Chapter 3 - Food

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Activity 1 To conduct qualitative tests for food

A qualitative test measures whether a substance is present or absent.

Activity 1a To test for reducing sugar

1. Dissolve glucose in water in a test tube.
2. Add an equal volume of Benedict's solution (which is blue).
3. In a second test tube mix equal volumes of water and Benedict's solution. This will act as a control.
4. Heat the test tubes in a boiling water bath.
5. If reducing sugar is present, the solution turns red (often called brick red).
6. If reducing sugar is not present the solution remains blue.

Note: Fehling's solution can be used instead of Benedict's solution.

3.18 *Benedict's test for reducing sugar: a negative result (blue) and a positive result (red-orange)*

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Starch

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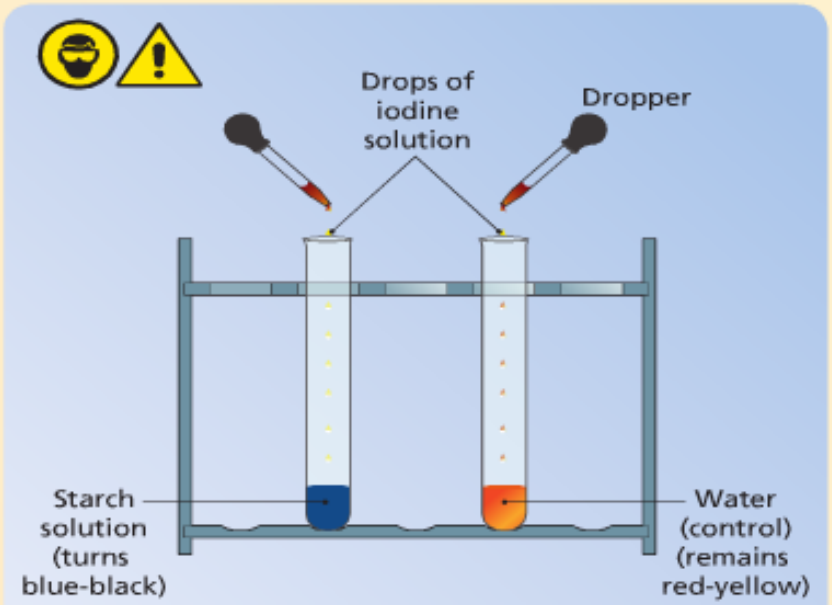
1. Add a few drops of iodine solution to some starch dissolved in water. (Iodine solution is a red-yellow colour.)
2. Add a few drops of iodine solution to some water. This acts as a control.
3. If starch is present the colour turns blue-black or purple.
4. If starch is absent the solution stays red-yellow.



3.21 *Testing for starch*

Activity 1c To test for lipid

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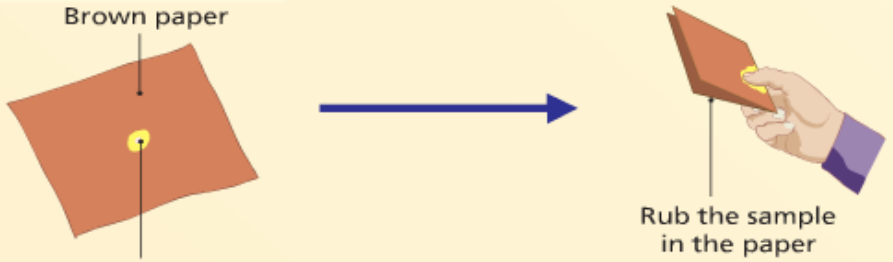
1. Label a piece of brown paper (or filter paper) as lipid.
2. Rub a small piece of butter or cooking oil (both lipids) on the paper.
3. Repeat the process using a few drops of water on a piece of paper labelled 'water'. (This acts as a control.)
4. Leave the two pieces of paper over a radiator to dry.
5. Lipid produces a permanent stain (or translucent spot) on the paper.
6. The water stain dries out.

Note: lipids can also be tested using Sudan III.



3.22 *Testing for lipid*

Activity 1d To test for protein

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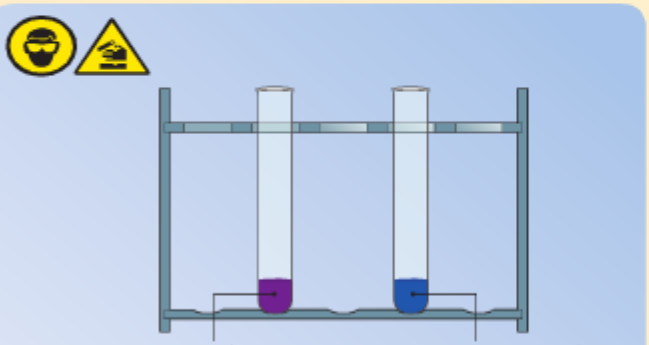
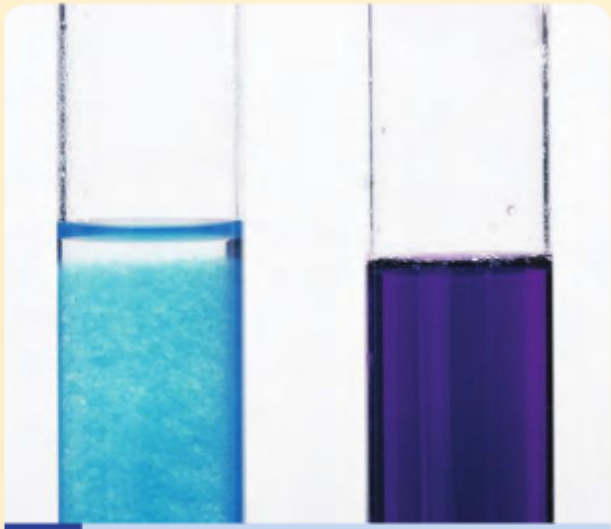
1. Dissolve a sample of soluble protein (e.g. egg white or milk) in water.
2. Add sodium hydroxide (colourless) until the solution clears.
3. Then add a few drops of dilute copper sulfate (blue).

Note: as an alternative to steps 2 and 3, add an equal volume of Biuret solution. This contains sodium hydroxide and copper sulfate and is blue.



3.23 *Testing for protein*



3.24 *Biuret test for protein: a negative result (blue) and a positive result (purple)*

4. As a control, add sodium hydroxide and copper sulfate (or Biuret solution) to water.
5. The appearance of a purple-violet colour shows that proteins are present.
6. If protein is not present the colour remains blue.

R. Cummins

Chapter 7 - The Cell

Activity 6 To be familiar with and to use a light microscope

Examine a range of prepared slides as follows:

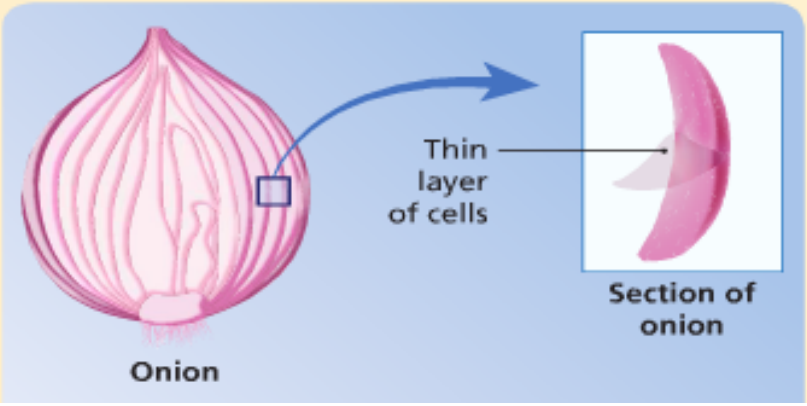
1. Make sure that the lenses are clean.
2. Lower the microscope stage as far as it will go.
3. Click the low-power objective lens into place.
4. Place a microscope slide on the stage. Ensure that the object to be viewed is in the centre of the opening in the stage.
5. Clip the slide into position.
6. View the stage from the side and use the coarse adjustment knob to move the low-power objective lens down so that it is just above the slide.
7. View the object through the microscope.
8. Adjust the coarse adjustment knob to move the stage down until the object is in focus. (Steps 6–8 prevent the slide from being damaged by the objective lens.)
9. Adjust the amount of light so that the object can be seen most clearly (this often involves reducing the amount of light). Depending on the type of microscope being used this may involve one or more of the following procedures:
   - Adjusting the condenser to focus light on the object
   - Adjusting the diaphragm to control the amount of light reaching the object
   - Adjusting the angle of the mirror
   - Using the concave side of the mirror
   - Placing a sheet of paper between the bulb and the microscope to cause the light to be diffused.
10. When the object is focused under low power, move the slide so that the part of the object you wish to view is in the centre of what you can see (called your field of view).
11. Click the high-power objective lens into place.
12. The object should be almost in focus. If it is not, use the fine adjustment knob to focus it correctly. Be careful not to move the objective lens too close to the slide (as this would crack the slide).

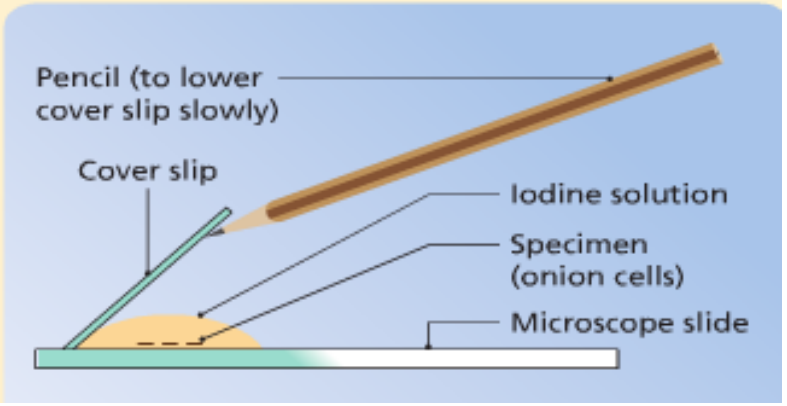
A Prepare the slide

1. Remove the outer, dry scaly leaves of an onion.
2. Use a forceps or your fingers to pull a strip of thin, transparent epidermis from the inner curve of a fleshy, inner leaf.
3. Place a small piece of the epidermal strip on a microscope slide.
4. Add a few drops of iodine solution. (This is a red-yellow stain. It stains the nucleus orange and the cytoplasm yellow. A mixture of potassium iodide and iodine gives a better result.)
5. Add a cover slip (this prevents the cells from drying out and prevents the lens from getting stained). Lower the cover slip at an angle (this eliminates air bubbles).
6. Blot off any surplus iodine, if necessary.
7. The cells can be viewed unstained by using a few drops of water instead of iodine solution at step 4 above.

B Examine under the microscope

1. The slide can be examined under the microscope in the same way as described in Activity 6.
2. The results will appear similar to those shown in diagram 7.18.
3. Draw diagrams of what you can see at low power and at high power.



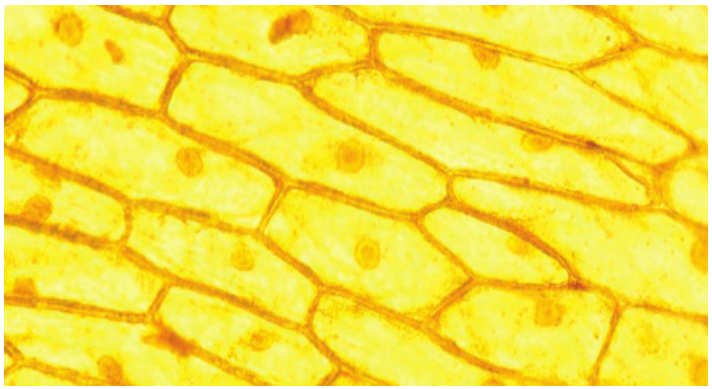


A Prepare the slide

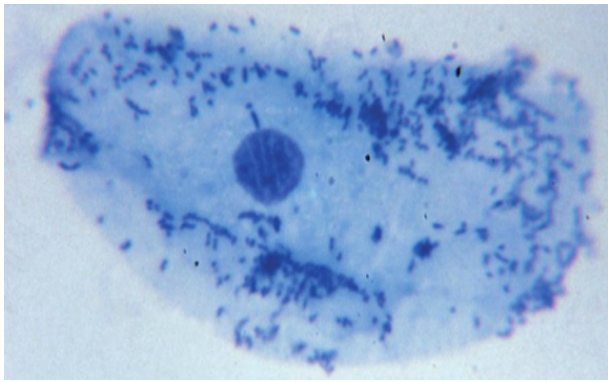
1. Rinse your mouth out with water.
2. Scrape the inside of your mouth with a lollipop stick or a cotton wool bud (this will collect many cheek cells).
3. Spread the smear of cells thinly onto a glass slide.
4. Add a few drops of methylene blue (this stains the nucleus dark blue and the cytoplasm pale blue) and leave for a few minutes.
5. Add a cover slip at an angle (to eliminate air bubbles).
6. Blot off excess stain, if necessary.
7. The cells can be viewed unstained by using water instead of methylene blue at step 4.

B Examine under the microscope

1. The slide can be examined under the microscope in the same way as described in Activity 6.
2. The result will appear as shown in diagram 7.21.
3. Draw diagrams of the cells at low power and at high power.



Onion Cell with Iodine



Human Cheek Cell with Methylene Blue

Chapter 9 - Enzymes

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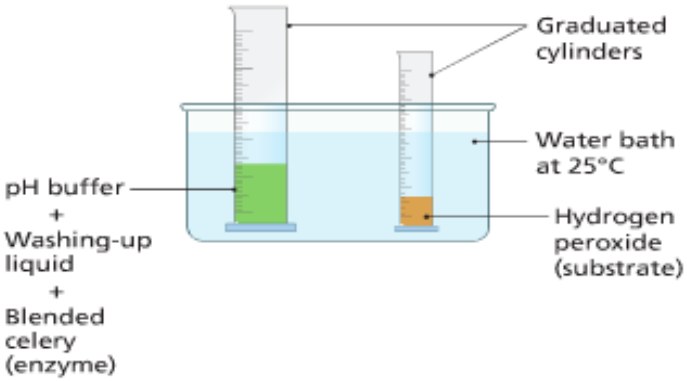
9.14 *Foam produced when hydrogen peroxide is broken down by catalase (in pieces of potato)*



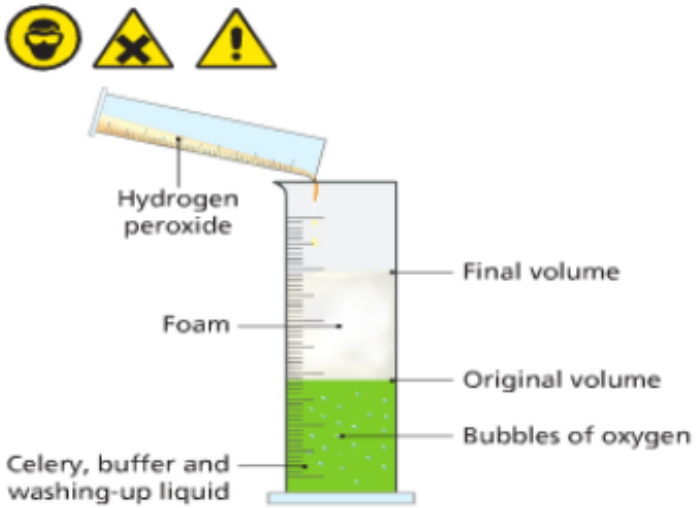
Introduction

Catalase is an enzyme that is found in a wide range of living things, e.g. liver, radishes, celery and potatoes. It converts the toxic substance hydrogen peroxide (H_2O_2) into water and oxygen.

When using catalase the oxygen forms foam (in association with washing-up liquid). The volume of the foam indicates the activity of the enzyme.

1. Place some pH buffer solution 4 in a graduated cylinder (pH buffer 4 ensures that the pH will remain at 4).
2. Using a dropper add one drop of washing-up liquid to the graduated cylinder (the washing-up liquid traps the oxygen that is released, forming foam).
3. Blend some stalks of celery in water in a blender. Filter this solution into a large beaker using coffee filter paper (filtration removes the blended cells and contents; coffee filter paper allows for fast filtration).
4. Add some of the filtrate to the graduated cylinder (the celery contains the enzyme catalase).
5. Add some hydrogen peroxide to a smaller graduated cylinder (hydrogen peroxide is the substrate).

9.15 *Investigating the effect of pH on enzyme activity*



9.16 *Measuring the rate of reaction*

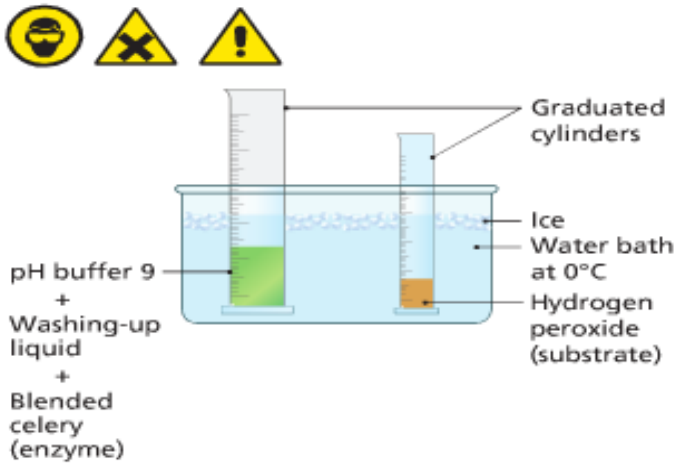
6. Stand both graduated cylinders in a water bath (or a beaker of water) at 25°C for a few minutes (this ensures a constant temperature).
7. Remove the graduated cylinders from the water bath and pour the hydrogen peroxide into the graduated cylinder containing the blended celery.
8. Note and record the volume at the top of the foam after 2 minutes.
9. Calculate the volume of foam produced. This is done by subtracting the original volume of liquid in the graduated cylinder from the volume at the top of the foam after 2 minutes (the volume of foam indicates the rate of the reaction).
10. Repeat steps 1–9 using pH buffers 7, 10 and 13.
11. Record the results as shown overleaf; the first set of figures is filled in as an example.

Activity 8 results				
pH buffer	**4**	**7**	**10**	**13**
Original volume (cm^3)	25			
Volume after 2 minutes (cm^3)	25			
Volume of foam (cm^3)	0			

12. Draw a graph of the results. Put pH on the horizontal axis and the volume of foam produced on the vertical axis. The graph should have a similar shape to that in diagram 9.7 (page 104).
13. Note that catalase is different to most enzymes as it has its maximum activity at pH 9 or 10.
14. As controls, repeat each procedure but do not add blended celery (i.e. no catalase is present) **or** add blended celery that has been boiled (to denature the catalase). In each case no foam is formed.

Activity 9 To investigate the effect of temperature on the rate of enzyme activity

1. Place some pH buffer 9 solution in a graduated cylinder (catalase works best at pH 9; the buffer ensures the pH remains constant at 9).
2. Using a dropper add one drop of washing-up liquid to the graduated cylinder (the washing-up liquid traps the oxygen that is released, forming foam).
3. Blend some stalks of celery in water in a blender. Filter this solution into a large beaker using coffee filter paper (filtration removes the blended cells and contents; coffee filter paper allows for fast filtration).
4. Add some of this solution to the graduated cylinder (the celery contains the enzyme catalase).



9.17 *Investigating the effect of temperature on enzyme activity*

5. Add some hydrogen peroxide to a smaller graduated cylinder (hydrogen peroxide is the substrate).
6. Stand the graduated cylinders in a large beaker of ice-cold water until they are at 0°C.
7. Remove the graduated cylinders from the water bath and pour the hydrogen peroxide into the graduated cylinder containing the blended celery.
8. Note and record the volume at the top of the foam after 2 minutes.
9. Calculate the volume of foam produced, as shown in diagram 9.16 in Activity 8.
10. Repeat steps 1–9 at 10°C, 20°C, 30°C, 40°C, 50°C and 60°C.
11. Record the results as shown; the first set of figures is given as an example.

Activity 9 results

Temperature (°C)	0	10	20	30	40	50	60
Original volume (cm^3)	25						
Volume after 2 minutes (cm^3)	25						
Volume of foam (cm^3)	0						

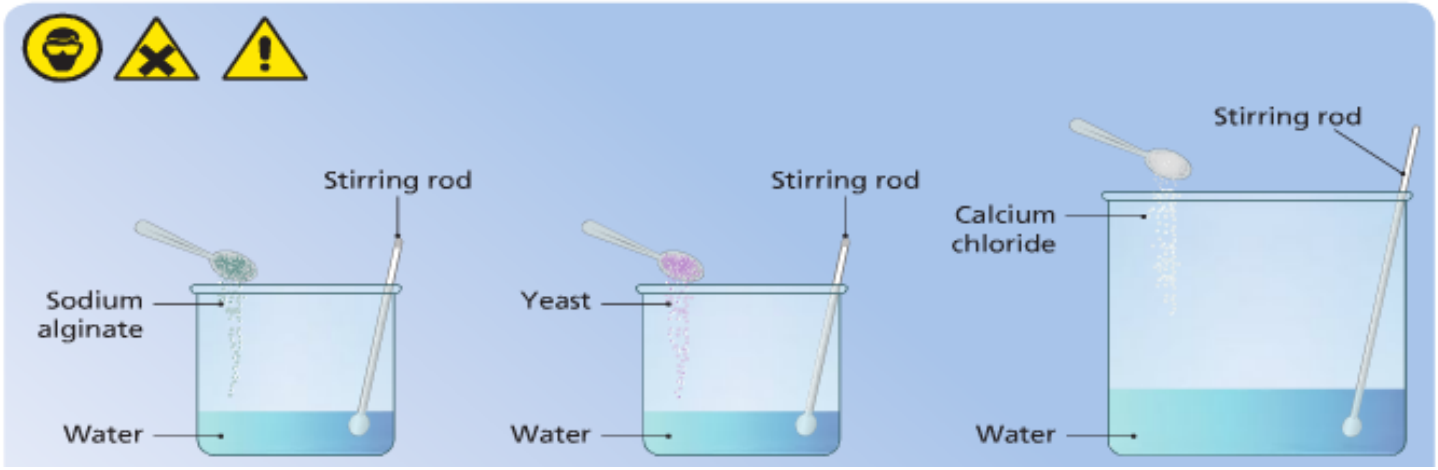
Activity 10 To prepare an enzyme immobilisation and examine its application

Preparing the immobilised enzyme

The formation of alginate beads is a delicate process. All equipment must be clean before use. If possible all the water used in this activity should be distilled water.

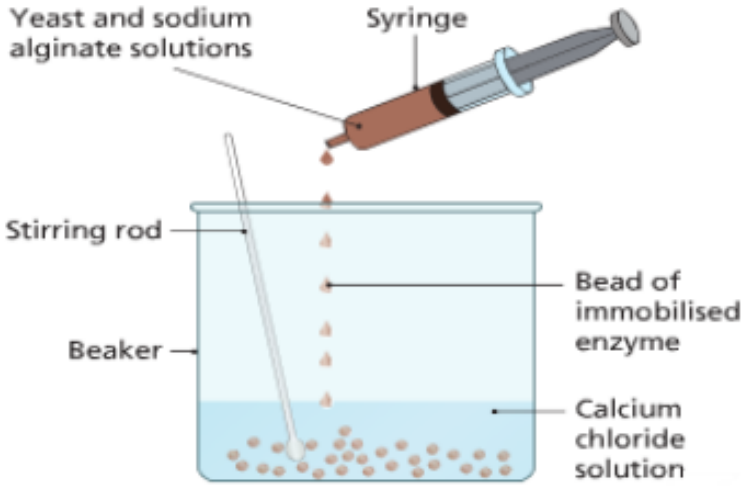
In this activity yeast is immobilised. Yeast contains the enzyme sucrase. This means that the enzyme that is immobilised is **sucrase**.

1. Add some sodium alginate to water in a beaker.
2. Stir the mixture with a glass rod until it forms a smooth paste and leave it to soak for 5 minutes (sodium alginate is used to immobilise the yeast (and the enzyme)).
3. Add some yeast to water in a second beaker (the yeast contains the enzyme (sucrase) that is to be immobilised).
4. Stir the yeast solution and leave it for 5 minutes.
5. Dissolve some calcium chloride in water in a larger beaker.



Continued...

6. Pour the yeast solution into the alginate paste and stir to mix thoroughly.
7. Draw some of the resulting mixture into a syringe (with no needle attached).
8. Slowly and steadily add a series of alginate and yeast drops from the syringe to the calcium chloride solution. Hold the syringe fairly high above the chloride solution and gently stir the solution as you add the drops (this prevents them from clumping). Beads of calcium alginate gel form, enclosing and immobilising some of the yeast cells.



9.19 *Immobilising an enzyme*

9. Leave the beads in the calcium chloride solution for 15 minutes (this allows the beads to harden).
10. Place the beads in a sieve and rinse them under a tap of running water (this removes any yeast cells from outside the hardened beads). If necessary, the beads can be stored in water or dried on filter paper and stored in a refrigerator.

Application of Immobilised Enzyme

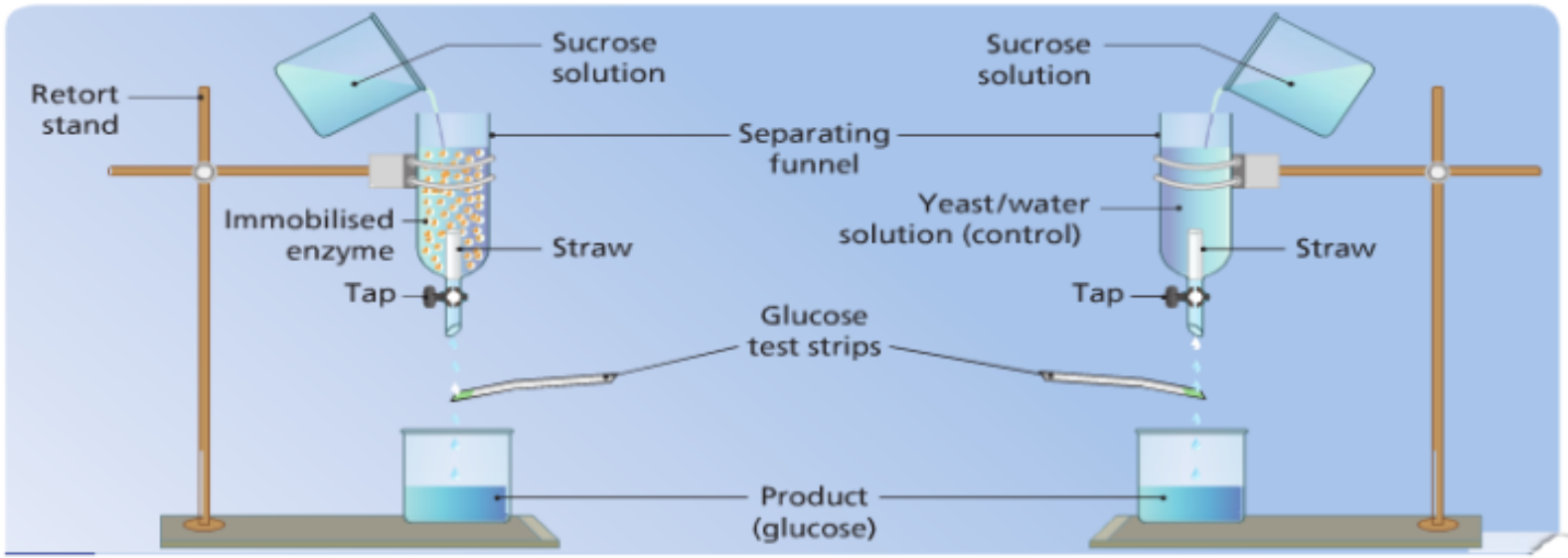
Examining the application of the immobilised enzyme

Yeast contains the enzyme sucrase. This enzyme converts sucrose into glucose and fructose. In this activity the ability of immobilised yeast and free yeast to convert sucrose into glucose is compared.

1. Pour the beads of immobilised enzyme into a separating funnel, as shown in diagram 9.21. A short piece of a drinking straw or a twisted-up paper clip should be used (to prevent the beads from blocking the outlet of the funnel).

9.20 *The conversion of sucrose to glucose and fructose*

2. Add some yeast to water in a beaker and stir.
3. Pour this solution into a second separating funnel.
4. Dissolve some sucrose in warm water.
5. Pour half of the sucrose solution into each separating funnel.
6. Test the products by letting them drip onto glucose test strips such as Clinistix or Diastrix.
7. Continue to test until glucose is found coming from each separating funnel.
8. Note and record the time taken for glucose to first form. Note that in most cases glucose is formed more quickly in the separating funnel containing the non-immobilised yeast (the control). Immobilised yeast is slower to **start** forming glucose. This is because it takes longer for the sucrose to penetrate the alginate beads and for the glucose to emerge from the alginate beads. However, once they start producing glucose the immobilised enzymes (or yeast) can be reused very easily.
9. Observe the products in each beaker. Compare the cloudiness of each solution. (The non-immobilised yeast solution contains many yeast cells and is very cloudy or turbid. The product of the immobilised yeast is much clearer because there are no yeast cells present.)
10. Present the results as shown below.



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Activity 10 results		
	Immobilised yeast (or enzyme)	**Non-immobilised yeast (or enzyme)**
Time taken (minutes) for glucose to form		
Cloudiness of product (cloudy or clear)		

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11 - Photosynthesis

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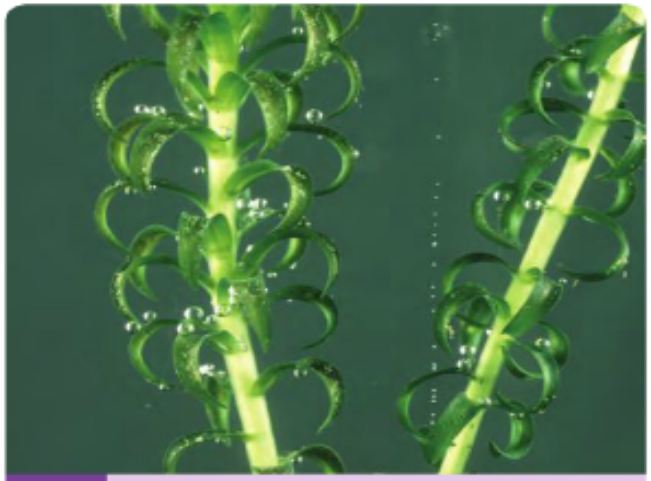
In this activity *Elodea* or pondweed is used. *Elodea* is an underwater plant, so it is possible to see the bubbles of oxygen released from the plant as they pass through the water.

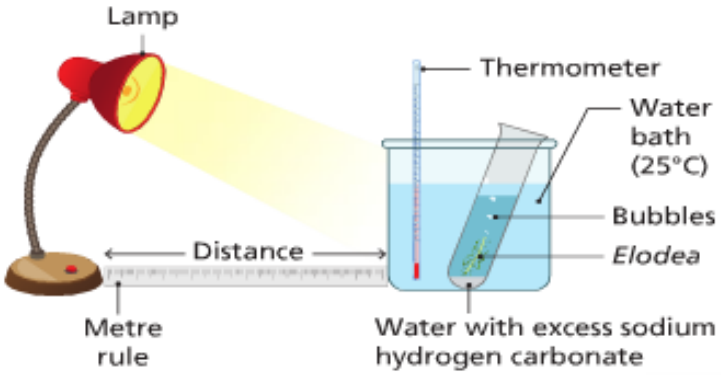
The rate of photosynthesis is measured by counting the number of bubbles released in a given time.

1. Add excess sodium bicarbonate (also called sodium hydrogen carbonate) to some water in a test tube. This means that water is saturated with carbon dioxide (which ensures a constant concentration of carbon dioxide during the experiment).
2. Cut a section of *Elodea* and place it (cut end upwards) in the test tube. Set up the apparatus as shown in diagram 11.20 in a darkened room. (The water bath ensures that the temperature stays constant.) The lamp should be 1 metre from the apparatus.
3. Leave the apparatus for 5 minutes (to allow the *Elodea* to adjust to the conditions).
4. Count the number of bubbles of oxygen coming from the cut end of the stem per minute.
5. Repeat step 4 twice more.

Syllabus There is a choice of activity here. This account refers only to the influence of **light intensity** on the rate of photosynthesis.



11.18 *Elodea: note the bubbles of oxygen*



6. Add the three numbers and divide by 3 to calculate the average number of bubbles per minute (this is a measure of the rate of photosynthesis).

11.19 *To investigate the effects of light intensity on the rate of photosynthesis*

7. Increase the light intensity by moving the lamp closer to the apparatus.
8. Repeat steps 3 to 6 each time the lamp is moved (i.e. at 80 cm, 60 cm, 40 cm and 20 cm).
9. Record your results as shown in the following table (some values are included as samples).

Activity 12 results		
Distance (cm)	**Number of bubbles/minute**	**Average number of bubbles/minute**
100	8, 10, 9	9
80	12, 14, 13	13
60		
40		
20		

10. You will see that as the lamp is moved closer to the apparatus the rate of bubble production increases. However, at some point, the rate of bubble production ceases to increase. The plant is then said to be saturated with light.

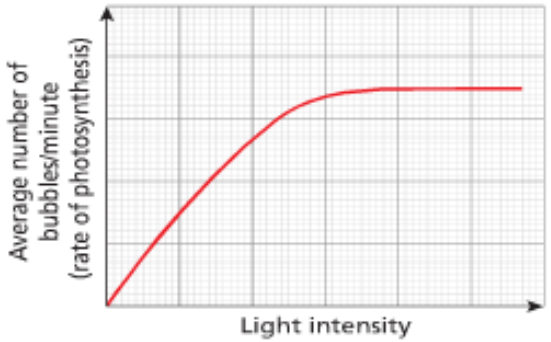
$$\text{Light intensity} \propto \frac{10\,000}{(\text{distance})^2}$$

11.20 *The formula to use to convert from distance (in cm) to light intensity where the symbol means 'is proportional to'.*

The final table of results (based on the figures in step 9) will appear as:

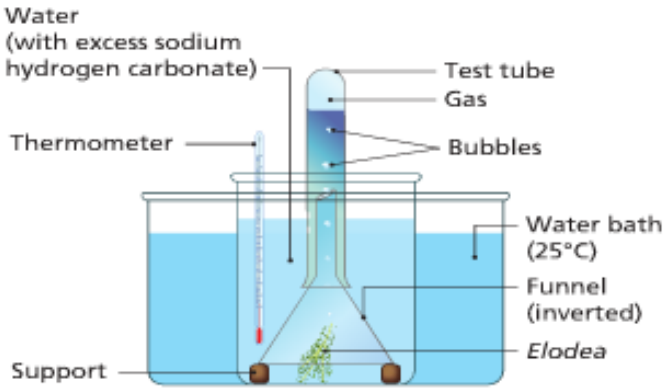
Distance (cm)	Light intensity	Average number of bubbles/minute
100	1	9
80	1.56	13
60	2.78	
40	6.25	
20	20	

11. Draw a graph of the average number of bubbles/minute vs. light intensity (putting light intensity on the horizontal axis). The graph should appear as shown below.



11.21 *Graph showing bubble rate against light intensity*

Note: This experiment can also be carried out using the apparatus shown in diagram 11.22.



11.22 *Alternative apparatus*

In this case the rate of photosynthesis can be calculated by:

- Counting the number of bubbles/minute
- Measuring the volume of oxygen gas collected in the top of the test tube after a suitable length of time (e.g. 15 minutes).

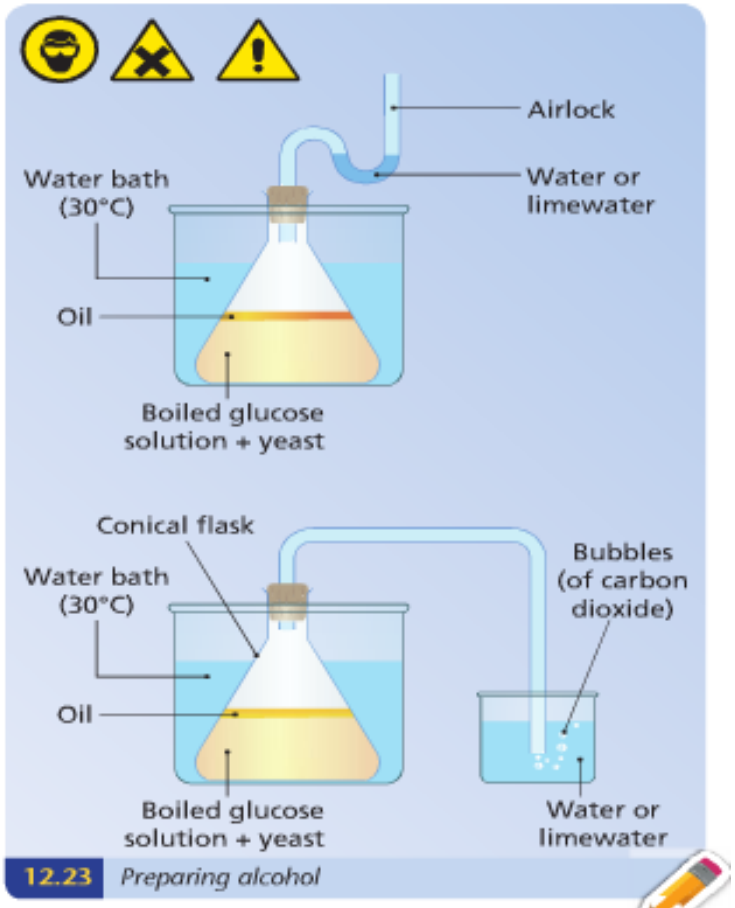
In addition, the gas collected in the test tube can be shown to be oxygen, as it rekindles a glowing splint.

12 - Respiration

Activity 13 To prepare and show the production of alcohol by yeast

Preparation of alcohol

1. Prepare a glucose solution by dissolving glucose in water.
2. Boil the solution in a conical flask for 5 minutes (this eliminates gases from the solution, forming anaerobic conditions).
3. When the solution cools, add some dried yeast.
4. Cover the liquid in the flask with oil (this prevents oxygen from re-entering the solution).
5. Set up either of the two pieces of apparatus as shown in diagram 12.23.
6. The airlock is needed to prevent micro-organisms entering and to allow carbon dioxide to pass out.
7. Limewater or water may be used in the airlock (limewater turns milky in the presence of carbon dioxide).
8. The apparatus is placed in a water bath at 30°C (this is an ideal temperature for the maximum rate of respiration).
9. Bubbles of carbon dioxide will be seen in the limewater. Fermentation is complete when the bubbles stop forming (often after a few days).
10. As a control, the same apparatus is used without adding any yeast cells (or adding boiled yeast). In this case, no bubbles form and the limewater remains clear.



12.23 *Preparing alcohol*

Test for Alcohol

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To show the production of ethanol (the iodoform test)

1. Filter the solution (to remove yeast cells).
2. Place some of filtrate into a test tube.
3. Add an equal volume of potassium iodide solution. Note this is a colourless solution.
4. Add sodium hypochlorite solution (note that the solution turns a brown-orange colour and then becomes colourless).
5. Place the test tube in a water bath at 50–60°C for 4 or 5 minutes.
6. Remove the test tube and allow it to cool.
7. The appearance of pale yellow crystals (of a chemical called iodoform) indicates that ethanol is present.
8. As a control use water instead of the filtered solution. Yellow crystals do not form.

To show that alcohol is produced

To test for alcohol, acidified potassium dichromate is added to the filtered solution. The test tube is placed in a warm water bath. If alcohol is present the colour changes from orange to green.

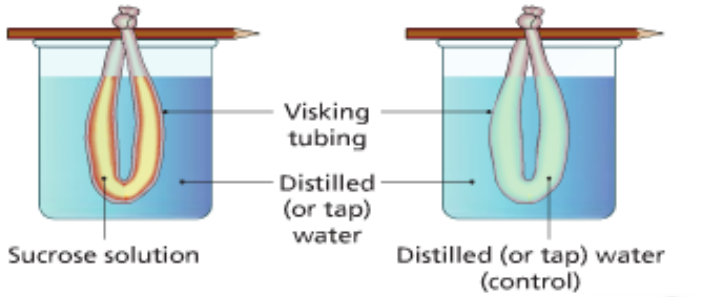
12.24 *Yeast fermenting: note the limewater has turned cloudy due to carbon dioxide*

Syllabus According to EU regulations the use of potassium dichromate should be discontinued in schools. This test remains as a theoretical part of the syllabus but students are not required to carry out the test.

Activity 14 To demonstrate osmosis

Note: *Visking tubing is a selectively permeable membrane. Water can pass freely through visking tubing, but sucrose cannot.*

1. Soak two strips of visking tubing in water (this softens them).
2. Tie a knot in one end of each of the strips.
3. Dissolve a large amount of sucrose in warm water in a beaker. This forms a concentrated sucrose solution (warm water is a better solvent than cold water).
4. Almost fill one piece of visking tubing with distilled (or tap) water. Tie a knot to seal the contents (this bag acts as a control).
5. Almost fill the second piece of visking tubing with sucrose solution. Tie a knot to seal the contents.
6. Dry each tube. Note the 'fullness' (or turgidity) of each tube and record its mass.
7. Place each tube of visking tubing in a container of distilled (or tap) water, as shown in diagram 13.13.
8. Leave the apparatus for about 30 minutes.
9. Remove the bags, dry them and note and record the 'fullness' and mass of each bag.



13.13 *To show osmosis*

10. Record the results as follows:

Contents of tube	Sucrose solution	Distilled or tap water
Mass at the start (g)		
Final mass (g)		
Change in fullness or turgidity (more, less or the same turgidity)		

The expected results are:

- The visking tubing containing the sucrose solution will have filled with water so that it has gained mass and is more full (this is due to water entering the tubing as a result of osmosis).
- The visking tubing containing the distilled water shows no change in mass or 'fullness' (i.e. it has not gained or lost water).

16 - DNA

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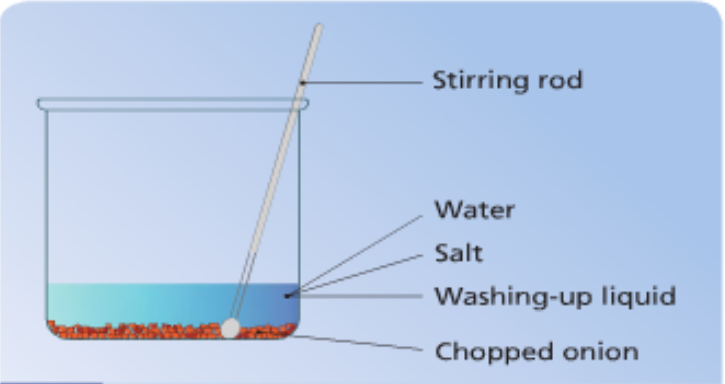
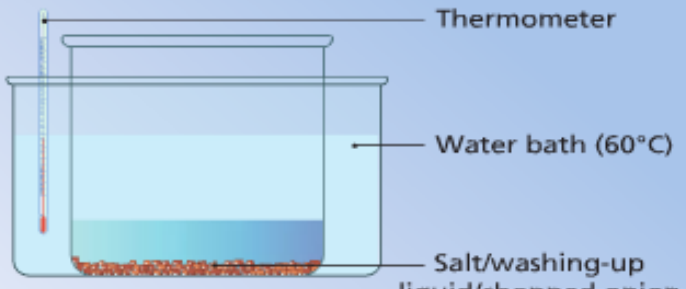
Activity 15 To isolate DNA from a plant tissue

1. Add some sodium chloride (salt) to a small volume of washing-up liquid dissolved in water (the salt will cause the DNA molecules to clump together, and the washing-up liquid will dissolve the cell and nuclear membranes and release DNA from the cells).
2. Cut an onion (or kiwi fruit) into small cubes (this allows the washing-up liquid to reach more cells).
3. Add the chopped onion to a beaker containing the salt/detergent solution and stir the mixture.



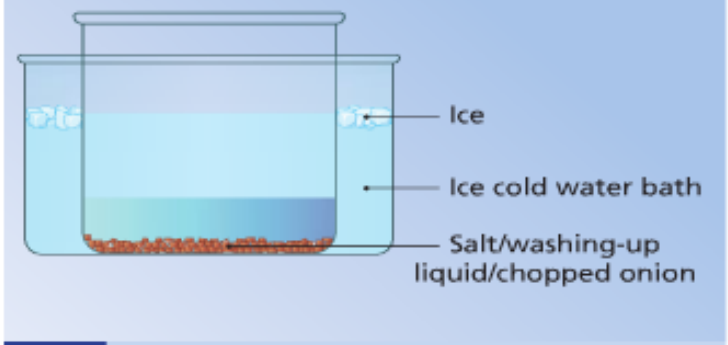
15.38 *Steps 1 to 3*

4. Put the beaker in a water bath at 60°C for 15 minutes. (This temperature inactivates (denatures) enzymes that would normally digest DNA. If left any longer than 15 minutes DNA itself would break down.)

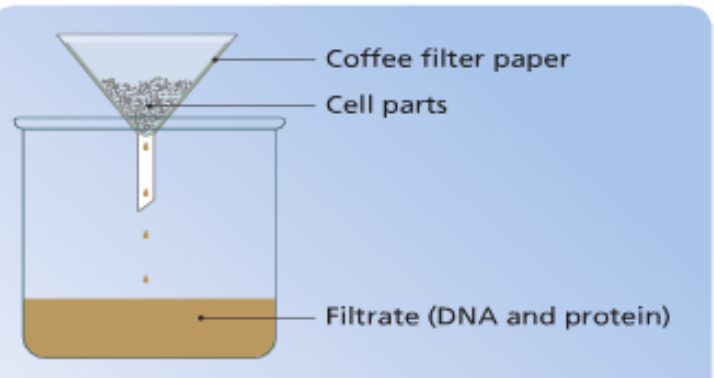


15.39 *Step 4*

5. Cool the mixture by placing the beaker in an ice water bath for 5 minutes, stirring frequently (this slows down the breakdown of the DNA).

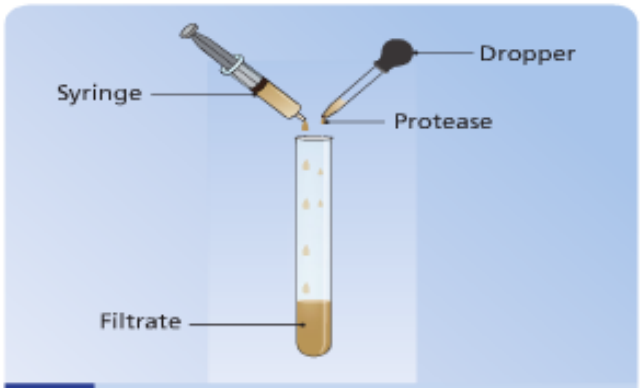


15.40 *Step 5*



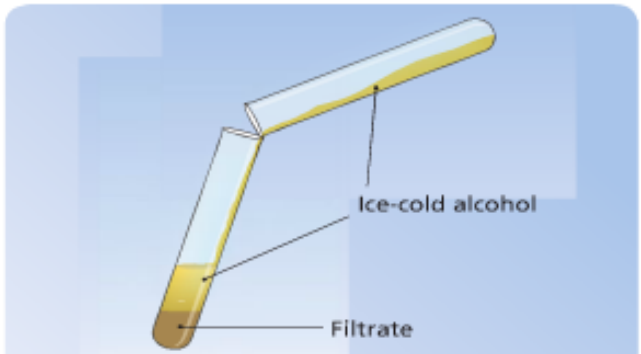
15.41 *Step 7*

6. Pour the mixture into a domestic food blender and blend it for only 3 seconds on high speed. (This breaks down the cell walls and releases DNA. Blending it for too long would break down the DNA strands.)
7. Filter the mixture through coffee filter paper into a second beaker. Do not add the foam from the top of the mixture to the filter paper. (Cell parts are retained in the filter paper. The filtered material, called filtrate, contains DNA and proteins. Normal laboratory filter paper is not used as its pores are too small and the process would be very slow.)
8. Use a syringe, without a needle, to place some of the onion filtrate into a boiling tube.
9. Add a few drops of protease enzyme (such as pepsin) to the contents of the boiling tube and mix well (the protease breaks down the proteins around the DNA).

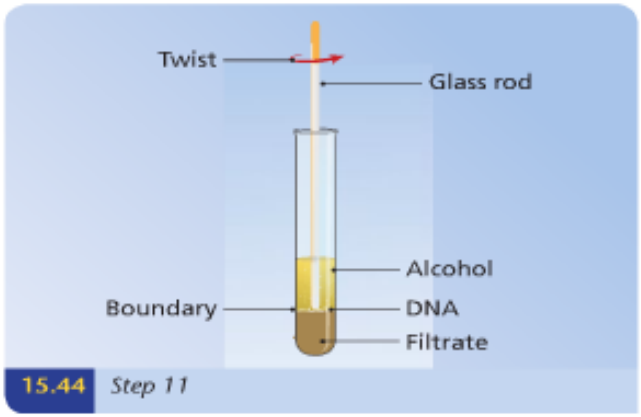


15.42 *Steps 8 and 9*

10. Pour some ice-cold ethanol or methylated spirits (stored in a freezer overnight) carefully down the side of the boiling tube. The ethanol should form a layer on top of the onion filtrate. (Alcohol removes water from DNA, which causes DNA to float to the top of the water. DNA is insoluble in ice-cold alcohol and so it precipitates at the alcohol–filtrate boundary. The DNA forms white threads at the alcohol–filtrate junction).



15.43 *Step 10*



15.44 *Step 11*

11. Gently twist a small glass rod or a wire loop in the alcohol. Strands of DNA should attach to the rod or wire. Do not mix the two layers or damage the DNA, which is very easily broken (DNA forms a clear mesh of what looks like stringy mucus, as shown in diagram 15.4 on page 171).

21 - Fungi

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General precautions when growing micro-organisms

Aseptic or **asepsis** means that measures are taken to exclude unwanted micro-organisms.

Sterile means that all micro-organisms are destroyed, i.e. there is nothing living.

In general you should assume that all micro-organisms are potentially harmful, unless it is stated or proven otherwise.

Aseptic techniques

Aseptic techniques involve the creation of a germ-free environment in as far as is possible. Aseptic methods include the following procedures:

1. Wash your hands before and after each experiment
2. Wash the bench with disinfectant before and after each experiment
3. Do not put fingers, food, drink or equipment in or near your mouth
4. Keep all containers closed where possible
5. (i) Open all containers for the shortest possible time and (ii) open lids the shortest possible distance (minimal opening)
6. When micro-organisms are in a petri dish, seal the dish with adhesive tape.

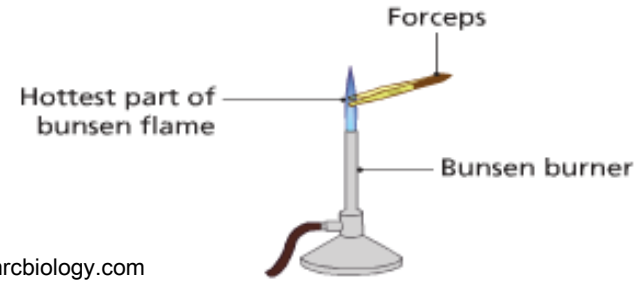
Sterile techniques

1. Sterilise all equipment before use or use equipment that is already sterile. This can be done by placing the equipment (except plastic) in a pressure cooker (or autoclave) at 120°C for 15 minutes or by placing it in an oven at 160°C for an hour.
2. Pass the neck of test tubes, needles or loops through the flame of a bunsen burner.
3. Flame all test tube necks, needles and loops again after they are used.
4. At the end of the experiment immerse all equipment and cultures in sterilising fluid.

The material can then be put in a dustbin or, in the case of glassware and metal, cleaned and reused as usual.

Activity 16 To investigate the growth of leaf yeast using agar plates

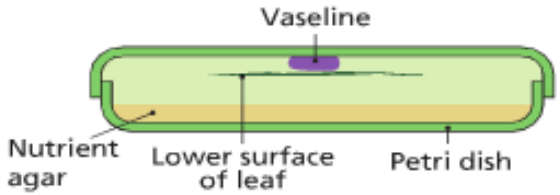
Micro-organisms are widely found in nature. This activity shows that, although they are not visible to the naked eye, leaves have many yeasts growing on their surfaces. These yeasts do not harm the leaves.



21.15 *Flaming a forceps*

1. Cut a small branch containing some leaves from an outdoor plant – privet, ash or sycamore leaves are ideal. (These will be tested for the presence of leaf yeasts.)
2. Wash your hands with an aseptic soap solution. (This reduces the chance of micro-organisms being on your hands.)
3. Wash the bench or worktop with disinfectant. (Again this eliminates micro-organisms.)
4. Sterilise a forceps by heating it in the flame of a bunsen burner for a few seconds. (This means there will be no micro-organisms on the forceps.)
5. Obtain two sterile petri dishes containing prepared sterile nutrient agar. (Agar is a material derived from seaweed. It is used to form a solid growth medium. The nutrient agar provides food for micro-organisms to grow.)
6. Use the forceps to pick up one of the leaves, which should be small enough to fit across a petri dish. (This prevents micro-organisms getting onto the leaf from your hands.) Alternatively, for large leaves, you may flame a cork borer or scissors, allow it to cool and use it to cut a number of leaf discs.

7. Place a small spot of petroleum jelly (such as Vaseline) on the inside lid of the petri dish. (This will be used to attach the leaf to the lid of the petri dish.)
8. Reflame the forceps and allow it to cool.
9. Barely open the lid of one of the petri dishes in terms of (a) the distance it is opened and (b) the time for which it is opened. Use the forceps to attach the **upper** surface of the leaf to the lid of the petri dish. Make sure that the leaf does not touch the agar. Close the lid of the petri dish. The lower surface of the leaf is now facing down onto the agar. (There are more micro-organisms on the lower surface of the leaf than on the upper surface. The upper surface is covered by a cuticle which prevents the growth of micro-organisms. Leaf yeasts can expel their spores onto the surface of the agar.)

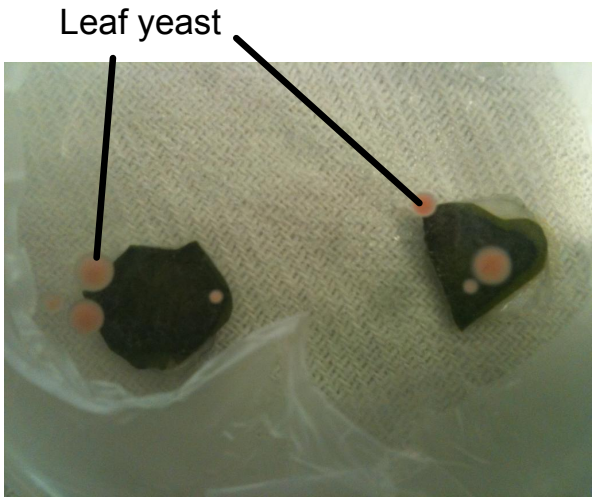


21.16 *Leaf attached to lid of petri dish*

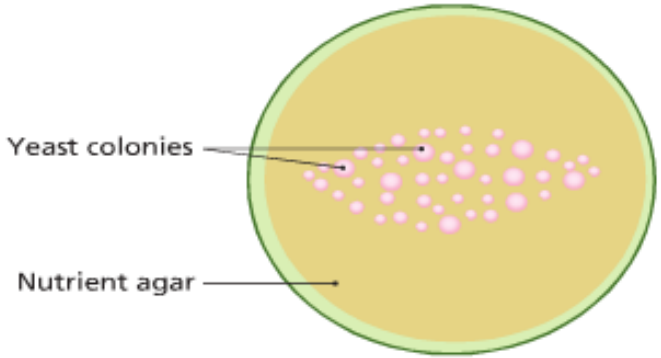
10. Reflame the forceps. (This will kill any micro-organisms on it.)
11. Seal a sterile nutrient agar petri dish containing no leaf. (This dish will act as a control or comparison. The only difference between the two petri dishes is that one contains a leaf and the other does not.)
12. Seal the petri dishes with tape or parafilm. (This prevents them from opening by accident.)
13. Label the petri dishes on the undersurface with a marker. (This allows the dishes to be identified, without further blocking the view of the agar surface.)
14. Leave the petri dishes at room temperature or in an oven or incubator at 25°C. (Leaf yeasts grow well at room temperature, but a higher temperature will speed up their growth.)
15. The dishes should be incubated upside down. (This prevents condensation forming on the lids.)
16. Observe the surfaces of the agar each day for three or four days (to see if any yeast colonies are forming.)

17. The expected results are:

- The dish with the leaf should show pink yeast colonies on the surface of the agar. These colonies may form a pattern similar to the shape of the leaf. Very few other micro-organisms will grow on the agar, unless part of the leaf is touching the agar. (The yeast can expel spores from a distance onto the agar; most other micro-organisms cannot grow across the space.)
- There should be no growth in the control dish. (This shows that the yeasts did not arise from any other source except the leaf.)



The growth of leaf yeasts is inhibited by air pollution. If the leaves are collected from a location with polluted air (such as a town or city) there may be few, or no, yeasts on the agar.



21.18 *Leaf yeast colonies growing on agar*

18. At the end of the experiment dispose of the agar and yeasts by sterilising it in an autoclave or pressure cooker for 15 minutes. Alternatively it can be immersed in sterilising fluid (such as Milton) and then put in a bin.

24 - Structure of Flowering Plants

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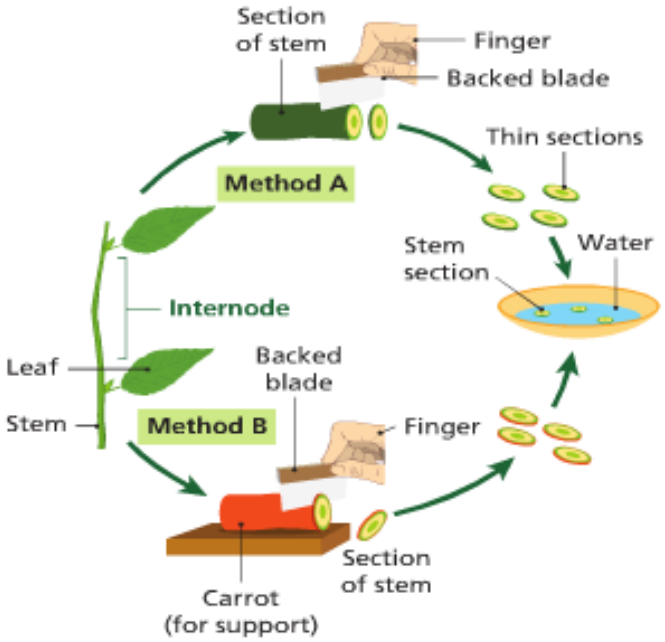
Activity 17 To prepare and examine a transverse section (TS) of a dicot stem

1. Plants that are suitable for this purpose are busy Lizzie, begonia, sunflower or celery (although celery is actually a petiole rather than a stem). As these are herbaceous (i.e. non-woody), they are easier to cut.
2. Cut out a short section of the stem between two nodes using a scalpel or backed blade.
3. Wet the blade (to reduce friction) and cut thin sections of the stem (cutting away from your fingers to prevent injury).
4. Cut the sections at right angles to the stem (i.e. try and avoid wedge-shaped sections). If the stem is too soft and flexible, it can be supported by placing it into a slit that is cut in some elder pith or carrot, which can then be sectioned as shown in diagram 23.25, method B.
5. Store the cut sections in a clock glass or petri dish of water (to prevent them dehydrating).



23.25 *Cutting sections of a stem for microscopic examination*

6. Transfer the thinnest sections onto a microscope slide using a forceps or small paint brush.
7. Add a few drops of water and a cover slip at an angle (to eliminate air bubbles).
8. Observe the section under low power and then under high power of the microscope (as explained in Activities 6 and 7 in Chapter 7) and compare them with diagram 23.20.
9. Draw a diagram of the TS of the stem. Label the position of the dermal tissue, ground tissue and vascular tissue.

27 - Heart and Blood Vessels

Activity 19a To investigate the effect of exercise on the pulse rate of a human

You have a choice between this activity and investigating the effect of exercise on the breathing rate of a human (Activity 19b on page 344).

1. Work in pairs, one person recording the results.
2. Sit down on a chair and rest for a few minutes.
3. Locate a strong pulse in your neck or wrist (just below the thumb).
4. Count the number of pulses per minute while at rest.
5. Repeat this two more times.
6. Calculate your average pulse rate per minute (measured in beats per minute, bpm) at rest by adding the three values and dividing the total by three. This is called the resting heart rate and is used as a control.
7. Walk slowly for 5 minutes.
8. Count your pulse rate per minute immediately after walking.
9. Walk briskly for 5 minutes.
10. Count your pulse rate per minute immediately after walking.
11. Exercise strenuously for 5 minutes (e.g. step up and down on a chair every 3 seconds or run).
12. Count your pulse rate per minute immediately after exercising.
13. Present your results in tables such as those shown below.

Heart rate before exercise					
Before exercise	**Trial 1**	**Trial 2**	**Trial 3**	**Total**	**Average**
Resting pulse rate (bpm)					

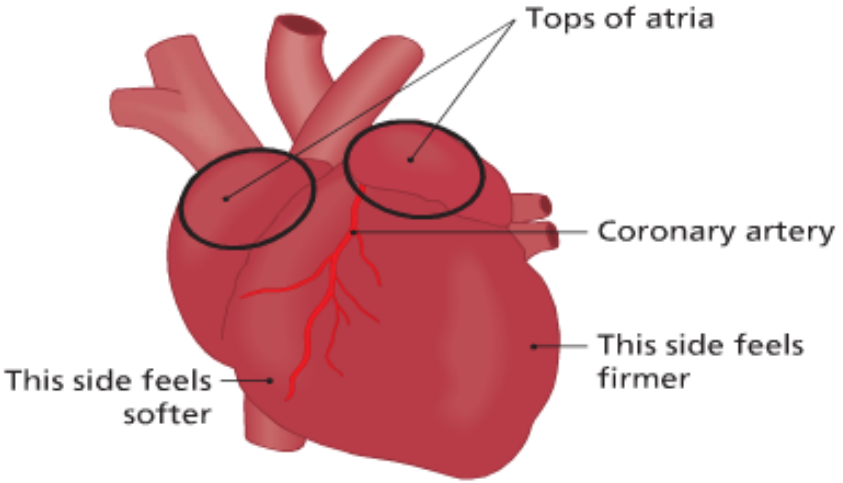
Heart rate after different types of exercise			
Activity	**Gentle walk**	**Brisk walk**	**Strenuous exercise**
Pulse rate (bpm)			

14. Compare the average resting rate with the rates after each type of exercise.

Activity 18 To dissect, display and identify the parts of a heart

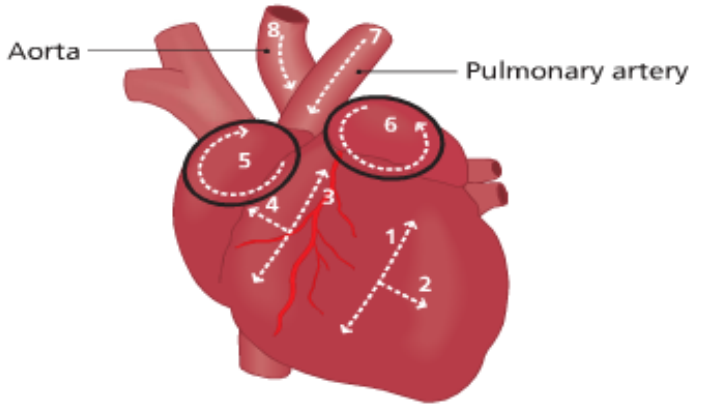
A sheep's heart is smaller than an ox's (or cow's) heart. Dissect an ox's or sheep's heart in the following manner.

1. Rinse the heart in cold water. Wash out any dark-coloured, jelly-like clumps of blood.
2. Place the heart on a dissecting board or tray.
3. Distinguish between the front (ventral) and back (dorsal) surface of the heart in one of the following ways:
   - The front is more rounded and the thick-walled arteries are on this side.
   - The lower part of the left side feels much firmer than the lower part of the right side.
   - One of the coronary arteries runs diagonally from the top right to the bottom left of the heart.

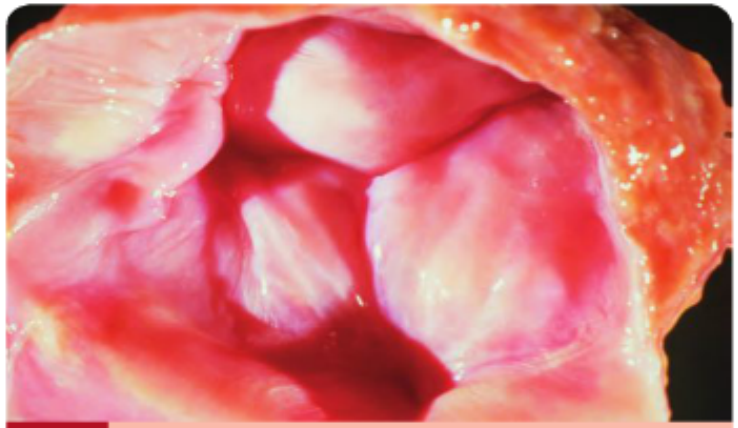


26.20 *Identifying the ventral surface of the heart*

4. Identify the four major blood vessels that enter and leave the heart. (Note that very often the butcher will have removed many of the vessels). Notice how thick-walled the arteries are compared with the veins. Notice the coronary arteries and veins on the surface of the heart. These vessels supply blood to the heart itself.
5. Locate the four chambers of the heart. (Note that the upper chambers or atria are quite small, are very high up and look like 'ears' on the outside of the heart.)
6. Draw a labelled diagram of the external structure of the un-dissected heart.
7. Make eight cuts in the front of the heart using a scalpel or scissors, in the positions shown in diagram 26.21.
8. Cuts 1 and 2 open up the left ventricle. In this chamber you should observe:
   - A very thick wall
   - White 'strings', which are the tendons that hold the valves in place
   - The two flaps of the bicuspid valve.
9. Cuts 3 and 4 open up the right ventricle. In this chamber you should observe:
   - A thinner wall
   - White 'strings', which are the tendons
   - The three flaps of the tricuspid valve.
10. Cuts 5 and 6 open up the atria. In these chambers you should observe:
    - Very thin walls
    - The bicuspid and tricuspid valves.



26.21 *Ventral view of the heart, showing the location of the cuts to be made*



26.22 *A semilunar valve: the three flaps are closed*

11. Cut 7 opens up the pulmonary artery. This should allow you to see:
- The three flaps of the semilunar valve (at the point where the artery emerges from the heart).

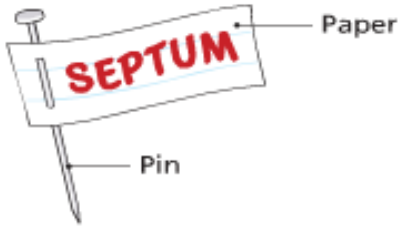
12. Cut 8 opens up the aorta. This should allow you to see:
- The second semilunar valve
- The origin or beginning of the coronary artery (just above the semilunar valve). If you squirt water (or a dye) into the coronary artery you will see it flow down to the heart.

13. Identify the septum between the ventricles.

14. Draw a diagram of the dissected heart. This will be similar to diagram 26.9.

15. Using small pins and paper labels, flag-label the parts you have identified.

16. Wash your hands and sterilise the board and dissecting instruments.



26.23 *A flag label* www.mrcbiology.com

31 - Human Breathing

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Activity 19b To investigate the effect of exercise on breathing rate

Syllabus You have a choice to carry out **either** this activity **or** Activity 19a on page 311.

1. Work in pairs, one person recording the results.
2. Sit down on a chair and rest for a few minutes.
3. Breathing in and breathing out is considered to be one breath.
4. Count the number of inhalations or exhalations per minute while at rest.
5. Repeat this two more times.
6. Calculate your average breathing rate per minute (measured in breaths per minute, or bpm) at rest by adding the three values and dividing the total by three. This is called the resting breathing rate and is used as a control.
7. Walk slowly for 5 minutes.
8. Count your breathing rate per minute immediately after walking.
9. Walk briskly for 5 minutes.
10. Count your breathing rate per minute immediately after walking.
11. Exercise strenuously for 5 minutes (e.g. step up and down on a chair every 3 seconds or run).
12. Count your breathing rate per minute immediately after exercising.
13. Compare your resting rate with the rate immediately after each type of exercise.
14. Present your results in tables such as those shown below.

Breathing rates before exercise					
Before exercise	**Trial 1**	**Trial 2**	**Trial 3**	**Total**	**Average**
Resting breathing rate (bpm)					

Breathing rates after different types of exercise			
Activity	**Slow walk**	**Brisk walk**	**Strenuous exercise**
Breathing rate (bpm)			

15. After exercise the rate of breathing often falls below the resting rate. This is due to deeper breathing.

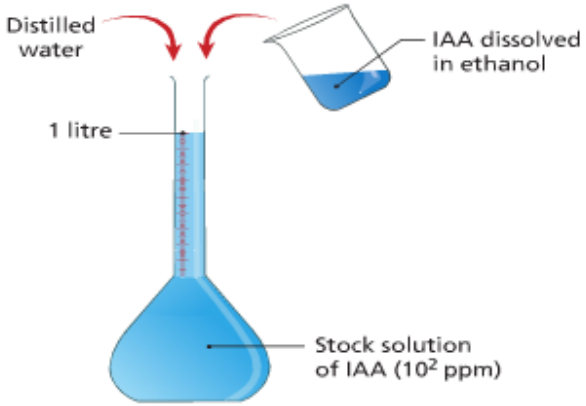
33 - Plant Responses

Activity 20 To investigate the effect of IAA growth regulator on plant tissue

Preparing a stock solution

This step is often carried out by the teacher, as IAA is irritant, toxic and may cause mutations.

1. Dissolve a small amount of IAA in a small volume of ethanol. Note that although pure IAA may be toxic, dilute solutions do not carry much risk. Ethanol is flammable. (IAA is dissolved in ethanol first because it does not easily dissolve in water.)
2. When the IAA is fully dissolved in the ethanol, bring the volume up to 1 litre using distilled water. If the correct weight of IAA is dissolved in 1 litre a stock solution of IAA is prepared with a concentration of 10^2 parts per million (ppm) (see diagram 32.8).



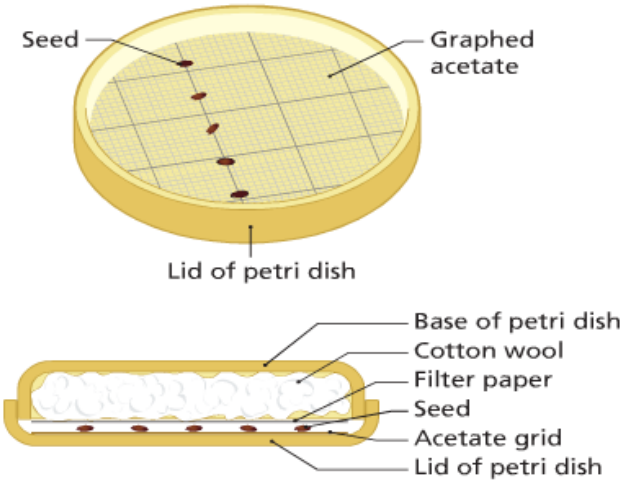
32.8 *Preparing a stock solution of IAA*

Carrying out a serial dilution

3. Label eight small bottles or petri dishes A to H.
4. **(a)** Transfer 10 ml of the stock solution into dish A.
   (b) Using a clean pipette, place 9 ml of distilled water into each of the dishes labelled B to H. (***Note:*** do not add any distilled water to dish A.)
   (c) Use a clean pipette to transfer 1 ml of IAA solution from dish A to dish B and stir to mix thoroughly.
   (d) Using a clean pipette each time and stirring to mix the contents thoroughly, transfer 1 ml of IAA solution from B to C, C to D, D to E, E to F and F to G (see diagram 32.9).
   (e) Remove 1 ml of solution from dish G and dispose of it down the sink. (The procedure carried out in step 4 is called a serial dilution. It produces a range of IAA solutions, each one of which is one-tenth the concentration of the previous solution.)
5. Do not transfer any IAA solution to dish H. It acts as a control (containing no IAA, only distilled water).

Investigating the effect of IAA on plant tissue

7. Photocopy a sheet of graph paper on to acetate sheets. (The acetate will be used to measure the length of the roots or shoots.)
8. Place a circular acetate grid in the **lid** of each of eight petri dishes.
9. Place five radish or cress seeds along one of the lines on each of the acetate sheets.
10. Place filter paper on top of the seeds in the lids of the petri dishes.
11. Use a clean dropper to add about one-quarter of each IAA solution to each filter paper.
12. Cover the filter papers with a layer of cotton wool.
13. Sprinkle the remaining IAA solutions onto each piece of cotton wool.

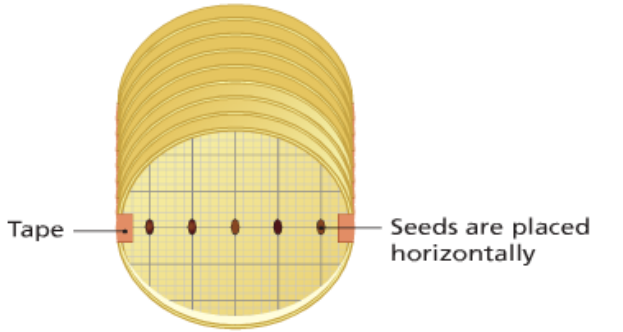


32.10 *The set-up in a petri dish*

14. Put the base of the petri dish over the cotton wool and tape it shut.
15. Stand the petri dishes on their edges with the seeds horizontal, as in diagram 32.11. (This ensures that the roots grow down and the shoots grow up.)
16. Leave the dishes in a warm place (20–25°C), such as an incubator, for 2 or 3 days.

Observing the results

17. If you can see the roots and shoots through the lid of the petri dish then you can leave the petri dish sealed shut. If not, remove the tape and the base of the petri dish. Carefully remove the filter paper (be careful that the plant tissue does not remain attached to the filter paper).
18. Use the acetate grid to measure the lengths of the roots and shoots of the seedlings in each dish. Record the results as shown in the table opposite. Use a similar table for the length of the shoots.



32.11 *Incubating the petri dishes*

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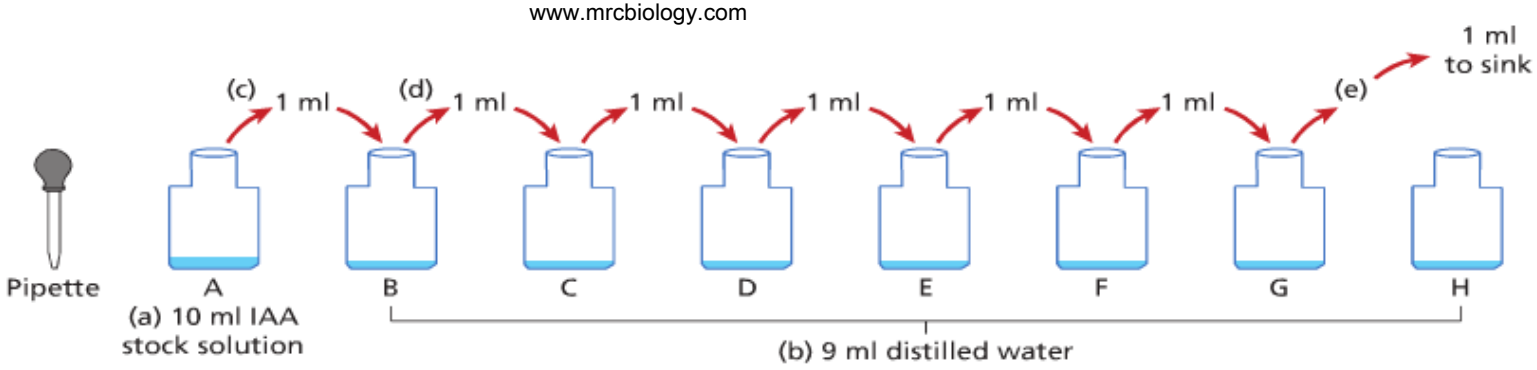
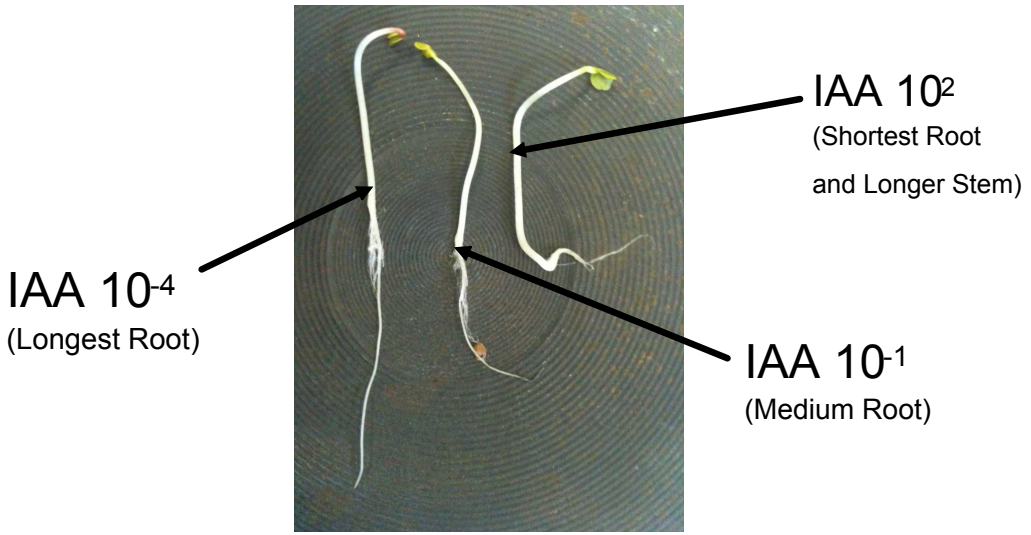


32.9 *Preparing a serial dilution of IAA*

6. The final concentration of IAA in each dish is shown in the table below.

Dish	A	B	C	D	E	F	G	H
Concentration of IAA (ppm)	10^{2}	10^{1}	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	0 (no IAA)



R. Cummins

40 - Plant Reproduction

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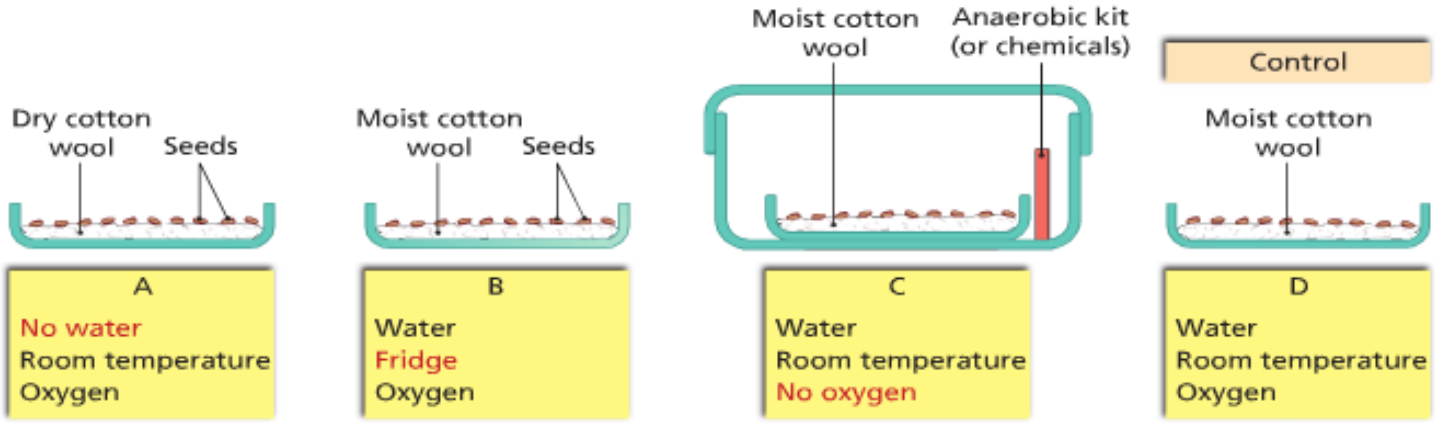
Activity 21 To investigate the effect of water, oxygen and temperature on germination

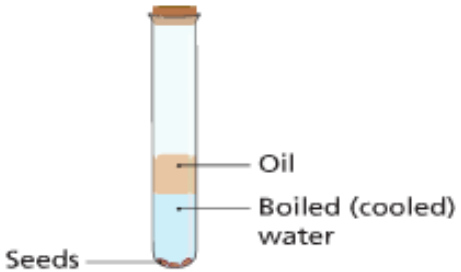


39.40 *To investigate the conditions needed for germination*

1. Place equal amounts of cotton wool in the base of four petri dishes.
2. Label the dishes A, B, C and D.
3. Add water to the cotton wool in dishes B, C and D. Leave dish A dry.
4. Place 10 small seeds (e.g. radish or cress) on the surface of the cotton wool in each dish.
5. Place dish B in a fridge (low temperature).
6. Place dish C in an anaerobic jar, activate the anaerobic-producing chemical and seal the jar.
7. Place dishes A, C (in the anaerobic jar) and D, which is the control, in an incubator at 25°C (or leave them at room temperature, 20°C).

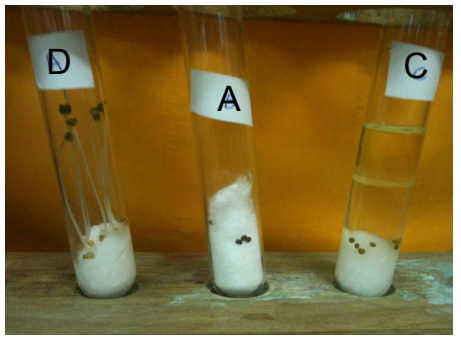
8. Check the dishes each day for 2 to 3 days. The seeds in the control dish should germinate, while those in the other dishes should not germinate as result of each dish missing one vital condition.
9. Record the results as shown below.

Results			
Dish	**Conditions present**	**Conditions absent**	**Germination (yes/no)**
A	Suitable temperature Oxygen	Water	
B	Water Oxygen	Suitable temperature	
C	Water Suitable temperature	Oxygen	
D	Water Suitable temperature Oxygen	None (control)	



39.41 *Creating anaerobic conditions*

Note 1: Test tubes can be used instead of petri dishes.
Note 2: Anaerobic conditions can be created in another way. To remove oxygen, boil water vigorously and allow it to cool. Place seeds in the boiled water and add a layer of oil to prevent oxygen diffusing into the water.



B - in fridge!

Activity 22 To use starch agar or skimmed milk plates to show digestive activity during germination

Note: This activity allows the use of starch agar or skimmed milk plates. Starch agar plates are used in the following account.

Seeds contain the enzyme amylase. In this activity, amylase breaks down starch in the agar plates.

1. Soak four large seeds (e.g. broad bean) in water for a day or two.
2. Wash the bench with disinfectant (to kill bacteria and fungi).
3. Kill two of the seeds by boiling them in water for 5 minutes (these will act as controls).
4. Use a backed blade (for safety) to split the four seeds in half. Cut away from your fingers (to prevent cutting yourself).
5. Sterilise the half seeds by soaking them in alcohol or mild disinfectant for 10 minutes.
6. Wash off the alcohol or mild disinfectant with water.
7. Flame a forceps using a bunsen burner and allow it to cool (this sterilises it).
8. Barely open a petri dish containing starch agar (to prevent bacteria or fungi from entering).
9. Using the forceps, place four of the cut, **unboiled**, half seeds face down onto the starch agar in one dish. (This allows the amylase in the seed to come in contact with starch in the petri dish). Label this dish A.
10. Re-flame the forceps and use it to place four cut, **boiled**, half seeds face down in another dish. Label this dish B. (These act as controls).
11. Place the covered dishes in a warm place for 2 days.
12. Remove the half seeds and add dilute iodine solution to the dishes (to test for starch).
13. After 2 minutes pour off the iodine.