of the dehydrogenase enzymes and their substrates increase, raising the likelihood of collisions resulting in enzyme–substrate complexes being formed. More hydrogen is removed from glucose and transferred to the methylene blue per unit time, resulting in faster decolourisation.

However, above a certain substrate concentration, there will be a dramatic fall in the rate of respiration as the dehydrogenase enzymes denature, losing the ability to catalyse reactions, and the yeast cells die.

Worksheet A: Answers



Worksheet B: Suggested points for discussion

The following methods could be used to measure the respiration rate of a yeast culture. However, some methods are much harder, or impossible, to carry out with a liquid culture.

Method	Suitability for yeast culture experiment
Rate of oxygen uptake	Can be measured for small invertebrates and seeds in a simple respirometer by including a carbon dioxide absorber such as potassium hydroxide, but this only works in air, not in a liquid culture.
Rate of glucose uptake	The presence of glucose could be detected by using Benedict's solution, but the test is not sensitive enough to follow the disappearance of glucose over time.
Rate of ethanol production	There is no simple method of measuring the concentration of ethanol.
Rate of pH decrease	This can be measured by using a pH indicator such as bromothymol blue. However, recording the colour changes only gives qualitative data and using the colour chart to convert to pH values will not give values to more than one significant figure. Some pH indicators are toxic to yeast so cannot be added directly to the culture. A pH probe linked to a data logger can be used to obtain more useful quantitative data.
Rate of carbon dioxide production	The volume of gas produced over time can be measured directly by collecting the carbon dioxide, by displacing liquid in an inverted container. Insufficient carbon dioxide is produced to collect in a gas syringe. Often only a small volume is produced, which can then be difficult to measure if the container is not graduated. Also, some of the carbon dioxide produced by the yeast will dissolve in the solution. Measurement of features of carbon dioxide production such as rate of foam build-up or rate of bubble formation can also be used, but these methods are less reliable. Bubbles can be different sizes and so counting bubbles is not necessarily an accurate method. The depth of foam measured in mm formed per minute can be measured, but the foam bubbles tend to burst as they form, so again, this is not necessarily an accurate method.

Worksheet C: Suggested / example answers

1. The volumes are divided by 20 to give a rate expressed as **mm^3 / min**.

Substrate	Fructose	Sucrose	Glucose	Maltose	Lactose
Volume of CO_2 / mm^3	390	930	1260	30	20
Rate of anaerobic respiration / mm^3 / min	**19.5**	**46.5**	**63.0**	**1.5**	**1.0**

2. Since the independent variable is categorical, the correct form of presentation is a bar chart (the bars should not touch).

3. Known volumes of air could be bubbled into a small test-tube inverted in a large beaker of water using a syringe and graduations drawn onto the test-tube with a marker pen. These graduations could be copied onto other small test-tubes.

4. A small quantity of oil could be added to the standard test-tubes to form an air-tight seal around the mouths of the inverted small test-tubes.

5. The temperature would have to be the same (temperature alters the rate of respiration and also might cause the small volume of gas to contract or expand). The concentration of the substrate solution in each tube would have to be the same because this might also alter the rate of respiration.

6. Some carbon dioxide would remain dissolved in the culture solution.

7. Higher temperatures would cause the trapped carbon dioxide bubbles to expand so the volumes of gas collected at each temperature would not be comparable.

Worksheet D: Suggested / example answers

1. Anaerobic respiration involves far fewer dehydrogenation reactions compared to aerobic respiration, so is much less effective at decolourising redox indicators. Therefore, in this type of experiment set-up, it's likely that only aerobic respiration will be detected using a redox indicator.

2.

Redox indicator	Advantage	Disadvantage
Methylene blue	Change from blue to colourless on reduction can be seen relatively easily in a cloudy yeast suspension, because it is the loss of dark colour against the beige liquid.	N/A
Triphenyltetrazolium chloride (TTC)	N/A	TTC changes from colourless to pink, which would be much harder to see, so the end-point would be much harder to judge.
Dichlorophenol indophenol (DCPIP)	Change from blue to colourless on reduction can be seen relatively easily in a cloudy yeast suspension, because it is the loss of dark colour against the beige liquid.	Oxidised DCPIP is pink in more acidic conditions, which would be the case for a rapidly respiring yeast suspension (carbon dioxide dissolves to form carbonic acid in solution). The loss of pink in a beige liquid would be harder to judge than the loss of blue.

3. This means that of the three redox indicators, methylene blue is the best option for use with a yeast suspension because the colour change is the easiest to see in a cloudy yeast suspension.

Worksheet F: Answers

Independent variable: which leaf surface is covered with grease
Dependent variable: final mass of the leaf
Raw data: which surface is greased; the initial mass of leaf; and final mass of leaf
Processed data: change in leaf mass; and the percentage mass loss.

leaf	greased surface	initial mass of leaf / g	final mass of leaf / g	change in mass / g	percentage mass loss /%
A1	neither surface	9.1	5.9	-3.2	35.2
A2	neither surface	9.3	6.2	-3.1	33.3
B1	upper surface	13.5	11.3	-2.2	16.3
B2	upper surface	13.6	11.2	-2.4	17.6
C1	lower surface	13.8	12.6	-1.2	8.7
C2	lower surface	13.8	12.7	-1.1	8.0
D1	both surfaces	14.4	14.2	-0.2	1.4
D2	both surfaces	14.2	14.1	-0.1	0.7

In the table above, processed data has been included (change in mass, and percentage mass loss) alongside raw data (greased surface; initial mass; and final mass). In many cases, this is the best way to present processed data, but it does require your learners to anticipate the need for additional columns and to design the table to include these at the start. Adding them as an afterthought often leads to unsatisfactory table presentation.

Note that the change in mass and percentage mass loss are only given to the same number of significant figures as the raw data. This is an important point of discussion to have with your learners, make sure they understand why.

One possible way to more clearly present the final outcome of the experiment would be to summarise the data in a separate table like that shown below.

greased surface of leaf	mean percentage mass loss /%
neither	34.3
upper	17.0
lower	8.4
both	1.0

A possible alternative would be to add a further column for the ‘mean percentage mass loss’ to the original table. However, because there are already a large number of figures in the table, and because of the way that the independent variable has been recorded in pairs, it would be more difficult to read across the table which mean value refers to which greased surface. So in this instance, a separate table makes the final results clearer.

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