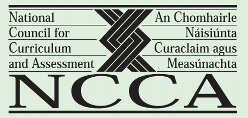
BIOLOGY

Leaving Certificate Ordinary Level and Higher Level

SUPPORT MATERIALS

Laboratory Handbook for Teachers



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The NCCA acknowledges the assistance of the

National Biology Support Service in the production of this material.

CONTENTS

1 SECTION ONE: INTRODUCTION 1

2 SECTION TWO: GUIDELINES FOR THE STUDENT NOTEBOOK OR FOLDER 7

3 SECTION THREE: PRESCRIBED ACTIVITIES 11

4 SECTION FOUR: MATERIALS AND EQUIPMENT 107

Section One
Introduction

1.	Introduction	2
2.	Syllabus aims	2
3.	Syllabus objective	2
4.	Practical activities	2
5.	Rationale for practical activities	3
6.	Student practical activity	3
7.	Skill attainment	4
8.	Laboratory organisation and maintenance	4
9.	Safety	5
10.	Assessment	5
11.	References	5

1. INTRODUCTION

This handbook offers guidance for teachers on the central issues of the prescribed practical activities in the Leaving Certificate Biology syllabus. The main focus of these activities for students is the attainment of practical skills. The emphasis is on the process rather than on product attainment alone. The intention is, that by focusing on a high level of practical skills, students will be better prepared for further education, training and employment in science related areas. The syllabus prescribes twenty two practical activities for students.

When arranging practical activities for students with special educational needs, including students with severe learning difficulties, it is important to break down the activities into small steps. You may also need advice and guidance on how apparatus and other facilities in the laboratory can be modified to provide maximum opportunity and safety for the students.

In all practical work, teachers are required to develop a positive attitude towards safety and instruct their students in the appropriate safety procedures.

2. SYLLABUS AIMS

The aims of the syllabus are to:

- Contribute to students' general education through their involvement in the process of scientific investigation
- Encourage in students an attitude of scientific enquiry, of curiosity and self-discovery through
    - (i) individual study and personal initiative
    - (ii) team work
    - (iii) class-directed work

3. SYLLABUS OBJECTIVE

The syllabus includes the following objective:

- To impart a knowledge and understanding of biological experimental techniques, including practical laboratory skills

4. PRACTICAL ACTIVITIES

- The primary emphasis of practical activities is skill attainment.
- In the course of their studies of the syllabus, students will follow a course of practical work, laboratory work and fieldwork that includes 22 prescribed activities.

5. RATIONALE FOR PRACTICAL ACTIVITIES

Natural science is the study and accumulation of knowledge about material things and events in the universe. Organisms form part of these "things" and "events". Biology, the study of life, is one of the disciplines within the natural sciences.

The method of scientific study falls within the framework described as the scientific method: making observations, formulating a hypothesis, designing a controlled experiment, collecting and interpreting data, reaching conclusions, placement of conclusions in the context of existing knowledge, reporting and publishing results, development of theory and principle. There should be an appreciation of the errors possible in activities and of the precautions or controls that can be applied to reduce such errors. Students should be aware that in their study of biology the value of the scientific method is limited by

(a) the extent of our basic knowledge,
(b) the basis of investigation,
(c) our ability to interpret results,
(d) its application to the natural world that is always subject to change and
(e) accidental discoveries.

As the study of biology is incomplete without the study and application of the scientific method, practical activity forms an essential and mandatory part of this course.

Students should be encouraged to integrate information and communication technologies (ICTs) into their study of biology.

6. STUDENT PRACTICAL ACTIVITY

Practical activity for students:

- Introduces them to a scientific method of investigation
- Allows for greater development of affective and psychomotor forms of learning
- Encourages accurate observation and careful recording
- Promotes simple, commonsense scientific methods of thought
- Develops manipulative skills
- Gives training in problem solving
- Elucidates the theoretical work so as to aid comprehension
- Verifies facts and principles already taught
- Arouses and maintains interest in biology
- Makes biological, chemical, and physical phenomena more real through actual experience

7. SKILL ATTAINMENT

The skills developed primarily through the practical activities include:

- Manipulation of apparatus
- Following instructions
- Observation
- Recording
- Interpretation of observations and results
- Practical enquiry and application of results

Manipulation of apparatus involves manual dexterity and efficiency e.g. using appropriate methods in collecting required specimens in an ecological study; being familiar with and using the light microscope to prepare and examine a plant cell; dissecting an ox's or a sheep's heart; conducting a series of laboratory activities and investigations safely and humanely.

Following instructions involves adherence to the instructed or recorded sequence of actions required to carry out an activity e.g. ability to follow instructions from a practical manual.

Observation is the most important tenet of the scientific method. All knowledge of biology is based on situations in which a biologist observes a particular event and records it. Instruments are used to extend our perceptual limits. Appropriateness, accuracy and completeness of observation need to be practised, monitored and guided from relevant theoretical criteria for their acquisition. These qualities will be identified from the student's notebook.

Recording is another essential tenet of the scientific method. Records are proof of what has been completed in the activity and what can be repeated with similar expected outcomes. Guidelines on the recording of practical activities are highlighted in Section three.

Interpretation of observations and results should ensue from the hypothesis being tested or the investigation undertaken. The final interpretation should be coherent and should explain clearly how conclusions are reached.

Practical enquiry and application of results e.g. if results are ambiguous, the student should be required to consider the results, and where necessary to repeat the activity or to design a new activity. The student may consider the results in a wider context and make suggestions or identify the activity as a scientific paradigm (an activity that serves as a model for further work).

8. LABORATORY ORGANISATION AND MAINTENANCE

The syllabus requires that students have access to a laboratory with supervision. The laboratory should be designed to enable the activities required by the syllabus and any other practical work to be carried out in a safe manner. The teacher should review the equipment and laboratory facilities available and then, in conjunction with the syllabus and the teacher guidelines, organise the students' activities, teacher demonstrations and any other activities.

It is important to maintain the laboratory equipment by ensuring that repairs are carried out as needed and that equipment is stored safely when not in use. It is also important to remind students that the final stage of laboratory work is cleaning up e.g. return all materials and solutions, clean up the work-bench and dispose of any waste materials carefully and efficiently, as directed by the teacher.

9. SAFETY

In all practical work safety must be a major concern. Teachers are encouraged to develop in their students positive attitudes and approaches to safety in the range of activities they encounter and to inculcate in them an awareness of the value of creating a safe working environment. Standard laboratory safety precautions should be observed and care taken when carrying out all activities. All legal and health regulations must be adhered to in activities involving live and dead organisms. Before rearing and maintaining organisms, detailed information on the appropriate methods for the rearing and maintenance of the organisms must be studied. These methods must be strictly adhered to during the activity.

The general principles of safe laboratory working procedures apply to Leaving Certificate Biology. The Department of Education and Science has published two booklets *Safety in School Science* (1996) and *Safety in the School Laboratory - disposal of chemicals* (1996). Specific reference to biological safety is highlighted under "Biological Hazards".

Teachers should draw up a list of laboratory rules. This list should be posted in a prominent place in the laboratory and students' attention should be drawn to it on a regular basis. Before starting a practical activity students should be made aware of the relevant safety considerations. Circular letter M24/04 contains further information on aspects of safety in school science laboratories, including a list of appropriate references. To read circular click here.

10. ASSESSMENT

Assessment of practical activities will be by means of a specific section of the terminal written examination paper. Practical work is an integral part of the study of biology.

A practical assessment component may be introduced as part of the overall assessment at a later stage.

11. REFERENCES

The National Commission for the Teaching of Biology, (1986) *Teachers' Handbook of Biology Practicals for Leaving Certificate Classes,* (Royal Irish Academy).

Irish Science Teachers' Association (1990) *Leaving Certificate Biology Practicals – a manual for teachers and students* (ISTA).

Ryder A.J. and Ryan J.O. (1987) *Laboratory Safety for Students* (Jay Cee Publishers).

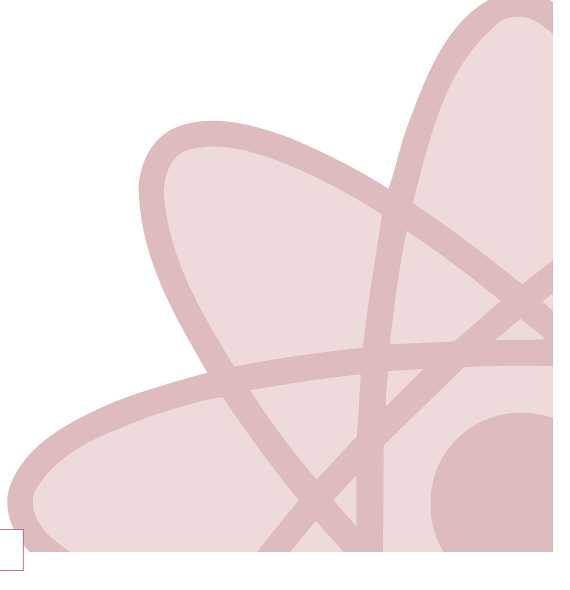
National Irish Safety Organisation (NISO) *Hazard Labelling of Laboratory Chemicals* - Leaflet.

Department of Education and Science (1996). *Safety in School Science* and *Safety in the School Laboratory - Disposal of Chemicals.*

Department of Education and Science (2004). Circular letter M24/04: *Aspects of Safety in Science Laboratories in Second Level Schools.*

Section Two
Guidelines for the student notebook or folder

1.	Introduction	8
2.	Title and date	8
3.	Description of procedure	8
4.	Data presentation	8
5.	Diagrams, calculations and graphs	9
6.	Results, observations and conclusions	9
7.	Application	9



1. INTRODUCTION

Each student should keep a record of her/his practical work. Legibility, spelling, correct use of symbols, neatness, and clarity of presentation should be emphasised.

A table of contents of all activities recorded should be placed at the beginning of the notebook or folder. Diagrams should be drawn with an HB pencil.

2. TITLE AND DATE

The purpose of the activity should be stated clearly.

3. DESCRIPTION OF PROCEDURE

A concise report in the student's own words including, where appropriate:

- A clearly labelled diagram of assembled apparatus
- The safety procedures undertaken
- Step by step description of the procedure
- Reference to essential adjustments to apparatus
- Details of outcomes noted, including measurements taken, colour changes observed etc.

4. DATA PRESENTATION

All measurements, with the appropriate units, should be recorded and presented in an orderly sequence and tabulated where appropriate.

Reference to a control should be stated when used.

5. DIAGRAMS, CALCULATIONS AND GRAPHS

Diagrams are worth a thousand words if clear, precise, well drawn and accurately labelled. Students should be encouraged to draw diagrams as frequently as possible during their course of study and during their practical activities.

The main steps in any *calculation* should be recorded with appropriate units so that the process can be easily followed to a final conclusion. Rough work need not be shown.

Graphs should be used (i) to illustrate the relationship between parameters and (ii) in the calculation of results. Suitable scales should be used when drawing graphs and graph axes should be labelled. Points should be plotted and circled clearly and curves drawn properly.

6. RESULTS, OBSERVATIONS AND CONCLUSIONS

Results from activities should be presented in a clear, methodical way.

Mathematical results should be expressed in a standard manner with correct use of units.

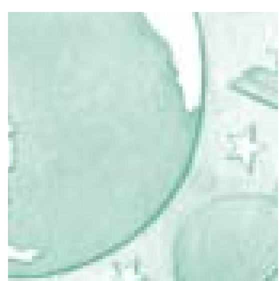
It is desirable to comment on the results obtained in an activity.

There should be an appreciation of the possible errors inherent in an activity and of the precautions that can be taken to reduce such errors.

Cross-referencing with other groups should be recorded, where appropriate, and conclusions drawn.

7. APPLICATION

Suggest any other application(s) of what was learnt in the activity.



Section Three
Prescribed activities

1. List of prescribed activities 12

As listed in the syllabus 12

Alternative list format 13

2. Detailed templates for each of the laboratory activities prepared by the National Biology Support Service 14

Materials/Equipment

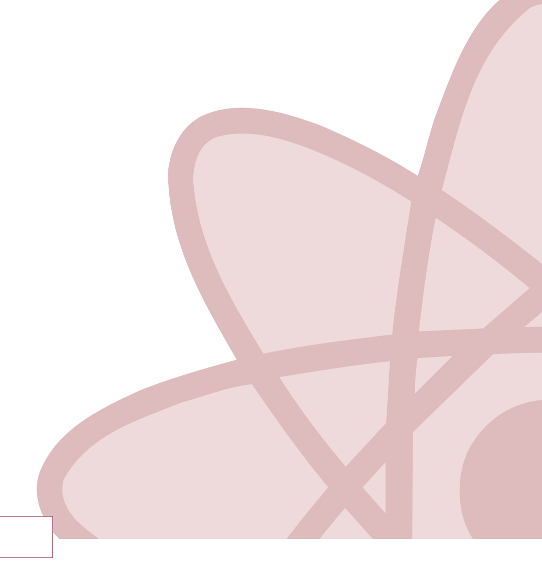
Procedure

Skill attainment

Background information

Advance preparation

Helpful hints



1. LIST OF PRESCRIBED ACTIVITIES

1. Conduct a qualitative test for: starch, fat, a reducing sugar, a protein.
2. Identify any five fauna and any five flora using simple keys. Identify a variety of habitats within the selected ecosystem.
3 Identify and use various apparatus required for collection methods in an ecological study.
4. Conduct a quantitative study of plants and animals of a sample area of the selected ecosystem. Transfer results to tables, diagrams, graphs, histograms or any relevent mode.
5. Investigate any three abiotic factors present in the selected ecosystem. Relate results to choice of habitat selected by each organism identified in this study.
6. Be familiar with and use the light microscope.
7. Prepare and examine one animal cell and one plant cell – unstained and stained – using the light microscope (×100, ×400).
8. Investigate the effect of pH on the rate of one of the following: amylase, pepsin or catalase activity.
9. Investigate the effect of temperature on the rate of one of the following: amylase, pepsin or catalase activity.
10. Prepare one enzyme immobilisation and examine its application.
11. Investigate the influence of light intensity or carbon dioxide on the rate of photosynthesis.
12. Prepare and show the production of alcohol by yeast.
13. Conduct any activity to demonstrate osmosis.
14. Investigate the effect of heat denaturation on the activity of one enzyme.
15. Isolate DNA from a plant tissue.
16. Investigate the growth of leaf yeast using agar plates and controls.
17. Prepare and examine microscopically the transverse section of a dicotyledonous stem (×100, ×400).
18. Dissect, display and identify an ox's or a sheep's heart.
19. Investigate the effect of exercise on the breathing rate or pulse rate of a human.
20. Investigate the effect of IAA growth regulator on plant tissue.
21. Investigate the effect of water, oxygen and temperature on germination.
22. Use starch agar or skimmed milk plates to show digestive activity during germination.

ALTERNATIVE LIST FORMAT

Fieldwork Activities

Students should:

1. Identify any five fauna and any five flora using simple keys. Identify a variety of habitats within the selected ecosystem.

2. Identify and use various apparatus required for collection methods in an ecological study.

3. Conduct a quantitative study of plants and animals of a sample area of the selected ecosystem. Transfer results to tables, diagrams, graphs, histograms or any relevant mode.

4. Investigate any three abiotic factors present in the selected ecosystem. Relate results to choice of habitat selected by each organism identified in this study.

Microscopy

Students should:

5. Be familiar with and use the light microscope.

6. Prepare and examine one animal cell and one plant cell – unstained and stained – using the light microscope (×100, ×400).

7. Prepare and examine microscopically the transverse section of a dicotyledonous stem (×100, ×400).

Dissection

Students should:

8. Dissect, display and identify an ox's or a sheep's heart.

Laboratory Activities
(Each conducted during one double class period)

Students should:

9. Conduct a qualitative test for: starch, fat, a reducing sugar, a protein.

10. Investigate the effect of pH on the rate of one of the following: amylase, pepsin or catalase activity.

11. Investigate the effect of temperature on the rate of one of the following: amylase, pepsin or catalase activity.

12. Investigate the effect of heat denaturation on the activity of one enzyme.

13. Investigate the influence of light intensity or carbon dioxide on the rate of photosynthesis.

14. Conduct any activity to demonstrate osmosis.

Laboratory Activities

(Some may be conducted over a series of class periods)

Students should:

15. Prepare one enzyme immobilisation and examine its application.
16. Prepare and show the production of alcohol by yeast.
17. Isolate DNA from a plant tissue.
18. Investigate the effect of exercise on the breathing rate or pulse rate of a human.
19. Investigate the effect of IAA growth regulator on plant tissue.
20. Investigate the growth of leaf yeast using agar plates and controls.
21. Investigate the effect of water, oxygen and temperature on germination.
22. Use starch agar or skimmed milk plates to show digestive activity during germination.

2. DETAILED TEMPLATES FOR EACH OF THE LABORATORY ACTIVITIES

PREPARED BY THE NATIONAL BIOLOGY SUPPORT SERVICE

The following materials were prepared by the National Biology Support Service and may be used as a template for conducting practical activities with students. They should not be read as the definitive prescribed procedures as alternative procedures are available from many other resources.

Conduct a qualitative test for: starch, fat, a reducing sugar, a protein. (Page 15).

Be familiar with and use the light microscope. (Page 22).

Prepare and examine one animal cell and one plant cell – unstained and stained – using the light microscope (×100, ×400). (Page 26, 29).

Investigate the effect of pH on the rate of one of the following: amylase, pepsin or catalase activity. (Page 33).

Investigate the effect of temperature on the rate of one of the following: amylase, pepsin or catalase activity. (Page 37).

Investigate the effect of heat denaturation on the activity of one enzyme. (Page 42).

Prepare one enzyme immobilisation and examine its application. (Page 46).

Investigate the influence of light intensity or carbon dioxide on the rate of photosynthesis. (Page 51, 54).

Prepare and show the production of alcohol by yeast. (Page 59).

Conduct any activity to demonstrate osmosis. (Page 64).

Isolate DNA from a plant tissue. (Page 68).

Investigate the growth of leaf yeast using agar plates and controls. (Page 72).

Prepare and examine microscopically the transverse section of a dicotyledonous stem (×100, ×400). (Page 77).

Dissect, display and identify an ox's or a sheep's heart. (Page 81).

Investigate the effect of exercise on the breathing rate or pulse rate of a human (Page 87, 90).

Investigate the effect of IAA growth regulator on plant tissue. (Page 93).

Investigate the effect of water, oxygen and temperature on germination. (Page 98).

Use starch agar or skimmed milk plates to show digestive activity during germination. (Page 101).

CONDUCT A QUALITATIVE TEST FOR: STARCH, FAT, A REDUCING SUGAR, A PROTEIN

Materials/Equipment

Starch solution (1%)
Iodine solution
Glucose solution (1%)
Benedict's reagent (qualitative)
Protein solution e.g. albumen (1%) or milk
Sodium hydroxide solution (10%), copper sulfate solution (1%) (or biuret reagent)
Vegetable oil
6 Boiling tubes
Beaker (250 cm^3)

Pipettes/droppers
Thermometer
Hot water bath (80 °C – 100 °C)
Test-tube holder
3 Test-tube racks
Disposable gloves
Brown paper
Scissors
Labels
Timer

(i) Test for starch

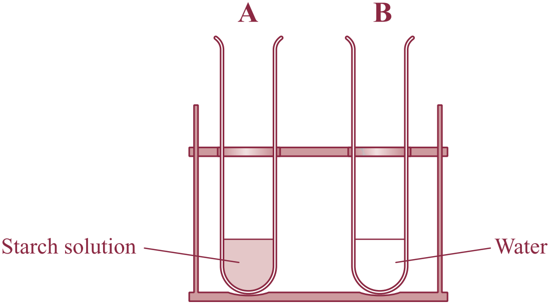


Fig. 1 Testing for starch

Procedure

1. Familiarise yourself with all procedures before starting.
2. Label boiling tube A 'starch solution' and boiling tube B 'water'.
3. Place 2 cm^3 of the starch solution into tube A.
4. Place 2 cm^3 of water into tube B. This acts as the control.
5. Add 2–3 drops of iodine solution to each tube.
6. Swirl each tube.
7. Record result.

Result

Sample	Initial colour	Final colour
A - Starch solution		
B - Water		

Conclusion/Comment

(ii) Test for fat

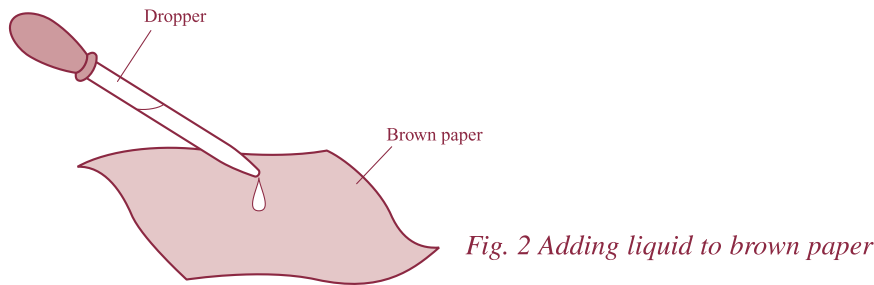


Fig. 2 Adding liquid to brown paper

Procedure

1. Familiarise yourself with all procedures before starting.
2. Cut two pieces of brown paper of similar size.
3. Place 2–3 drops of oil on one piece of paper and label it ‘oil’.
4. Place 2–3 drops of water on the other piece of paper and label it ‘water’. This acts as the control.
5. Leave both aside to dry.
6. Hold both pieces of paper up to the light.
7. Record result.

Result

Conclusion/Comment

Sample	Translucent spot	
	Before drying	After drying
Oil		
Water		

(iii) Test for a reducing sugar

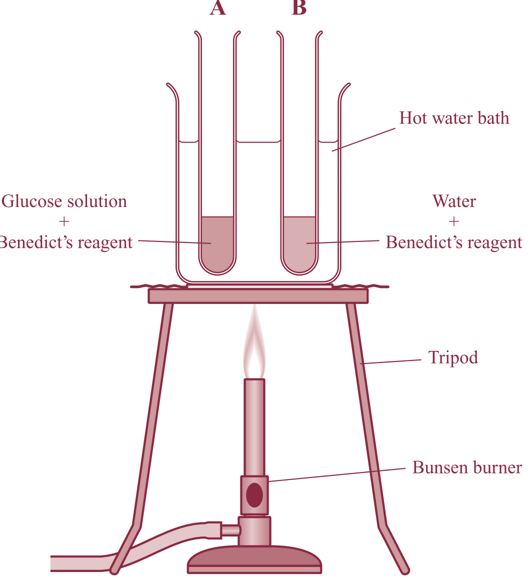


Fig. 3 Testing for a reducing sugar

Procedure

1. Familiarise yourself with all procedures before starting.
2. Label boiling tube A 'glucose solution' and boiling tube B 'water'.
3. Place 2 cm^3 of the glucose solution into tube A.
4. Place 2 cm^3 of water into tube B. This acts as the control.
5. Add 2 cm^3 of Benedict's reagent to each tube.
6. Swirl each tube.
7. Place both tubes in the hot water bath and heat for 5 minutes.
8. Using the test-tube holder, carefully remove both tubes from the water bath and place in the test-tube rack.
9. Record result.

Result

Sample	Initial colour	Final colour
A - Glucose solution		
B - Water		

Conclusion/Comment

(iv) Test for a protein

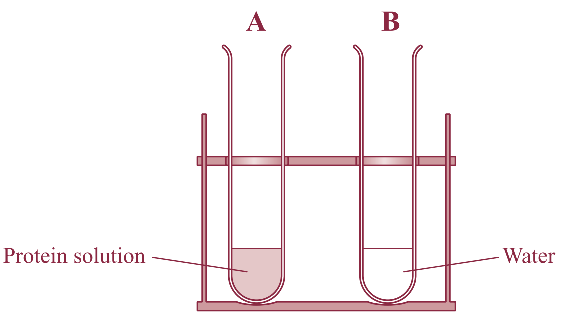


Fig. 4 Testing for a protein

Procedure

1. Familiarise yourself with all procedures before starting.
2. Label boiling tube A 'protein solution' and boiling tube B 'water'.
3. Place 2 cm^3 of the protein solution into tube A.
4. Place 2 cm^3 of water into tube B. This acts as the control.
5. Add 2 cm^3 of sodium hydroxide solution to each tube followed by 2–3 drops of copper sulfate solution (or add 2 cm^3 of biuret reagent to each tube).
6. Swirl both tubes.
7. Record result.

Result

Sample	Initial colour	Final colour
A - Protein solution		
B - Water		

Conclusion/Comment

SKILL ATTAINMENT

CONDUCT A QUALITATIVE TEST FOR: STARCH, FAT, A REDUCING SUGAR, A PROTEIN

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Fill the pipette

Set up the water bath

Use the test-tube holder

Use the timer

Label the tubes

Swirl the tubes

Observation

Observe a colour change

Note the appearance of the translucent spot

Appreciate the significance of heat

Notice the effect of swirling

Recording

Write up the procedure

Record colour change/translucent spot

Tabulate results

Compare with controls

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work as part of a group or team

Label as appropriate

Work in an organised and efficient manner

Clean up after the practical activity

Background information

Starch

Starch is a complex, sparingly soluble, polysaccharide of plants. It consists of two main components: amylose and amylopectin. The amylose stains blue-black with iodine solution.

The solution of iodine used to test for starch contains potassium iodide and water in addition to iodine. The potassium iodide is necessary to dissolve iodine in water. This reagent changes from a brownish or yellowish colour to blue-black when starch is present, but there is no colour change in the presence of monosaccharides or disaccharides.

Fat

Fat does not evaporate from brown paper, but instead leaves a translucent spot.

Reducing sugar

Carbohydrates with a free or potentially free aldehyde (RCHO) or ketone (RCOR') group have reducing properties in alkaline solution. For monosaccharides, the aldo- and keto- groups carry out reducing sugar reactions. Common disaccharides such as lactose and maltose have at least one exposed aldo- or keto- group and can give a positive reducing sugar reaction also.

When a reducing sugar solution, such as glucose or maltose, is mixed with Benedict's reagent and heated, the reaction reduces the blue copper (II) ion to form a brick red precipitate of copper (I) ion. The colour of the reagent changes from blue to green to yellow to reddish-orange, depending on the amount of reducing sugar present. Orange and red indicate the highest proportion of these sugars. However, sucrose is not a reducing sugar because it is a particular kind of disaccharide in which both the aldo- and keto- groups of glucose and fructose (its contributory monomers) are locked into a covalent bond. As a result the aldo- and keto- groups are not free to react in a reducing sugar reaction. Therefore, when sucrose is tested in a reducing sugar reaction, a negative result is obtained.

Protein

Protein molecules are long chains of amino acids joined by peptide bonds. The biuret reagent reacts with any compound containing two or more peptide bonds to give a violet-coloured complex. Any compound containing two carbonyl groups (C=O) linked through either a nitrogen or a carbon atom will give a positive reaction. Therefore this test is not fully specific for proteins. However, the intensity of the reaction is an indication of the number of peptide bonds present in a protein.

The biuret reagent, which is blue in colour, contains a strong solution of sodium or potassium hydroxide (NaOH or KOH) and a smaller amount of dilute copper sulfate solution. The name of the test actually comes from another compound called biuret ($H_2NCONHCONH_2$), which also gives a positive reaction. The biuret compound itself is not part of the reagent, but just gives the test its name. The reagent changes colour in the presence of proteins or peptides because the amino group (H_2N-) of the protein or peptide chemically combines with the copper (II) ions in the biuret reagent. A purple/violet copper (II) complex indicates a positive result. This reagent changes to pink when combined with short-chain polypeptides. A negative result occurs with free amino acids because there are no peptide bonds present.

Advance preparation

- Set the water bath and check the temperature with the thermometer.
- Prepare the following solutions: starch solution (1% w/v), iodine solution, glucose solution (1% w/v), albumen solution (1% w/v), sodium hydroxide solution (10% w/v), copper sulfate solution (1% w/v).

Helpful hints

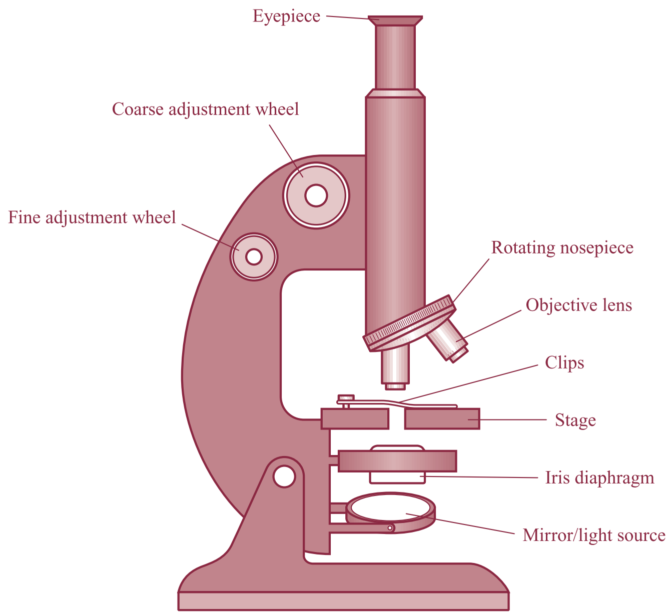
- Starch solution should always be freshly prepared to avoid fungal contamination.
- To quickly dry the brown paper in the fat test place it on a radiator for a minute or two.
- Filter paper may be used instead of brown paper in the fat test.
- When using Benedict's reagent it is essential to use the qualitative rather than quantitative type to obtain a brick-red colour with a reducing sugar.
- To reduce staining of boiling tubes they should be washed as soon as possible after a result is obtained, preferably using warm soapy water.
- Commercially available glucose test strips specifically test for glucose and not for other reducing sugars.
- When carrying tubes a test-tube holder tends to be better than a tongs.
- Biuret reagent tests for proteins in solution only.

BE FAMILIAR WITH AND USE THE LIGHT MICROSCOPE

Materials/Equipment

Microscope
Prepared microscope slides

Note: Study your microscope carefully and compare it with the diagram below. Yours may be slightly different. Identify each of the labelled parts.



The light microscope

Procedure

1. Familiarise yourself with all procedures before starting.
2. Switch on the light source and remove the eyepiece cover, if present.
3. Rotate the nosepiece so that the low power lens is used.
4. Put a prepared microscope slide on the stage of the microscope.
5. Move the slide until the object is above the hole in the stage.
6. Use the stage clips to hold the slide in place.
7. Using the coarse adjustment wheel, ensure that the low power lens is at the closest setting to the slide.
8. Look down the eyepiece. Keep your eye about 2 cm from the eyepiece. Adjust the iris diaphragm so that the field of vision is bright but not dazzling. Adjust the position of the slide, if necessary.
9. Use the coarse adjustment wheel to focus the object as sharply as possible. Then use the fine adjustment wheel to sharpen the focus. If necessary, readjust the iris diaphragm so that the specimen is correctly illuminated.
10. To increase the magnification, rotate the nosepiece so that the next highest power objective lens is above the specimen.
11. Refocus using the fine adjustment wheel. Readjust the illumination if necessary.

12. To further increase the magnification, rotate the nosepiece again so that the highest power objective lens is immediately above the specimen.
13. Focus using the fine adjustment wheel only.
14. Magnification is calculated by multiplying the magnification of the objective lens by the magnification of the eyepiece. The magnification of microscope lenses is engraved on the lens casing.
15. Draw labelled diagrams of your observations under low power (L.P.) and high power (H.P.).

Result

Conclusion/Comment

SKILL ATTAINMENT

BE FAMILIAR WITH AND USE THE LIGHT MICROSCOPE

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Provide a light source
Remove eyepiece cover
Rotate nosepiece so that the low power objective lens is being used
Place the slide on the stage
Use the stage clips
Use the coarse adjustment wheel
Use the iris diaphragm
Use the fine adjustment wheel
Use the high power objective lens
Refocus using the fine adjustment wheel

Observation
See a clear image
Appreciate the importance of the correct placement of the slide
Notice the effect of magnification
See the effect of adjusting the iris diaphragm
Appreciate the use of the coarse adjustment wheel
Appreciate the use of the fine adjustment wheel

Recording
Write up the procedure
Draw labelled diagrams of your observations

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Microscopes are fundamental biological tools. There are two basic types of light microscope, the compound microscope and the stereoscopic microscope. The compound microscope is used to examine cellular material and gives a magnification of up to ×400. The specimen has to be translucent. The stereoscopic microscope is used to view whole structures in 3 dimensions.

The most important feature of any lens system is its resolving power. The resolving power of a lens system is the smallest distance separating two objects that can be distinguished by the lens system and that allows them to be seen as two distinct objects rather than as a single entity. For example, most humans see two fine parallel lines as two distinct markings if they are separated by 0.1 mm. If they are closer together we see them as a single line. Thus the resolving power of the human eye is 0.1 mm.

The light microscope has a resolving power of about 0.0002 mm so it gives useful views of cells and can reveal features of some of the sub-cellular contents of eukaryotic cells.

Most microscopes are fitted with three objective lenses, which magnify ×4, ×10 and ×40.
The eyepiece lens is usually ×10. The total magnification is obtained by multiplying the number on the eyepiece by the number on the objective lens.

Helpful hints

- It is advisable to clean the lenses of the microscope regularly using lens tissue. This minimises confusion between debris and unstained cells.
- The objective lenses are preferably cleaned with ethanol-diethyl ether (30:70) on lens tissue or with lens cleaning tissue.
- A common mistake is to have too much light coming through the specimen particularly when viewing under low power. Opening or closing the iris diaphragm will vary the light intensity.
- To locate small objects e.g. single cells, reduce the light intensity by closing the iris diaphragm and traverse the slide methodically.
- Never remove a slide while the high power objective lens is in position. Turn back to the low power first.
- Always treat the microscope with great care. Carry it in both hands and cover when not in use. Allow the lamp to cool before covering and storing.
- Students who wear glasses can remove them for viewing, as microscope adjustments will accommodate most deficiencies in eyesight (except astigmatism). This is more comfortable and stops the spectacle lenses being scratched by the eyepiece holders.

PREPARE AND EXAMINE ONE ANIMAL CELL, UNSTAINED AND STAINED, USING THE LIGHT MICROSCOPE (×100, ×400)

Materials/Equipment

Microscope	Filter paper/absorbent paper
Disinfectant	Disposal jar for inoculating loops/swabs
2 Microscope slides	2 Disposable inoculating loops/mouth swabs
Labels	Methylene blue stain (1%)
2 Cover slips	Timer
Wash bottle	Seeker/mounted needle
Disposal jar for slides	Disposable gloves

Procedure

Unstained

1. Familiarise yourself with all procedures before starting.
2. Set up the microscope.
3. Swab inside cheek surface with a disposable inoculating loop and transfer the sample to the slide. Put the loop into the disposal jar.
4. Cover the sample with one drop of water.
5. Apply the cover slip to this preparation as follows:
   a) Place the cover slip at the edge of the water at an angle of 45° to the slide.

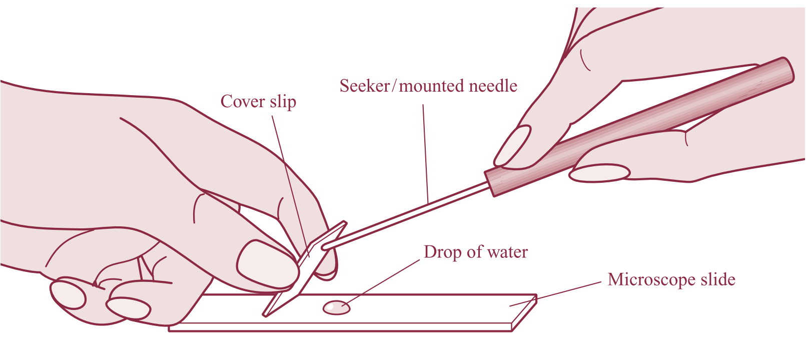


Fig. 1

Application of cover slip

   b) Slowly lower the cover slip onto the water, supporting it with the seeker/mounted needle, until it is in place. This helps to avoid trapping air bubbles.
6. Dry the slide if necessary and label it.
7. Examine under the microscope following the usual procedure.
8. Draw labelled diagrams of what you see at ×100 and at ×400.

Result

Conclusion/Comment

Stained

1. Swab inside cheek surface again using a disposable inoculating loop/swab and transfer the sample onto the second slide. Put the loop/swab into the disposal jar.
2. Air dry the slide.
3. Cover the sample with one drop of methylene blue solution.
4. Allow to stand for one minute.
5. Gently, using a wash bottle, wash excess stain from the slide.
6. Apply a cover slip to this preparation as in Fig. 1.
7. Dry the slide carefully with filter paper/absorbent paper and label it.
8. Examine under the microscope following the usual procedure.
9. Draw labelled diagrams of what you see at ×100 and at ×400.

Result

Conclusion/Comment

SKILL ATTAINMENT

PREPARE AND EXAMINE ONE ANIMAL CELL, UNSTAINED AND STAINED, USING THE LIGHT MICROSCOPE (×100, ×400)

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the light microscope effectively
Use the disposable inoculating loop to gather cheek cells
Transfer the sample to the slide
Cover the sample with water
Apply the cover slip
Apply the stain from the dropper
Gently wash excess stain off the sample

Observation
Locate cells
View cells under different magnification
Differentiate between cells and debris
Appreciate the limitation of the unstained preparation
Appreciate the value of the stained preparation

Recording
Write up the procedure
Draw labelled diagrams

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

PREPARE AND EXAMINE ONE PLANT CELL, UNSTAINED AND STAINED, USING THE LIGHT MICROSCOPE (×100, ×400)

Materials/Equipment

Microscope
2 Microscope slides
2 Cover slips
Beaker for used slides
Iodine stain
Petri dish
Onion
Chopping board
Small scissors
Small paintbrush
Sharp knife
Labels
Seeker/mounted needle
Filter paper/absorbent paper
Dropper
Disposable gloves

Procedure

Unstained

1. Familiarise yourself with all procedures before starting.
2. Set up the microscope.
3. Place a drop of water on the slide.
4. Cut the onion in half.
5. Separate two fleshy leaves and locate the epidermis between them.
6. Peel off the epidermis and cut it into small pieces.
7. Put these pieces into water in a petri dish.
8. Transfer one piece into the drop of water on the slide, using the small paintbrush.
9. Apply the cover slip.
10. Dry the slide if necessary and label it.
11. Examine under the microscope following the usual procedure.
12. Draw labelled diagrams of what you see at ×100 and ×400.

Result

Conclusion/Comment

Stained

1. Prepare another slide as above and stain as follows.
2. Place a drop of iodine solution at one side of the cover slip and draw it across the plant tissue by placing the edge of a piece of filter paper at the opposite side of the cover slip.

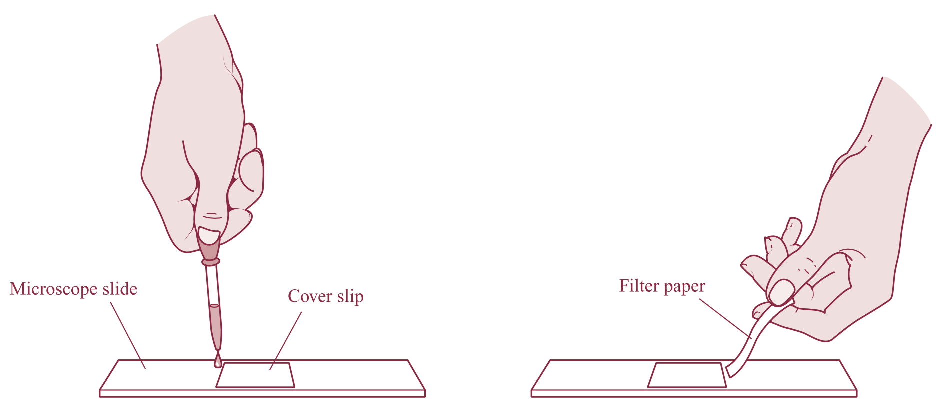


Fig. 2 Drawing stain under cover slip

3. Dry the slide carefully with filter paper/absorbent paper and label it.
4. Examine under the microscope following the usual procedure.
5. Draw labelled diagrams of what you see at ×100 and ×400.

Result

Conclusion/Comment

SKILL ATTAINMENT

PREPARE AND EXAMINE ONE PLANT CELL, UNSTAINED AND STAINED, USING THE LIGHT MICROSCOPE (×100, ×400)

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Use the light microscope

Remove some of the onion epidermis

Transfer small pieces of the epidermis to water in the petri dish

Put a piece of the epidermis in a drop of water on the slide

Apply the cover slip

Draw the iodine across under the cover slip, using the filter paper

Observation

Locate the epidermis between the fleshy leaves of the onion

Locate cells under the microscope

View cells under different magnification

Appreciate the limitations of the unstained preparation

Appreciate the value of a stained preparation

Recording

Write up the procedure

Draw labelled diagrams

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Label as appropriate

Work as part of a group or team

Clean up after the practical activity

Background information

The human eye has a resolving power of 0.1 mm. Most eukaryotic cells are between 0.01 mm and 0.03 mm in diameter some 3 to 10 times below the resolving power of the human eye. Most of our current knowledge of cell structure has been gained with the assistance of microscopes.

We are asked to view animal and plant cells at ×100 and ×400. The cheek cell, taken from the loose tissue on the inside of the mouth, provides us with a good example of a typical animal cell. The cells and tissues under the microscope may lack contrast with their surroundings. They are often nearly invisible. Stains provide contrast. Methylene blue stain is an aqueous solution that stains the nucleus a dark colour.

After a specimen has been treated with a staining substance all of the stain that does not adhere to the structures must be washed away. To prevent the cells being washed away or damaged they may need to be fixed. Fixing may be carried out by chemicals or by heat but in the experiment above air drying for one minute is sufficient.

The onion cell provides us with a good example of a typical plant cell. However, the onion cells lack chlorophyll as the onion is an underground organ. Chloroplasts can be seen in *Elodea* or moss leaf cells. Iodine stain is used to stain plant cells. The starch in some plant cells turns the iodine a blue-black colour.

Advance preparation

- Prepare methylene blue stain.
- Prepare iodine stain.

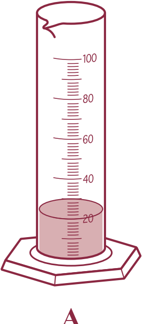
Helpful hints

- Gloves should be worn by students when using stains.
- Place the slides on a rack over a small tray when staining to avoid spillages on the desk. Such a rack may be made with two 10 cm^3 pipettes linked together at each end with rubber tubing.
- Ethanoic acid (10%) may be used on the unstained animal cell to highlight the nucleus. Ethanoic acid is not a stain.
- Used inoculating loops/swabs and slides with cheek cells should be sterilised with disinfectant and then disposed of at the end of the activity.
- Cut small pieces of the onion epidermis while still on the onion.
- Multiple nucleoli (two) may be seen in onion nuclei.

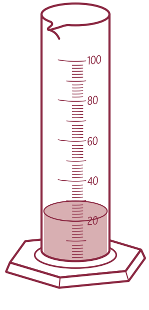
INVESTIGATE THE EFFECT OF pH ON THE RATE OF CATALASE ACTIVITY

Materials/Equipment

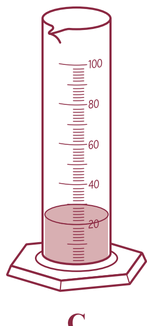
Enzyme source e.g. radishes
Hydrogen peroxide (20% or less)
Range of buffer solutions – acidic, neutral and alkaline
pH paper
Washing-up liquid
Large beaker of water at 25 °C
Graduated cylinders (100 cm^3)
Syringe
Boiling tubes
Thermometer
Knife
Chopping board
Electronic balance
Weigh boats
Disposable gloves
Labels
Timer
Dropper



A
20 cm^3 buffer solution one
1 drop washing-up liquid
Enzyme source
2 cm^3 H_2O_2



B
20 cm^3 buffer solution two
1 drop washing-up liquid
Enzyme source
2 cm^3 H_2O_2



C
20 cm^3 buffer solution three
1 drop washing-up liquid
Enzyme source
2 cm^3 H_2O_2

Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 20 cm^3 of one of the selected buffers to a graduated cylinder.
3. Using the dropper, add one drop of washing-up liquid.
4. Add 5 g of finely chopped radish to the cylinder.
5. Add 2 cm^3 of hydrogen peroxide to a boiling tube.
6. Stand the cylinder and the boiling tube in the beaker of water at 25 °C.
7. Pour the hydrogen peroxide into the cylinder.
8. Note the volume in the cylinder immediately and record.
9. Read the volume again after a measured amount of time e.g. 2 minutes, and record.
10. Subtract the initial volume from the final volume to get the volume of foam and record.
11. Repeat the procedure from step 3 for each of the other buffer solutions.
12. A graph should be drawn of enzyme activity (volume of foam) against pH. Put pH on the horizontal axis.

Result

pH of buffer	Initial volume (cm^3)	Final volume (cm^3)	Volume of foam produced (cm^3)

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF pH ON THE RATE OF CATALASE ACTIVITY

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Maintain constant temperature (25 °C) in the beaker

Obtain enzyme extract

Use the syringe

Use the graduated cylinder

Measure pH

Use the timer

Use the electronic balance

Observation

Appreciate the use of washing-up liquid

Note the evolution of bubbles in the mixture

Note the rise of foam in the cylinders

Note the colours on the pH papers before and after dipping in solutions

Recording

Write up the procedure

Record the volume of foam per unit time

Tabulate the results

Record the pH in each cylinder

Draw a graph with labelled axes

Interpretation

Draw conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Label as appropriate

Work as part of a group or team

Clean up after the practical activity

Background information

In higher organisms, catalase occurs in cell organelles called peroxisomes. It is one of the fastest reacting enzymes known. It catalyses the breakdown of hydrogen peroxide to water and oxygen.

Superoxide radicals (O_2^-) are generated during aerobic respiration when a small amount of the oxygen that normally forms water gains an electron. Excess superoxide may be converted to more damaging hydroxyl radicals. Within cells the enzyme superoxide dismutase removes superoxide by converting it to hydrogen peroxide. Hydrogen peroxide is toxic to the body and is broken down by catalase. Hydrogen peroxide is also produced by white blood cells. They produce it during phagocytosis to kill microorganisms.

Enzymes function over a narrow pH range. Each enzyme has an optimum pH and as the pH goes above or below this the activity of the enzyme drops. Extremes of pH denature enzymes.

Changes in pH alter the ionic charge of the acidic and basic groups that exist on an enzyme and on its substrate. In an enzyme these charges help to maintain the shape of its active site. Changes in pH influence the formation and decomposition of the enzyme-substrate complex and thus the activity of the enzyme.

Advance preparation

- Prepare or obtain buffer solutions.
- Obtain fresh radishes.

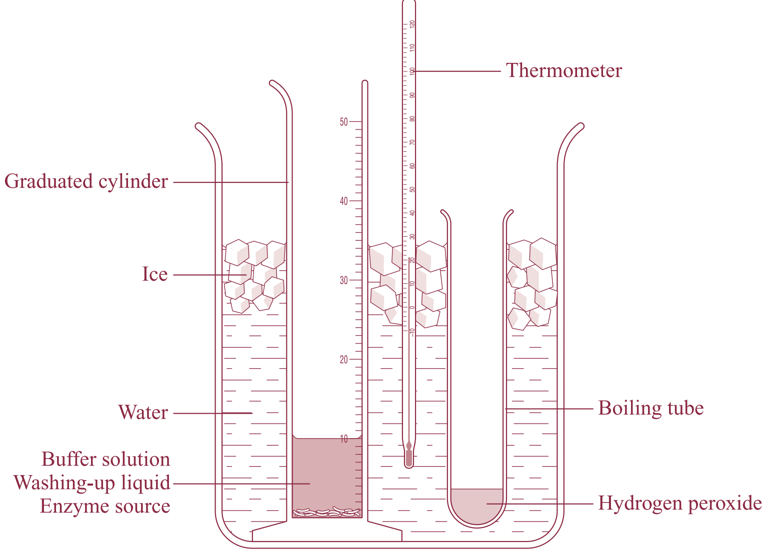
Helpful hints

- The enzyme source must be fresh.
- Besides radish there are many other good sources of catalase. Liver is the best source but celery and potato are also good. These can be used chopped, as described in the investigation, or extracts can be prepared. To prepare celery extract, chop 3 stalks and macerate them in a blender in 100 cm^3 of distilled water. Filter through coffee filter. If using liver, macerate 5 g in 100 cm^3 of distilled water and strain through a household sieve. Use 1 cm^3 of these extracts in each cylinder. Increase this volume if activity is low.
- Enzyme preparations lose activity very quickly. Therefore, the enzyme extract must be prepared immediately before use.
- If activity is high, e.g. when using liver, use larger cylinders.
- When using radish or potato as a source of catalase, lack of uniformity in the surface area of the pieces can be reduced by cutting the vegetable in slices and using a cork borer to divide each slice up into equal sized pieces. Use an equal number of pieces from each slice in each cylinder.
- Use a large beaker as a water bath. The catalase reaction is exothermic and the large volume of water will act as a heat sink. It will also protect the bench top from spillages and overflowing foam.
- The concentration of hydrogen peroxide may be expressed both as percentage and volume e.g. 30% (100 vol.).
- 6% (20 vol.) hydrogen peroxide is available in pharmacies. Other percentages are available from laboratory suppliers. Concentrations below 20% are irritants, concentrations above this are corrosive and cause burns.
- Hydrogen peroxide should be stored in a lightproof bottle because light accelerates its decomposition.
- Buffer tablets and capsules are available. Make these up with distilled water according to the instructions on the container. Liquid buffers may also be purchased.
- Higher concentrations of hydrogen peroxide than indicated in the investigation may require a greater volume of buffer to maintain pH.
- A trial run of the investigation should be carried out in advance to determine the best conditions.

INVESTIGATE THE EFFECT OF TEMPERATURE ON THE RATE OF CATALASE ACTIVITY

Materials/Equipment

- Enzyme source e.g. radishes
- Hydrogen peroxide (20% or less)
- Buffer solution (pH 9)
- Washing-up liquid
- Boiling tubes
- Syringe
- Graduated cylinders (100 cm^3)
- Water baths (0 °C – 60 °C)
- Thermometer
- Knife
- Chopping board
- Electronic balance
- Weigh boats
- Dropper
- Test-tube holder
- Disposable gloves
- Timer
- Test-tube rack



Reactants cooling to 0 °C

Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 20 cm^3 of the buffer to the graduated cylinder.
3. Using the dropper, add one drop of washing-up liquid.
4. Add 5 g of finely chopped radish to the cylinder.
5. Add 2 cm^3 of hydrogen peroxide to the boiling tube.
6. Stand the cylinder and the boiling tube in an ice-cold water bath until the desired temperature (0 °C) is reached.
7. Pour the hydrogen peroxide into the cylinder.
8. Note the volume in the cylinder immediately and record.
9. Read the volume again after a measured amount of time, e.g. 2 minutes, and record.
10. Subtract the initial volume from the final volume to get the volume of foam and record.
11. Repeat the procedure from step 3 for at least four more temperatures, to include a sample in the 50 °C – 60 °C range.
12. A graph should be drawn of enzyme activity (volume of foam after 2 minutes) against temperature. Put temperature on the horizontal axis.

Result

Temperature (°C)					
Initial volume (cm^3)					
Final volume (cm^3)					
Volume of foam produced (cm^3)					

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF TEMPERATURE ON THE RATE OF CATALASE ACTIVITY

Following instructions

Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus

Prepare the enzyme source
Use the syringe
Use the graduated cylinder
Use the thermometer
Use the electronic balance
Set up and maintain the water baths
Use the test-tube holder
Use the timer

Observation

Observe the evolution of bubbles in the mixture
Observe the rise of foam in the cylinders

Recording

Write up the procedure
Record the volume of foam
Tabulate results
Record the temperature each time the procedure is repeated
Draw a graph with labelled axes

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Effect of temperature on enzymes

Each enzyme has its own optimum temperature, that is the temperature at which it works most efficiently. This optimum temperature may vary, for a given enzyme, depending on its source.

Below the optimum temperature, increasing temperature will increase the rate of the reaction. This is because temperature increases the kinetic energy of the system, effectively increasing the number of collisions between the substrate and the enzyme's active site. The effect of temperature on the rate of a chemical reaction is described by the term "temperature coefficient" (Q_{10}).

$$Q_{10} = \frac{\text{rate of reaction at } (T + 10)\ °C}{\text{rate of reaction at } T\ °C}$$

T = Temperature

Many enzymes have a Q_{10} of between two and three which means that, provided that the temperature is not so high that it causes denaturation, an increase in temperature of 10 °C will speed up the reaction by a factor of two to three i.e. double or treble it (Q_{10} rule).

Catalase

All enzymes have a characteristic turnover number, which is the number of substrate molecules converted to product per second. One of the fastest known enzymes is catalase, which converts up to 10,000,000 hydrogen peroxide molecules per second into water and oxygen. The bubbles that appear in the foam are pure oxygen bubbles generated by the catalase.

For further background information refer to the investigation on the effect of pH on the rate of catalase activity.

Advance preparation

- Prepare or obtain the buffer solution.
- Buy fresh radishes.
- Set up the water baths and check the temperatures with a thermometer.

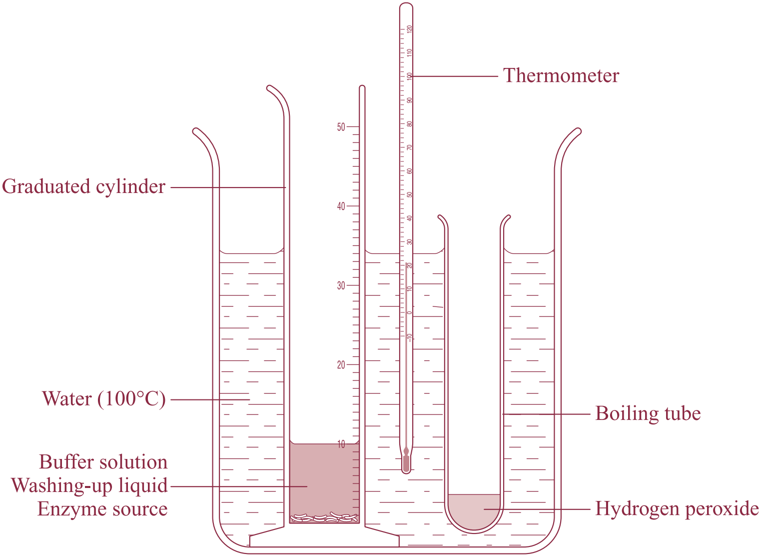
Helpful hints

- The enzyme source must be fresh.
- Besides radish there are many other good sources of catalase. Liver is the best source but celery and potato are also good. These can be used chopped, as described in the investigation, or extracts can be prepared. To prepare a celery extract, chop three stalks and macerate them in a blender in 100 cm^3 of distilled water. Filter through a coffee filter. If using liver, macerate 5 g in 100 cm^3 of distilled water and strain through a household sieve. Use 1 cm^3 of these extracts in each cylinder. Increase this volume if activity is low.
- A more accurate value for optimum temperature may be obtained by carrying out the investigation at more than the minimum five temperatures mentioned above.
- Enzyme preparations lose activity very quickly. Therefore, the enzyme extract must be prepared immediately before use.
- If activity is high, e.g. when using liver, use larger cylinders.
- When using radish or potato as a source of catalase, lack of uniformity in the surface area of the pieces can be reduced by cutting the vegetable in slices and using a cork borer to divide each slice up into equal sized pieces. Use an equal number of pieces from each slice in each cylinder.
- The concentration of hydrogen peroxide may be expressed both as percentage and volume e.g. 30% (100 vol.).
- 6% (20 vol.) hydrogen peroxide is available in pharmacies. Other percentages are available from laboratory suppliers. Concentrations below 20% are irritants, concentrations above this are corrosive and cause burns.
- Hydrogen peroxide should be stored in a lightproof bottle because light accelerates its decomposition.
- Buffer tablets and capsules are available. Make these up with distilled water according to the instructions on the container. Liquid buffers may also be purchased.
- Higher concentrations of hydrogen peroxide than indicated in the investigation may require a greater volume of buffer to maintain pH.
- A trial run of the investigation should be carried out in advance to determine the best conditions.

INVESTIGATE THE EFFECT OF HEAT DENATURATION ON CATALASE ACTIVITY

Materials/Equipment

Enzyme source e.g. radishes
Hydrogen peroxide (20% or less)
Buffer solution (pH 9)
Boiling tubes
Washing-up liquid
Syringe
2 Water baths (25 °C, 100 °C)
Graduated cylinders (100 cm^3)
Test-tube holder
Knife
Chopping board
Electronic balance
Weigh boats
Dropper
Thermometer
Disposable gloves
Timer
Test-tube rack



Denaturing catalase enzyme

Procedure

1. Familiarise yourself with all procedures before starting.
2. Place 5 g of finely chopped radish into a boiling tube (without water) and place the boiling tube into the water bath at 100 °C for ten minutes.
3. Remove the boiling tube and allow it to cool.
4. Add 20 cm^3 of the buffer to the graduated cylinder.
5. Using the dropper, add one drop of washing-up liquid.
6. Add 5 g of the heated radish to the cylinder.
7. Add 2 cm^3 of hydrogen peroxide to the boiling tube.
8. Stand the cylinder and the boiling tube in the water bath until the desired temperature (25 °C) is reached.
9. Pour the hydrogen peroxide into the cylinder.
10. Note the presence or absence of foam formation and record.
11. Repeat the procedure from step 5 using an unheated radish sample.

Result

//////////////////////////////	Unheated enzyme	Heated enzyme
Foam formation		

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF HEAT DENATURATION ON CATALASE ACTIVITY

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Prepare the enzyme source

Use the syringe

Use the graduated cylinder

Use the thermometer

Use the electronic balance

Set up and maintain the water baths

Use the test-tube holder

Use the timer

Observation

Observe the presence or absence of foam formation

Recording

Write up the procedure

Tabulate the results

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Label as appropriate

Work as part of a group or team

Clean up after the practical activity

Background information

An enzyme is a protein composed of a chain of amino acids, joined together by covalent bonds called peptide bonds. The actual activity of an enzyme is derived from the way this protein (polypeptide) chain is folded into a three dimensional structure (tertiary structure). When this three dimensional structure is destabilised by heat, typically at temperatures over 50 °C, denaturation is said to have occurred. This means that the linear sequence of amino acids unique to that protein (its primary structure) still exists but the enzyme has lost its activity. Enzymes catalyse reactions by forming a complex with the substrate (known as the enzyme-substrate complex) at a specific region of the enzyme called the active site. Denaturation occurs because the hydrogen bonds and the disulfide bonds which determine the tertiary structure of the enzyme, and consequently help to maintain the shape of the active site, are broken and do not reform with cooling. Thus enzyme-substrate complexes can no longer be formed and enzyme activity is lost.

Advance preparation

- Prepare the buffer solution.
- Buy fresh radishes.
- Set up the water baths and check the temperatures with a thermometer.

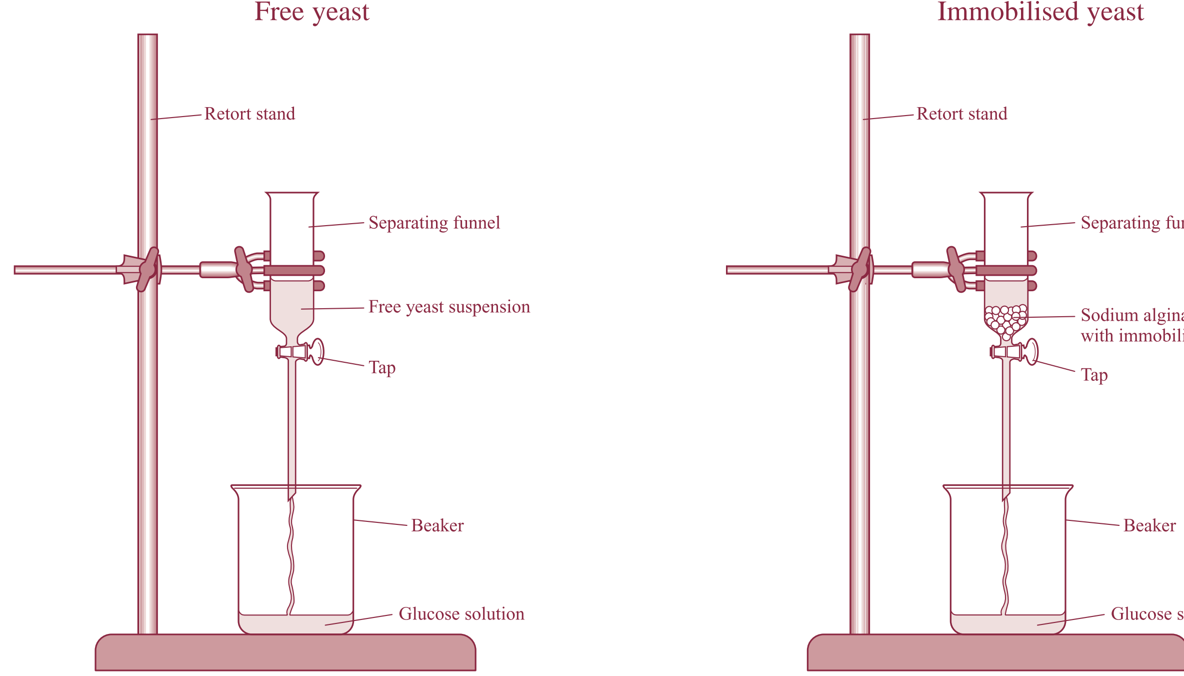
Helpful hints

- The enzyme source must be fresh.
- Besides radish there are many other good sources of catalase. Liver is the best source but celery and potato are also good. These can be used chopped, as described in the investigation, or extracts can be prepared. To prepare celery extract, chop three stalks and macerate them in a blender in 100 cm^3 of distilled water. Filter through a coffee filter. If using liver, macerate 5 g in 100 cm^3 of distilled water and strain through a household sieve. Use 1 cm^3 of these extracts in each cylinder. Increase this volume if activity is low.
- Enzyme preparations lose activity very quickly. Therefore, the enzyme extract must be prepared immediately before use.
- The concentration of hydrogen peroxide may be expressed both as percentage and volume e.g. 30% (100 vol.).
- 6% (20 vol.) hydrogen peroxide is available in pharmacies. Other percentages are available from laboratory suppliers. Concentrations below 20% are irritants, concentrations above this are corrosive and cause burns.
- Hydrogen peroxide should be stored in a lightproof bottle because light accelerates its decomposition.
- Buffer tablets and capsules are available. Make these up with distilled water according to the instructions on the container. Liquid buffers can also be purchased.

PREPARE ONE ENZYME IMMOBILISATION AND EXAMINE ITS APPLICATION

Materials/Equipment

- Sachet of yeast (without $CaSO_4$)
- Sodium alginate
- Calcium chloride powder
- Sucrose
- Distilled water
- Glucose test strips
- Hot water (30 °C – 40 °C)
- 2 Graduated cylinders (100 cm^3)
- 4 Beakers (100 cm^3)
- Beaker (500 cm^3)
- 2 Separating funnels
- 3 Glass rods
- 3 Thermometers
- 2 Retort stands
- Electronic balance
- Weigh boats
- Syringe (20 cm^3)
- Sieve
- Labels
- 3 Spatulas
- Wash bottle
- Timer



Application of the immobilised enzyme

Procedure

1. Familiarise yourself with all procedures before starting.

Preparation of the immobilised enzyme

2. Add 0.4 g of sodium alginate to 10 cm^3 of distilled water in a 100 cm^3 beaker. Mix thoroughly.
3. Mix 2 g of yeast in 10 cm^3 of distilled water in a 100 cm^3 beaker.
4. Prepare 100 cm^3 of a 1.4% w/v calcium chloride solution in the large beaker.
5. Add the yeast suspension to the sodium alginate solution and mix thoroughly with a glass rod.
6. Draw all of the mixture into a 20 cm^3 syringe.
7. From a height of 10 cm release the mixture from the syringe, one drop at a time, into the calcium chloride solution. Beads containing yeast cells will form.
8. Leave the beads to harden for at least 10 minutes.
9. Filter the beads through a sieve and rinse with distilled water.

Application of the immobilised enzyme – production of glucose from sucrose

10. Mix another 2 g of yeast in 10 cm^3 of distilled water.
11. Pour this yeast suspension into a separating funnel labelled ‘Free yeast’ as shown in the diagram.
12. Pour the beads into another separating funnel labelled ‘Immobilised yeast’.
13. Prepare 100 cm^3 of 1% w/v sucrose solution with water warmed to about 40 °C.
14. Pour 50 cm^3 of the sucrose solution into the yeast in each of the separating funnels.
15. Using glucose test strips, immediately test samples from each funnel for glucose.
16. Repeat the test at two-minute intervals until glucose appears in both.
17. Record result.
18. Run off the remaining product from each funnel into the beakers as shown in the diagram.
19. Compare the turbidity of the solutions from both funnels.
20. Replicate the investigation or cross reference your results with other groups.

Result

Time (minutes)	Free yeast – presence of glucose	Immobilised yeast – presence of glucose
0		
2		
4		
6		
8		
10		

//////////////////////////////	Free yeast	Immobilised yeast
Turbidity of solution		

Conclusion/Comment

SKILL ATTAINMENT

PREPARE ONE ENZYME IMMOBILISATION AND EXAMINE ITS APPLICATION

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the balance
Use a graduated cylinder to measure volumes
Prepare solutions and mixtures
Draw the mixture of alginate solution and yeast suspension into the syringe
Release the mixture drop by drop into the calcium chloride solution
Transfer the beads to the separating funnel
Filter the beads
Rinse the beads with distilled water
Set up the separating funnels
Warm water to about 40 °C
Use a thermometer
Pour 50 cm^3 of sucrose solution into each funnel
Use glucose test strips to test for glucose
Repeat at two-minute intervals using the timer

Observation
Note the clarity of both sucrose solutions
See beads forming
Observe any colour change using glucose test strips
Compare the turbidity of both end products

Recording
Write up the procedure
Tabulate the results
Record the time

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

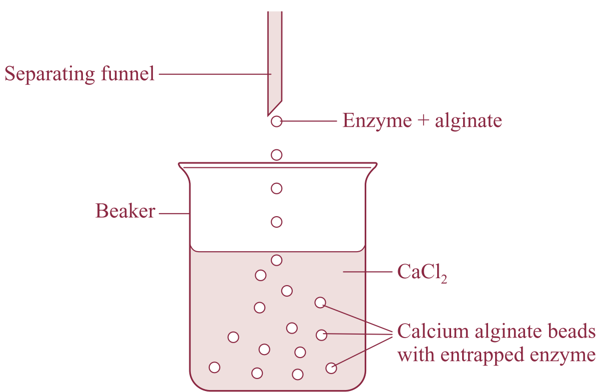
Alginates

Most alginates are produced by brown algae (Phaeophyceae, mainly *Laminaria spp.*) whereas agars are produced from red algae (Rhodophyceae).

Immobilised enzymes

An immobilised enzyme is one that has been made insoluble or held in place so that it can be reused many times. Once immobilised, an enzyme's stability is increased, possibly because its ability to change shape is reduced. One method of immobilisation is an entrapment method, in which the enzyme is enclosed within a gel (e.g. alginate beads). The openings in the encapsulating material have to be of sufficient size to permit the substrate or reactant to pass through to the enzyme without allowing the enzyme to leak out. The most common entrapment method is the formation of calcium alginate beads. The enzyme is mixed with sodium alginate, an acidic polysaccharide, and the mixture is dropped into a solution of calcium chloride. The calcium ions replace the sodium ions and cross-link the polysaccharide. The result is the production of insoluble calcium alginate beads containing trapped enzymes. Calcium alginate gel entrapment is a suitable method for this activity because it is simple to prepare and allows high enzyme activity and stability.

$$2\ Na(Alginate) + Ca^{++} \rightarrow Ca(Alginate)_2 + 2\ Na^{+}$$



Advance preparation

- Prepare the hot water required to make the sucrose solution.

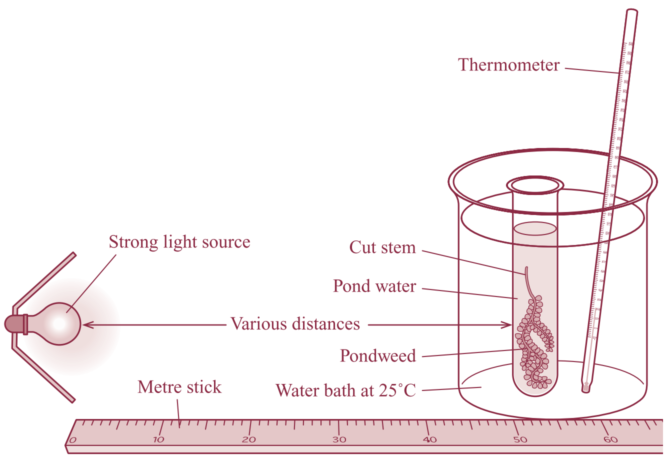
Helpful hints

- Sodium alginate is not readily soluble in water. Sodium alginate solution is best prepared by adding the powder to agitated water, rather than vice versa to avoid the formation of clumps.
- If left to stand, sodium alginate will absorb water and will dissolve more readily.
- Beads may be stored under distilled water for two to three days until ready to use.
- To avoid beads blocking the separating funnel, a plastic disposable pipette or straw may be inserted into the funnel before adding the beads.
- When using glucose test strips make sure to read instructions carefully as the procedure varies depending on the brand used.
- Only use yeast which does not contain calcium sulfate.
- For a better visual display, calcium chloride solution may be transferred to a clear graduated cylinder before adding yeast/alginate mixture.
- Cut glucose test strips in two, lengthwise, to make the supply last longer.
- Use separate weigh boats for the different chemicals to avoid calcium coming in contact with the yeast and alginate.
- Separating funnels with Teflon taps, rather than glass taps, are preferable as they are less likely to stick.
- Use wide-topped separating funnels as it is easier to introduce beads.

INVESTIGATE THE INFLUENCE OF LIGHT INTENSITY ON THE RATE OF PHOTOSYNTHESIS

Materials/Equipment

Fresh *Elodea*
Boiling tube
Large beaker of water at 25 °C
Strong light source
Metre stick
Pond water
Timer
Thermometer
Scissors
Forceps
Light meter (optional)



Illuminating pondweed

Procedure

1. Familiarise yourself with all procedures before starting.
2. Obtain a fresh shoot of *Elodea*.
3. Cut the stem at an angle. Remove several leaves from around the cut end of the stem.
4. Fill a boiling tube with pond water.
5. Place the plant into the boiling tube, cut end pointing upwards.
6. Place this tube into the water bath.
7. Switch on the light source.
8. Place the boiling tube containing the pondweed at a measured distance from the light source e.g. 15 cm.
9. Allow the plant to adjust for at least 5 minutes and observe bubbles being released from the cut end of the stem.
10. Count and record the number of bubbles released per minute. Repeat twice.
11. Calculate and record the average number of bubbles released per minute.
12. Measure the light intensity at this distance using the light meter or calculate the light intensity by using the formula: light intensity = $1/d^2$, where ‘d’ represents the distance from the light source. Record result.
13. Repeat the procedure from step 9 at other measured distances e.g. at 30 cm, 45 cm, 60 cm, 75 cm.
14. A graph should be drawn of rate of bubble production against light intensity. Put light intensity on the horizontal axis.

Note:
During this investigation only one factor (light intensity) should be varied – temperature and carbon dioxide concentration must be kept constant.

To keep the temperature constant, use a water bath at 25 °C.

To keep the carbon dioxide concentration constant use pond water and complete the investigation over a short period of time.

Result

Distance from light source (cm)	Light intensity or $1/d^2$	Trial 1 (No. of bubbles/min)	Trial 2 (No. of bubbles/min)	Trial 3 (No. of bubbles/min)	Average (No. of bubbles/min)

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE INFLUENCE OF LIGHT INTENSITY ON THE RATE OF PHOTOSYNTHESIS

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Carefully use the scissors to cut the end of the plant

Place the plant in the boiling tube, cut end pointing upwards

Use the metre stick to measure distances of plant from light source

Use the thermometer

Use the timer

Use the light meter (optional)

Observation

Observe bubbles being released

After allowing the plant to adjust, observe a steady stream of bubbles

Observe the number of bubbles being released per minute at each distance

Recording

Write up the procedure

Record the distance of the plant from the light source

Record the number of bubbles being liberated per minute at each distance

Record the average number of bubbles being liberated per minute at each of the given distances

Record the light intensity or $1/d^2$

Draw a graph with labelled axis

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Label as appropriate

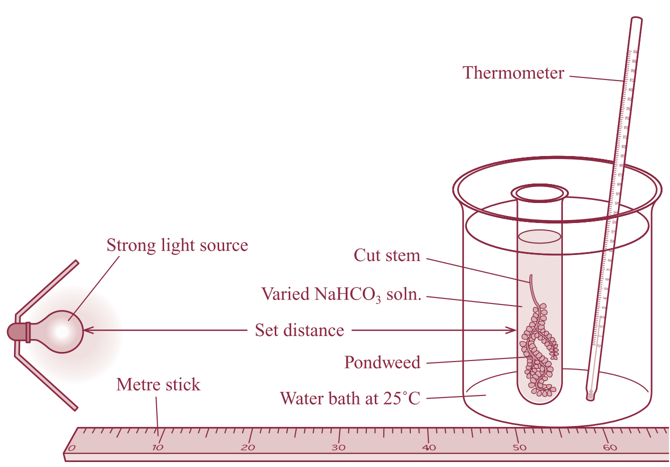
Work as part of a group or team

Clean up after the practical activity

INVESTIGATE THE INFLUENCE OF CARBON DIOXIDE ON THE RATE OF PHOTOSYNTHESIS

Materials/Equipment

Fresh *Elodea*
Sodium hydrogencarbonate solutions of various concentrations e.g. 0.02% - 1%
5 Boiling tubes
Large beaker of water at 25 °C
Funnel
Strong light source
Metre stick
Timer
2 Thermometers
Scissors
Forceps
Test-tube rack



Illuminating pondweed

Procedure

1. Familiarise yourself with all procedures before starting.
2. Fill each boiling tube with a different concentration of sodium hydrogencarbonate, label and place in the water bath. Leave to warm to 25 °C.
3. Obtain a fresh shoot of *Elodea.*
4. Cut the stem at an angle. Remove several leaves from around the cut end of the stem.
5. Switch on the light source.
6. Put the plant into the boiling tube with the lowest concentration of sodium hydrogencarbonate e.g. 0.02%, cut end pointing upwards and stand this boiling tube in the beaker as shown in the diagram.
7. Place this boiling tube at a measured distance from the light source e.g. 15 cm.
8. Allow the plant to adjust for at least 5 minutes and observe bubbles being released from the cut end of the stem.
9. Count and record the number of bubbles released per minute. Repeat twice.
10. Calculate and record the average number of bubbles released per minute.
11. Using the same piece of pondweed repeat steps 7 to 11 with the other concentrations of sodium hydrogencarbonate.
12. A graph should be drawn of the rate of bubble production against sodium hydrogencarbonate concentration. Put the sodium hydrogencarbonate concentration on the horizontal axis.

Note:
During this experiment only one factor (carbon dioxide concentration) should be varied – light intensity and temperature must be kept constant.

To keep the light intensity constant, keep the boiling tube at a constant distance from the light source.

To keep the temperature constant use a water bath at 25 °C.

Result

$NaHCO_3$ concentration	Trial 1 (No. of bubbles/min)	Trial 2 (No. of bubbles/min)	Trial 3 (No. of bubbles/min)	Average (No. of bubbles/min)

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE INFLUENCE OF CARBON DIOXIDE ON THE RATE OF PHOTOSYNTHESIS

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Carefully use the scissors to cut the end of the plant

Place the plant in the boiling tube, cut end pointing upwards

Use the metre stick to measure the distance of the plant from the light source

Use the thermometer

Use the timer

Observation

Observe bubbles being released

After allowing the plant to adjust, observe a steady stream of bubbles

Observe the number of bubbles being released per minute at each of the given concentrations

Recording

Write up the procedure

Record each concentration used

Record the number of bubbles being released per minute at each of the given concentrations

Record the average number of bubbles being released per minute at each of the given concentrations

Draw a graph with labelled axes

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Label as appropriate

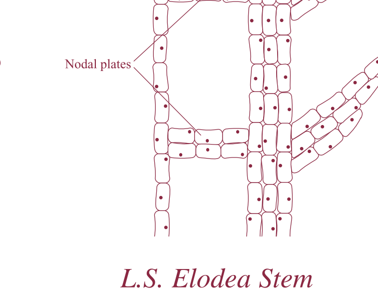
Work as part of a group or team

Clean up after the practical activity

Background information

Elodea spp.

Elodea is a hydrophyte, a plant adapted to living in water. One important adaptation of freshwater aquatic plants is the formation of aerenchyma. This is parenchyma tissue with large intercellular air spaces that help to keep the plant buoyant.



L.S. Elodea Stem

Elodea has a large proportion of the stem occupied by these structured airspaces, which are separated from one another by nodal plates. These 'cross' plates prevent free movement of large bubbles of gas. When the oxygen is produced in the leaves during photosynthesis it diffuses into the air spaces and accumulates there. This is easier than diffusing into the surrounding water.

Oxygen can form up to 33% of this trapped air. Eventually the pressure becomes so great that bubbles of gas are forced out of the cut end of the stem when the plant is actively photosynthesising.

Factors affecting the rate of photosynthesis

There are certain factors which affect the rate of photosynthesis e.g. light intensity, temperature, and carbon dioxide concentration. The maximum rate of photosynthesis will be constrained by a limiting factor – any factor that is in short supply. This factor will prevent the rate of photosynthesis from rising above a certain level, even if the other conditions needed for photosynthesis are improved. It is therefore necessary to control these factors throughout the experiment and to keep them constant so as not to let them affect the integrity of the investigation.

(i) Light intensity

Increasing the light intensity will increase the rate of photosynthesis provided there is sufficient carbon dioxide available and provided the temperature is warm enough to allow the reactions to occur. Light intensity is proportional to the inverse of the square of the distance from the lamp ($1/d^2$) rather than just the distance because it depends on the area of the beam of light falling on the plant. The further the distance from the lamp the greater the area being lit by it.

(ii) Carbon dioxide concentration

Provided the other factors are available in sufficient quantities, increasing the concentration of carbon dioxide will increase the rate of photosynthesis until the plant is photosynthesising at its maximum rate (it has become saturated). If there is too little carbon dioxide, it can become the limiting factor, thus impeding the viability of the investigation. However, as long as the investigation is completed in a short period of time, the amount of carbon dioxide used up by the plant will not be sufficient to cause the carbon dioxide concentration to become the limiting factor.

(iii) Temperature

Enzymes are required for photosynthesis. Therefore, increasing the temperature will increase the rate of photosynthesis, until a point at which the enzyme activity decreases or the enzymes become denatured. Thus the experiment is carried out at a constant optimum temperature for the plant enzymes i.e. 25 °C.

Advance preparation

- Collect *Elodea* and pondwater from a pond or canal or purchase from a 'fish tank' suppliers or garden centre.
- Try out the experiment beforehand to determine
  (i) the distance(s) suited to the light source
  (ii) the concentration of sodium hydrogencarbonate to get a steady (but not too fast) stream of bubbles.
- Make up sodium hydrogencarbonate solutions (if doing the 'carbon dioxide concentration' investigation).
- Set the water bath at 25 °C and check the temperature with a thermometer.

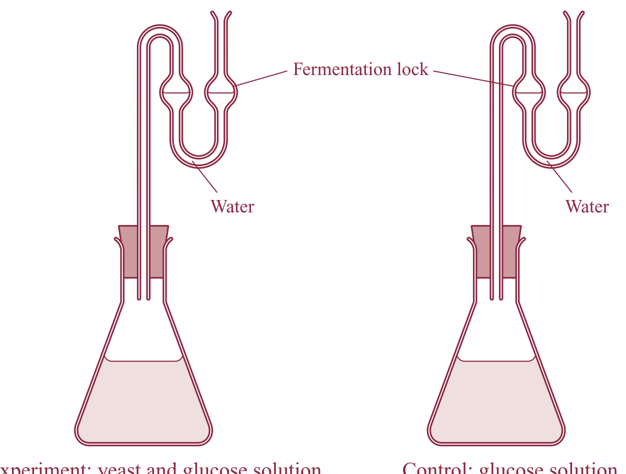
Helpful hints

- Ensure that the *Elodea* is fresh and well illuminated before the experiment. If the plant has to be stored, leave it in an aquarium and bubble air through it.
- Use a very bright light e.g. that from a projector or a daylight bulb (can be purchased from art material suppliers or some garden centres). If you use a Halogen lamp make sure to place an open glass container of water in front of it in order to absorb some of the heat emitted.
- Try out a few different sprigs of *Elodea* to see which one is bubbling best and then use this one for the investigation.
- If the bubbles stop, or do not start, cut the stem again and lightly crush between your fingers. This seems to allow the bubbles out past the nodal plate (see the diagram in background information). If bubbles are too fast, a pinch with the forceps sometimes helps to slow them down.
- Keep the pondweed down at the bottom of the boiling tube. It is easier to count the bubbles travelling through a long column of water or solution. If the pondweed tends to float, it can be weighed down with a paper clip.
- *Cabomba* is an excellent aquatic plant to use in place of *Elodea,* it is available from suppliers of tropical fish.
- *Scenedesmus,* a green alga, may be immobilised and used with hydrogencarbonate indicator and a colorimeter.
- A conical flask can be substituted for the beaker as this will keep the boiling tube steadier, making it easier to move along the bench.
- The experiment seems to work best in May and September as the plant is photosynthesising more actively.
- A solution of 0.2% sodium hydrogencarbonate can be used instead of pondwater. Tapwater can be used if dechlorinated.
- Count the bubbles for 15 seconds and multiply by 4 to get the number of bubbles per minute.
- When moving the *Elodea* from tube to tube, do it gently so as not to damage the plant. If there is a risk of damage, leave the *Elodea* in one tube, pour off the sodium hydrogen-carbonate solution just tested and refill with a solution of a higher concentration.
- When the student is drawing the graph using the formula $1/d^2$, multiply by 1000 to make the figures easier to handle i.e. use $1000/d^2$ as the light intensity.

PREPARE AND SHOW THE PRODUCTION OF ALCOHOL BY YEAST

Materials/Equipment

Yeast
Glucose
Distilled water
Sodium hypochlorite solution (15% w/v)
Potassium iodide solution (10% w/v)
2 Conical flasks (250 cm^3)
Graduated cylinder (250 cm^3)
2 Graduated cylinders (50 cm^3)
2 Beakers (250 cm^3)
2 Beakers (50 cm^3)
2 Boiling tubes
Thermometer
Funnel
Filter paper
Timer
Labels
2 Syringes (5 cm^3)
2 Rubber bungs
2 Fermentation locks
Electronic balance
Weigh boats
Incubator (25 °C – 30 °C)
Water bath (50 °C – 60 °C)



Production of alcohol by yeast

Procedure

1. Familiarise yourself with all procedures before starting.

To produce alcohol using yeast

2. Prepare 500 cm^3 of a 10% w/v glucose solution.
3. Into each of the two conical flasks, add 250 cm^3 of the 10% w/v glucose solution.
4. To one, add 5 g of yeast and swirl. Label this ‘yeast + glucose solution’.
5. The second flask acts as the control (has no yeast). Label as ‘control’.
6. Attach a fermentation lock (half-filled with water) to each flask.
7. Place both flasks in the incubator at 30 °C overnight.

To show the presence of alcohol: Iodoform test for alcohol

8. Remove both flasks from the incubator and filter the contents of each into separate beakers and label as before.
9. Transfer 3 cm^3 of the yeast and glucose filtrate into a test tube and label.
10. Transfer 3 cm^3 of the control filtrate into another test tube and label.
11. To each test tube, add 3 cm^3 of the potassium iodide solution and 5 cm^3 of the sodium hypocholorite solution.
12. Warm gently for 4 – 5 minutes in the water bath.
13. Allow to cool and observe any change(s).
14. Record and compare results.
15. Replicate the investigation or cross reference your results with other groups.

Result

Flask	Original colour of filtrate	Final colour of filtrate	Other change(s)
Yeast and glucose solution			
Control (no yeast)			

Conclusion/Comment

SKILL ATTAINMENT

PREPARE AND SHOW THE PRODUCTION OF ALCOHOL BY YEAST

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the graduated cylinder to measure the volumes of glucose solution
Use the syringe for measurement of small volumes
Use the electronic balance
Attach the fermentation locks to the conical flasks
Filter the suspension
Set the incubator
Set and maintain the water bath
Use the timer

Observation
Observe bubbles of carbon dioxide being liberated
Observe the effect of filtering
Observe colour changes during the iodoform test
Observe the presence/absence of yellow crystals

Recording
Write up the procedure
Record any colour changes during the iodoform test
Record the presence/absence of yellow crystals

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Alcohol production by fermentation is a well established technology which has long been practised throughout the world. Continuous alcohol production with the use of immobilised yeasts, gives rise to more efficient fermentation.

Alcohol is produced by yeast fermentation of the sugars of various plants. Yeasts ferment simple sugars (monosaccharides) into carbon dioxide and ethanol under anaerobic conditions. Yeasts are single-celled fungi. The genus *Saccharomyces* is the one most commonly used due to its efficient alcohol production and tolerance of high alcohol levels. Some yeasts can live until the alcohol concentration reaches 18%.

Iodoform test

Alcohol groups when treated with potassium iodide (KI) and sodium hypochlorite (NaOCl) readily yield iodoform (CHI_3).

There are three stages in this reaction:

a) Oxidation:

aqueous sodium hypochlorite oxidises the potassium iodide to potassium hypoiodite, this then oxidises the alcohol to an aldehyde.

$$C_2H_5OH \rightarrow CH_3CHO$$

ethanol → ethanal

b) Substitution:

potassium hypoiodite then iodinates the aldehyde to tri-iodoethanal (iodal).

$$CH_3CHO \rightarrow CI_3CHO$$

ethanal → iodal

c) Hydrolysis:

aqueous sodium hypochlorite always contains sodium hydroxide, which converts the iodal to tri-iodomethane (iodoform) and sodium methanoate

$$CI_3CHO + NaOH \rightarrow CHI_3 + HCOONa$$

iodal + sodium hydroxide → iodoform + sodium methanoate

Formation of solid iodoform (yellow crystals) is a positive result.
Iodoform is a disinfectant and can be used as an external antiseptic.

Advance preparation

- Set the incubator.
- Set the water bath.
- Check the temperatures of the incubator and water bath with a thermometer.
- Prepare the following solutions: glucose solution (10% w/v), sodium hypochlorite solution (15% w/v), potassium iodide solution (10% w/v).

Helpful hints

- Immobilised yeast may be used instead of dried yeast - this eliminates the need to filter.
- In the iodoform test the sodium hypochlorite used must contain some sodium hydroxide. Use commercial bleach and add sodium hydroxide if necessary.
- Use pure ethanol to observe positive result in the iodoform test.
- Use a cork borer when inserting tubing into bungs.
- Using long necked conical flasks and setting the incubator at 25 °C would help to prevent overflow during fermentation.
- A Bunsen valve can be used instead of a fermentation lock during fermentation.

To construct a Bunsen valve

a) Insert a short piece of glass tubing into a single holed bung.

b) Attach approximately 4 cm of rubber tubing to the glass tubing.

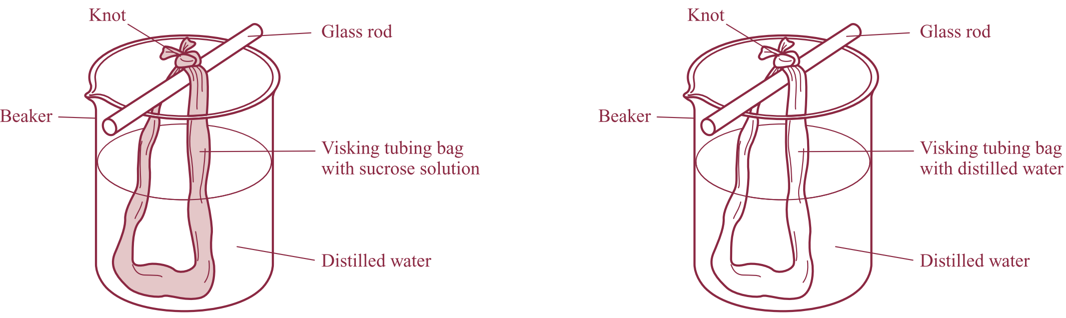
c) Using a scalpel blade, carefully cut a small vertical slit in the rubber tubing. This allows the gas to escape but will prevent air from entering the flask.

d) Use a Hoffman clip to seal the end of the rubber tubing (above the slit).

CONDUCT ANY ACTIVITY TO DEMONSTRATE OSMOSIS

Materials/Equipment

Distilled water	Electronic balance
Sucrose solution (80%)	Scissors
2 Beakers	Ruler
2 Glass rods	Labels
Small funnel	Paper towels
Length of dialysis tubing (80 cm)	



Osmosis in an artificial cell

Procedure

1. Familiarise yourself with all procedures before starting.
2. Soften two 40 cm strips of dialysis tubing by soaking them in water.
3. Tie a knot at one end of each strip.
4. About half-fill one piece of tubing with the sucrose solution and the other with distilled water.
5. Eliminate as much air as possible from the tubes and tie a knot at the open end of each tube. Then attach the two ends of each tube as in the diagram.
6. Wash off any excess sucrose solution from the outside of the sucrose tube and pat dry both tubes with the paper towels.
7. Observe and record the turgidity of each tube.
8. Find the mass of each tube and record.
9. Suspend the tube containing the concentrated sucrose solution, by means of a glass rod, in a beaker of distilled water and label it ‘sucrose solution’.
10. Similarly, place the tube containing the distilled water into a second beaker of distilled water and label it ‘distilled water’. This acts as the control.
11. Allow the tubes to stand for at least 15 minutes.
12. Remove the tubes and dry as before.
13. Observe and record the turgidity of each tube.
14. Again find the mass of each tube and record.
15. Replicate the investigation or cross reference your results with other groups.

Result

Tube contents	Turgidity at start	Turgidity after test period	Mass at start (g)	Mass after test period (g)
Sucrose solution				
Distilled water				

Conclusion/Comment

SKILL ATTAINMENT

CONDUCT ANY ACTIVITY TO DEMONSTRATE OSMOSIS

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Soften the tubing in water
Tie leak-proof knots
Wash off excess sucrose
Dry the tubes
Use the electronic balance
Suspend the tubes from the rods

Observation
Observe the appearance of the tubes at the start
Observe the appearance of the tubes after the test period

Recording
Write up the procedure
Tabulate the results

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Diffusion and osmosis

Molecules and ions in solution can move passively in a particular direction as a result of diffusion. *Diffusion is the movement of molecules or ions from a region of their high concentration to a region of their low concentration down a concentration gradient.* It is possible for the net diffusion of different types of molecules or ions to be in different directions at the same time, each type moving down its own concentration gradient. Thus in the lungs, oxygen diffuses into the blood at the same time as carbon dioxide diffuses out into the alveoli.

Osmosis is a special type of diffusion. Osmosis is *the passage of water molecules from a region of their high concentration to a region of their low concentration through a partially permeable membrane.* **Water** *is* the solvent in all biological systems**.** Unlike active transport, diffusion and osmosis are passive, i.e. do not require the expenditure of energy.

Certain membranes, previously known as semi-permeable membranes, allow the passage of *solvent molecules only* and completely exclude solute molecules or ions. The membranes of living cells, however, allow the passage of certain solute molecules or ions in a selective manner depending on the nature of the membrane. They are therefore best described as **partially permeable** rather than semi-permeable.

Advance preparation

- Make up an 80% w/v solution of sucrose.

Helpful hints

- For best results, use dialysis tubing about 2 cm wide.
- Treacle or golden syrup could be used instead of the 80% sucrose solution.
- If using treacle or golden syrup, heat it in a beaker in a hot water bath to decrease its viscosity. This makes it easier to add to the dialysis tubing bag.
- Syringes could be used to fill the dialysis tubing bags.
- Make sure to wash any sucrose solution off the knots.
- A useful activity might be to measure and record the mass of the tubes at five-minute intervals throughout the activity.

ISOLATE DNA FROM A PLANT TISSUE

Materials/Equipment

Onion
Washing-up liquid (not the concentrated type)
Blender
Distilled water
Sharp knife
Table salt (3 g)
Chopping board
Protease enzyme e.g. trypsin (1%)
Coffee filter paper
Ethanol at freezer temperature
Droppers
Water bath (60 °C)
Spatula
Ice-water bath
Plastic syringe (10 cm^3)
2 Beakers (250 cm^3)
Timer
Boiling tube
Test tube rack
Graduated cylinder (100 cm^3)
Retort stand
Glass rod/wooden skewer/wire loop
Electronic balance
Large funnel
Weigh boat
Glass stirrer
Plastic syringe
Disposable gloves
Thermometer

Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 3 g of table salt to 10 cm^3 of washing-up liquid in the beaker and make up to 100 cm^3 with distilled water.
3. Chop the onion into small pieces.
4. Add the chopped onion to the beaker with the salty washing-up liquid solution and stir.
5. Put the beaker in the water bath at 60 °C for exactly 15 minutes.
6. Cool the mixture by standing the beaker in the ice-water bath for 5 minutes, stirring frequently.
7. Pour the mixture into the blender and blend it for no more than 3 seconds.
8. Carefully filter the mixture into the second beaker.
9. Transfer about 10 cm^3 of this filtrate into the boiling tube.
10. Add 2 – 3 drops of protease to the filtrate and mix gently.
11. Trickle about 10 cm^3 of the ethanol, straight from the freezer, down the side of the boiling tube, to form a layer on top of the filtrate. Leave the tube for a few minutes without disturbing it.
12. Observe any changes that take place at the interface of the alcohol and the filtrate.
13. Using the glass rod, gently draw the DNA out from the alcohol.
14. Record the result.

Result

Conclusion/Comment

SKILL ATTAINMENT

ISOLATE DNA FROM A PLANT TISSUE

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the electronic balance
Set and maintain the water bath at the correct temperature
Use the thermometer
Use the timer
Use the blender for the correct length of time
Filter the mixture into a clean beaker
Transfer the filtrate to the boiling tube
Add the correct amount of protease to the filtrate
Trickle the ethanol down the side of the boiling tube
Draw out the DNA

Observation
Accurately read the temperature of the water bath
Observe two distinct layers of liquid after the addition of the alcohol
Notice a change in viscosity at the interface of the two liquids
Notice cloudy matter appearing at the interface of the two liquids
Observe the appearance of the DNA

Recording
Write up the procedure
Record the result

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

DNA is isolated from cells by taking advantage of the different chemical and physical properties of the molecules and structures within the cell.

Reasons for the main steps in the activity:

Chopping the onion

The physical chopping breaks the cell walls and allows the cytoplasm to leak out.

Adding the washing-up liquid

Breaks down the lipids in the phospholipid bilayer and causes the protein in the membranes to break apart. This results in the release of the nuclear material from the cell.

Adding the salt

Once the cell is destroyed, the ion levels within the cell change. The proteins in the membranes, which have been exposed by the detergent, are now positively charged. These naturally attract the negatively charged phosphate groups in the DNA. This causes a problem in extracting the DNA. The salt is added to minimise the attractive forces between the DNA and the protein by shielding the DNA molecules, causing them to clump together.

Heating the mixture to 60 °C for exactly fifteen minutes

Causes DNases, released from the lysosomes, to be broken down. After fifteen minutes DNA itself will be broken down.

Cooling the mixture

Decreases the rate of the chemical reactions, slowing the action of any remaining enzymes before they destroy the DNA.

Blending

Further destroys cell walls and membranes. Causes DNA to be released. Blending for more than three seconds shears the fragile DNA strands.

Adding protease

Breaks down the proteins associated with DNA.

Filtering

Strains all the large cellular debris out of the mixture. DNA passes through the filter with the liquid.

Using cold ethanol

Ethanol forms a layer on top of the onion filtrate. The alcohol tends to draw the water out of the DNA molecule, making it less dense. It is now found at the interface of the two liquids. DNA is insoluble in freezing cold ethanol but soluble in alcohol at room temperature.

Testing for DNA

Currently, there is no satisfactory method of testing for DNA available for use in second level laboratories.

Advance preparation

- Set up the water bath.
- Place the ethanol in a freezer *at least* 24 hours before carrying out this activity.

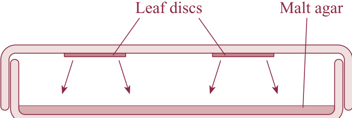
Helpful hints

- Use a medium-sized onion.
- Some alternatives to onions are: tomatoes, peas, peaches, nectarines and kiwi fruits.
- Use a cheap brand of washing-up liquid. However, it is important not to use the bacteriocidal washing-up liquid as this contains an enzyme which causes the breakdown of DNA.
- It is vital to blend **for no more than three seconds.** If the DNA appears fluffy, it was sheared in the extraction process. It should appear as thin threads.
- Since the ethanol must be very cold for this procedure to work, it must be kept in a freezer overnight. Dispense the ethanol into small bottles, one for each student group. Remove the bottles directly from the freezer, just before the students use them.
- Industrial methylated spirits may be used as a cheaper alternative to ethanol.
- If the DNA remains at the interface, place the boiling tube back into the ice bath.
- Other sources of protease include fresh pineapple juice, contact lens protein removal tablets and meat tenderiser.

INVESTIGATE THE GROWTH OF LEAF YEAST USING AGAR PLATES AND CONTROLS

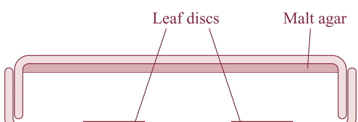
Materials/Equipment

- Fresh leaves (e.g. common ash)
- 2 Sterile malt agar plates
- Petroleum jelly
- Disinfectant
- Thermometer
- Incubator (18 °C – 20 °C)
- Cork borer (approx. 1 cm diameter)
- Chopping board
- Bunsen burner
- Matches/lighter
- Marker/labels
- Forceps
- Paper towels
- Masking tape/parafilm



First 24 hours - leaf discs suspended over agar. Spores drop onto the agar.

Fig. 1



After 24 hours - invert the plates and incubate upside down for 3 days. Yeasts grow on the agar.

Fig. 2

Procedure

1. Familiarise yourself with all procedures before starting.
2. Swab the laboratory bench with disinfectant.
3. Seal one sterile malt agar plate and label as ‘control’. Initial and date.
4. Flame the cork borer and allow to cool.
5. Cut five discs from the leaves using the sterilised cork borer.
6. Take the second plate and place it upside down on the bench. (The malt agar is now in the top half.)
7. Lift the base of this plate (containing the agar) and place it open-side facing down on the bench. This will reduce potential contamination.
8. Flame the forceps to sterilise it and allow to cool.
9. Using the forceps, smear five small amounts of petroleum jelly (well spaced) on the inside of the lid.
10. Clean the forceps and re-flame it.
11. Use the forceps to attach a leaf disc to each of the blobs of petroleum jelly.
12. Replace the agar-containing base of the plate in the lid.
13. Clean and re-flame the forceps.
14. Seal the plate and label as ‘experiment’. Initial and date.
15. Re-swab the bench with disinfectant.
16. Invert this plate so that the leaf discs are uppermost and over the malt agar (Fig. 1). Leave the plate like this for approximately 24 hours so that spores can drop onto the agar from the leaf discs.
17. After 24 hours invert this plate (Fig. 2).
18. Incubate both plates (experiment and control) upside down, at 18 °C – 20 °C, for 3 days.
19. Compare the experiment plate with the control plate. Record the result. Leaf yeasts *(Sporobolomyces roseus)* will grow as pink glistening colonies.
20. Replicate the investigation or cross reference your results with other groups.

Result

Agar plate	Appearance of colonies
Control	
Experiment	

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE GROWTH OF LEAF YEAST USING AGAR PLATES AND CONTROLS

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Label the plates

Swab the bench

Use the cork borer

Place the base of the experiment plate upside down on the bench

Sterilise the forceps and the cork borer

Smear the petroleum jelly on the lid

Seal the plates

Invert the experiment plate for approximately 24 hours

Re-invert this plate for 3 days

Use the thermometer

Incubate the plates

Observation

Observe the appearance of the colonies if present

Observe any differences between the experiment and the control plates

Recording

Write up the procedure

Tabulate the results

Record the appearance of the experiment plate

Record the appearance of the control plate

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Label as appropriate

Work as part of a group or team

Use aseptic technique throughout

Clean up after the practical activity

Background information

Sporobolomyces roseus

This species is the most common of the leaf yeasts. It grows on both the upper and lower surfaces of plant leaves. Its presence is influenced by factors such as leaf exudates, light intensity and temperature. The age of the leaf and its position on the tree also play a part in determining the presence and abundance of the yeast. Furthermore, the type and amount of wax, the number and position of stomata on the leaf surface and the presence of epicuticular hair will also play a role. This species grows as pink colonies and its abundance has been used to monitor air quality. It is known as a 'mirror' yeast because it can forcibly discharge its spores which then grow on the agar forming a mirror image. It is thought that the leaf yeasts overwinter on grasses, especially on ryegrass and meadow fescue. These then provide the source of the initial populations in the Spring/Summer.

S. roseus has evolved efficient air uptake mechanisms and as a result, where the air contains poisonous pollutants, particularly sulfur dioxide, the number of leaf yeast colonies per disc is greatly reduced. Since leaf yeast cells have a rapid life cycle, changes in the leaf populations can be used to monitor short-term changes in air quality. It is not possible to count yeast cells directly on the leaf surface but an indirect measure of population density can be obtained by measuring the number of colonies that can be isolated on agar plates from a given area of the leaf. This would be difficult to do with most leaf-surface fungi but the fact that *S. roseus* shoots basidiospores into the air where they can be intercepted, means that relatively simple techniques can be used. The number of colonies will reflect the health of the yeast populations and also the quality of the air.
Large-scale comparative studies, carried out by school children in several European countries, have established that the lowest numbers of leaf yeast colonies correlate well with higher levels of sulfur dioxide pollution. Eanna Ní Lamhna of An Foras Forbartha co-ordinated the Irish studies undertaken from 1982 to 1986. Secondary school students from the mid west, the south coast, Cork city and the east coast used lichens, leaf yeasts (from the underside of ash tree leaves), and the acidity of the rainfall to monitor the air quality. While the lichens reflected the air quality in the few years prior to the study, the results for the leaf yeast study depended on the air quality in the weeks prior to the investigation.

Common ash – *Fraxinus excelsior*

The common ash is a large familiar tree with a long silvery stem. The 20 cm – 30 cm leaves are pinnate with 9 – 13 toothed oval leaflets arranged in pairs with a single one at the tip.

In April, the flowers appear before the leaves – the flowers are green in colour, are small and inconspicuous having neither a calyx nor a corolla.

In Autumn, the leaves turn a muddy brown or yellow colour and are shed in October. The ash tree is hardy enough to survive almost anywhere. In Winter, clusters of black velvety buds will help to identify the tree.

Advance preparation

- Collect fresh leaves.
- Set the incubator and check the temperature with a thermometer.
- Prepare/purchase malt agar plates.

Helpful hints

- Leaves from common ash, lilac, sycamore, red alder or hawthorn are generally suitable for use in this investigation. Ash leaves are particularly good as they are widely available, easy to identify and have good yeast populations. Ash trees can be found in parks and roadsides and also in hedges used to mark field boundaries. Clover leaves and cherry laurel leaves release cyanide thus inhibiting the growth of *S. roseus*.
- The investigation is best conducted in September when the leaves have been growing for a few months and the yeasts have had time to colonise and grow.
- Take leaves from the base of long shoots as these are the older leaves and have been on the tree since Spring. New young leaves from the tips of the shoots have fewer yeasts on them.
- After collecting the leaves keep them in a rigid container e.g. a plastic box, to prevent the leaves being crushed and the leaf yeasts from being rubbed off.
- After heavy rainfall or high wind, wait a few days to collect leaves as rain or wind may remove some of the leaf yeasts.
- Process the samples the same day that you collect them.
- Take care not to get petroleum jelly on the side of the leaf discs from which the spores are to be collected.
- Malt agar plates can be made up if they are not readily available.
- Variations on the investigation could be carried out as project work e.g. compare the leaf yeast populations from the upper and the lower leaf surfaces or from trees growing in different areas.

PREPARE AND EXAMINE MICROSCOPICALLY A TRANSVERSE SECTION OF A DICOTYLEDONOUS STEM

Materials/Equipment

Dicotyledonous stems
Microscope slides
Cover slips
Beaker for used slides
Petri dish
Microscope
Backed blade
Small paintbrush
Dropper
Labels
Filter paper/absorbent paper
Mounted needle

Procedure

1. Familiarise yourself with all procedures before starting.
2. Cut a short length of wet stem using the blade. Cut across at right angles to the stem, away from the body, to get a very thin transverse section.
3. Repeat several times, placing each transverse section in the petri dish of water.
4. With the paintbrush, remove the thinnest sections from the water and place them on a microscope slide in a drop of water. Blot off excess water.
5. Add a coverslip and label the slide.
6. Examine under the microscope.
7. Draw labelled diagrams of what is seen.

Result

Conclusion/Comment

SKILL ATTAINMENT

PREPARE AND EXAMINE MICROSCOPICALLY A TRANSVERSE SECTION OF A DICOTYLEDONOUS STEM

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the blade to cut the section
Transfer sections using the paintbrush
Use the dropper
Manipulate the cover slip
Use the microscope

Observation
Observe the appearance of the sections under different magnifications
Observe any differences between the sections

Recording
Write up the procedure
Draw and label a tissue plan

Interpretation
Draw reasonable conclusions from your observations and results

Applications
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work as part of a group or team
Work in an organised and efficient manner
Label as appropriate
Clean up after the practical activity

Background information

To enhance microscope images staining of samples may be carried out. This is not specifically mentioned in the syllabus in relation to this activity and, therefore, is not prescribed. The use of appropriate stains can highlight particular tissues or structures.

Phloroglucinol stain works very well with transverse sections of dicot. stems. It stains lignin a red colour. It is taken up very well by sclerenchyma fibres, by secondary xylem and, to a lesser extent by primary xylem.

The procedure for staining with phloroglucinol is as follows:

- Wearing disposable gloves, use a dropper to add phloroglucinol to cover each section and leave for two minutes.
- Soak up the excess phloroglucinol with filter paper.
- Add a drop of HCl to cover each section. Leave for one minute. Soak up the HCl and add a drop of water.

Advance preparation

- Select and gather a range of stems e.g. buttercup, sunflower, winter rose, ivy, busy lizzy, geranium, others as available.
- If staining sections, prepare the following solutions: phloroglucinol solution, HCl 20% w/v solution.

Helpful hints

- A microtome, if available, may be used to cut thin sections of stem material. Inexpensive hand held microtomes are available from laboratory suppliers. Instructions regarding the type of wax to use should be followed.
- A simple microtome may be made from a nut and bolt (Fig. 1). Almost remove the bolt from the nut and fill the hole with hot wax. Place the sample in the wax and allow to cool. Then screw the bolt into the nut, raising the wax and allowing a thin section to be cut.

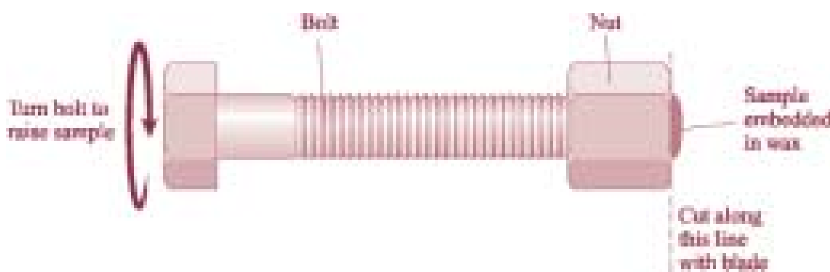


Fig. 1 A

simple microtome

- A carrot may be used to support a stem while cutting. Slit along the diameter at one end as shown, cut a groove and insert the stem. Bring the carrot to a point like a pencil and cut (Fig. 2). Small polystyrene blocks, potatoes or elder pith may be used in this way also.

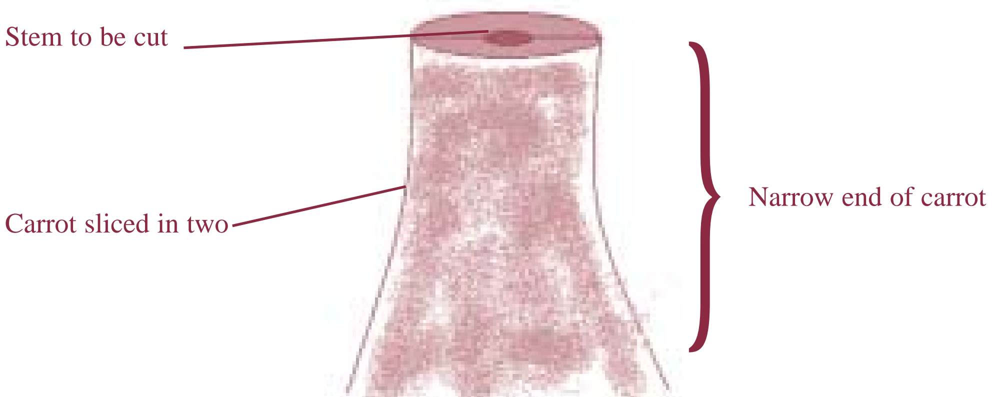


Fig. 2 Using a carrot to help cut a section

- Fresh green stems, with no secondary thickening, are easiest to cut. The various tissues are more obvious in such young specimens – dermal, ground and vascular tissues should be identified.
- Keep the stems wet, as turgid material is easier to cut thinly. Cutting under water can also help with difficult specimens.
- All cuts should be made at an internode.
- Some samples will turn out to be slightly wedge-shaped but may still be useful as they will be very thin near one edge.
- A seeker may be used instead of a paintbrush to transfer the sections from the petri dish to the microscope slide.

DISSECT, DISPLAY AND IDENTIFY AN OX'S OR A SHEEP'S HEART

Materials/Equipment

Ox's/sheep's heart
Dissecting board/white tray
Scalpel
Seeker
Fine scissors
Forceps
Flag labels
Disposable gloves
Paper towels

Fig. 1 Identifying ventral side

Procedure

1. Familiarise yourself with all procedures before starting.
2. Wash the heart with cold water. Drain and dry it with paper towels.
3. Place the heart on the dissecting board/white tray so that the front (ventral) side is facing up. The front of the heart is recognised by feeling the sidewalls. The left side will feel much firmer than the right side. To further identify the front of the heart observe a groove that extends from the right side of the broad end of the heart diagonally downward. This groove is the location of a coronary vessel. (Fig.1)
4. Locate the following chambers of the heart:
   left atrium – upper chamber on your right
   left ventricle – lower chamber on your right
   right atrium – upper chamber on your left
   right ventricle – lower chamber on your left (Fig. 2).
5. Note the main blood vessels located at the broad end of the heart.
6. Draw a labelled sketch of the external structure of the heart.

Fig. 2 Chambers of the heart

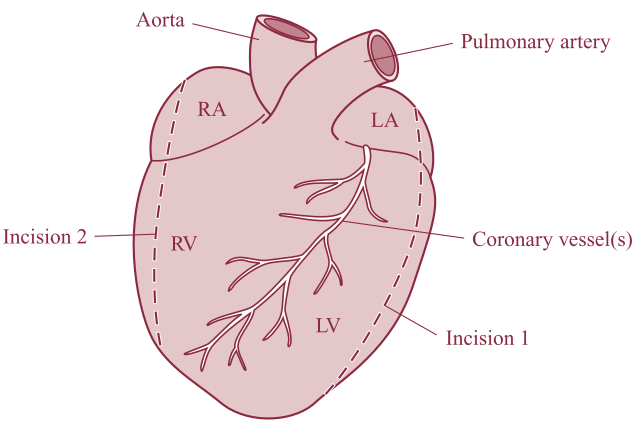


Fig. 3 Points of incision

7. Carefully make a shallow cut in the left ventricle and left atrium following the lines in Fig. 3.
8. Using your fingers, push open the heart at the cut to examine the internal structure. If there is blood inside the chambers, rinse out the heart.
9. Observe the different sizes of the chambers.
10. Locate the bicuspid valve between the left atrium and left ventricle. This valve consists of two flaps.
11. Insert the forceps under the chordae tendinae and notice that they extend from the valve to the papillary muscles.
12. Repeat steps 8 to 10 for the other side of the heart.
13. Note the difference in thickness between the walls of the left and right ventricles.
14. Locate the tricuspid valve between the right atrium and the right ventricle. This valve consists of three flaps.
15. Find the septum, a thick muscular wall, which separates the right and left ventricles.
16. Insert the seeker (or your finger) through the arteries and veins in order to identify them.
17. Using the scalpel cut open the aorta and observe the semi-lunar valve. Note the three half-moon shaped flaps of this valve.
18. Find two small openings at the base of the aorta just above the semi-lunar valve. These lead into the coronary arteries. Insert the seeker into a coronary artery to trace its pathway.
19. Flag label the parts identified and draw a labelled diagram of the internal structure of the heart.
20. Wash and sterilise the dissecting instruments after use.

Result

Chamber	Size – small/large	Wall – thin/thick
Left atrium		
Right atrium		
Left ventricle		
Right ventricle		

Valve type	Number of flaps
Bicuspid	
Tricuspid	
Semi-lunar	

Conclusion/Comment

SKILL ATTAINMENT

DISSECT, DISPLAY AND IDENTIFY AN OX'S OR A SHEEP'S HEART

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Handle the heart with respect
Use the forceps
Use the scissors
Use the scalpel
Explore using the seeker
Flag label the parts of the heart

Observation
Appreciate the significance of washing the heart
Observe the thickness of the left and right sides of the heart
Locate the ventral side of the heart
Identify the coronary groove
Observe the four heart chambers
Observe the position of the main blood vessels
Compare the different chamber sizes
Distinguish between the left and right ventricles
Identify the septum
Observe the bicuspid valve
Locate the chordae tendinae and papillary muscles
Observe the tricuspid valve
Note the positions of the blood vessels going to and coming from the heart
Identify the semi-lunar valve
Locate the openings of the coronary arteries
Examine the parts of the heart

Recording
Write up the procedure
Tabulate results
Draw labelled diagrams

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Heart chambers

All mammals have a four-chambered heart and two-circuit circulatory system. Both birds and mammals have hearts with two atria and two ventricles. Birds and mammals descended from different reptilian ancestors and their four-chambered hearts evolved independently – an example of convergent evolution. Amphibians and reptiles do not have completely separate atria and ventricles. In the four-chambered system the left side of the heart pumps oxygenated blood while the right side pumps deoxygenated blood. Because of the two-circuit circulatory system and no mixing of the blood, the delivery of oxygen to all cells is enhanced.

Phylum Chordata - Class	Number of chambers
Fish	2
Amphibians	3
Reptiles	3-4 (iguanas have 3, crocodiles have 4)
Birds	4
Mammals	4

Hole in the heart

During foetal development the lungs do not function as the placenta oxygenates the blood. Blood is diverted away from the lungs through two short circuits:

The *ductus arteriosus* is a short vessel between the pulmonary artery and the aorta.

The *foramen ovale* is a hole between the right atrium and the left atrium.

Both of these pathways usually close within the first few days after birth and seal permanently within the first weeks.

If the *foramen ovale* fails to close the infant is said to have "a hole in the heart".

Volumes of blood pumped

Every day the heart pumps 7,600 litres of blood, beating about 100,000 times.

The heart beats more than 2000 million times during the average human life span, and in that time will pump 500 million litres of blood. Even during sleep, the fist-sized heart of an adult pumps about 340 litres an hour – enough to fill an average car's petrol tank every seven minutes. It generates enough muscle power every day to lift an average-sized car about 15 m (50 ft)!

Coronary vessels

The coronary arteries are two blood vessels that branch off from the beginning of the aorta. The right coronary artery supplies oxygenated blood to the walls of the ventricles and the right atrium. The left coronary artery supplies oxygenated blood to the walls of the ventricles and left atrium. They both have two major branches. The coronary arteries branch into capillaries that course throughout the heart walls and supply the heart muscle with oxygenated blood.

The coronary veins return blood from the heart muscle, but instead of emptying it into another larger vein, they empty directly into the right atrium.

Coronary bypass

This is a procedure that re-routes blood circulation from blocked coronary arteries.
Obstruction of these arteries results in the muscles of the heart not receiving enough blood. This condition is called *angina pectoris.*
Veins from the leg and/or arteries behind the breastbone are used as replacement tubes to bypass the blocked coronary arteries.

Moderator band

The moderator band *(septomarginal trabecula)* is found in sheep hearts and is formed from papillary muscles. It is a muscular band that runs from the anterior papillary muscle to the interventricular septum. Its function is to prevent over dilation/expansion of the right ventricle.

Advance preparation

- Order hearts with vessels attached.

Helpful hints

- To locate the blood vessels before cutting:
  - (a) Insert a rubber tube, attached to a tap, into part of the vena cava and gently turn on the tap. The water comes out through the pulmonary artery.
  - (b) Repeat this for the pulmonary vein. The water flows out of the aorta.
- The sino-atrial node, atrioventricular node and Purkinje fibres are not seen in the dissection.
- To show the pathway of blood through a coronary artery, dye may be injected using a plastic dropper.
- As an alternative to injecting dye, air may be pumped in by using the dropper to make the arteries pulsate.
- The coronary artery at the front of the heart shows the dye better. Leave one heart aside for dye/air injection. This may be demonstrated at the start of the activity as sometimes the arteries are severed during the dissection.
- To make flag labels use long pins and paper. Write with a pencil, as ink tends to run.

INVESTIGATE THE EFFECT OF EXERCISE ON THE PULSE RATE OF A HUMAN

Do not attempt this activity if you have a health problem or if you are recovering from an illness

Materials/Equipment

Pulse monitor (optional)
Timer

Procedure

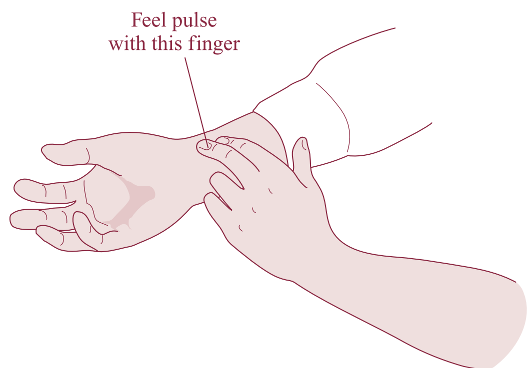
1. Familiarise yourself with all procedures before starting.
2. Sit down comfortably on a chair. Take 5 minutes to settle. Locate your pulse. If, at this point, you are using a pulse monitor, follow the manufacturer's instructions.
3. Count the number of pulses per minute and record.
4. Repeat twice and calculate the average number of pulses per minute and record. This is called the resting heart rate.
5. Stand up. Immediately measure your pulse rate and record.
6. Walk gently for 5 minutes. Immediately measure the pulse rate and record.
7. Walk briskly for 5 minutes. Immediately measure the pulse rate and record.
8. Run for 5 minutes. Immediately measure the pulse rate and record.
9. Compare the pulse rates after the different levels of exercise.
10. Replicate the investigation or cross reference your results with other groups.
11. A bar chart of the results should be drawn.

Result

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

Activity	Standing	Gentle walking	Brisk walking	Running
Pulse rate (bpm)				

Conclusion/Comment



To locate and measure your pulse

The most common place for you to feel a pulse is on your wrist. The pulse on your wrist is called a radial pulse because you measure the pulse near a bone called the radius. You can feel your radial pulse on either wrist, about an inch below your thumb.

If you do not use a pulse monitor, it is usually not possible to measure your pulse rate accurately while participating in most activities. However, an estimate of your exercise pulse rate can be obtained if your pulse rate is measured immediately after exercising. If you do not start counting within 10 seconds after stopping the exercise, the result is likely to be inaccurate.

With the palm of one hand facing upwards, hold the index and middle fingers of the other hand together and press lightly on the pulse site as in the diagram. You should feel an indented area. You may need to change the position of your fingers in order to feel your pulse really well.

Count the number of pulses in 10 seconds and multiply this value by 6 to convert it to beats/min. Do not count for a longer time because your heart rate begins to slow down as soon as you stop exercising. The fitter you are, the quicker your heart rate will decrease after exercise.

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF EXERCISE ON THE PULSE RATE OF A HUMAN

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the timer
Attach the pulse monitor (if in use) to the body

Observation
Find a pulse
Observe changes in the pulse rate
Observe the effect of exercise on the pulse rate

Recording
Write up the procedure
Record the average resting pulse rate
Record the pulse rate after standing
Record the pulse rate after gentle walking
Record the pulse rate after brisk walking
Record the pulse rate after running
Draw a bar chart

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Work as part of a group or team
Clean up after the practical activity

INVESTIGATE THE EFFECT OF EXERCISE ON THE BREATHING RATE OF A HUMAN

Do not attempt this activity if you have a health problem or if you are recovering from an illness

Materials/Equipment

Timer

Procedure

1. Familiarise yourself with all procedures before starting.
2. Sit down comfortably on a chair. Take 5 minutes to settle.
3. Count the number of breaths per minute and record.
4. Repeat step 4 twice and calculate the average.
   This is the resting breathing rate.
5. Stand up. Immediately measure the breathing rate and record.
6. Walk gently for 5 minutes. Immediately measure the breathing rate and record.
7. Walk briskly for 5 minutes. Immediately measure the breathing rate and record.
8. Run for 5 minutes. Immediately measure the breathing rate and record.
9. Compare the breathing rates after the different levels of exercise.
10. A bar chart of the results should be drawn.

Result

Before exercise	Trial 1	Trial 2	Trial 3	Total	Average
Resting breathing rate (breaths/min)					

Activity	Standing	Gentle walking	Brisk walking	Running
Breathing rate (breaths/min)				

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF EXERCISE ON THE BREATHING RATE OF A HUMAN

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Use the timer

Observation

Observe changes in breathing

Observe the effect of exercise on the breathing rate

Recording

Write up the procedure

Record the average resting breathing rate

Record the breathing rate after standing

Record the breathing rate after gentle walking

Record the breathing rate after brisk walking

Record the breathing rate after running

Draw a bar chart

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Work as part of a group or team

Clean up after the practical activity

Background information

It is important to remember that your heart rate varies depending on a number of different factors such as age, sex and exercise. It also depends on when it is measured and what you were doing immediately before measuring it. Allowing for this, the heart rates listed are considered to be average. Adults have a heart rate of 60 – 90 bpm (beats per minute) while resting. In the first year of life, the average heart rate is 120 – 160 bpm, and by the age of l2, it has usually fallen to 70 – 80 bpm. A child's heart rate continues to fall as he or she reaches adulthood.

Vigorous exercise accelerates heart rate in two ways:

(i) Increased carbon dioxide production, due to respiration in muscle cells, causes a decrease in blood pH. This change in pH is detected by chemoreceptors in the walls of various blood vessels such as the aorta, carotid artery and vena cava. Impulses are sent from these receptors to the cardiovascular centre of the medulla oblongata, which also possesses receptors. Impulses are sent from the medulla oblongata, through the sympathetic nervous system, to the sino-atrial node (pacemaker) where the production of noradrenaline by the accelerator nerve brings about an increase in heart rate.

(ii) As muscular activity increases, more blood is driven back to the right atrium. The atrium becomes distended with blood, thus stimulating stretch receptors in its wall. The stretching of the heart wall makes the heart pump more blood into the circulation.

The fastest heart rate that you can reach is known as the **maximal heart rate.** It is highest in childhood and gets lower with age. The maximal heart rate of a 20 year old when exercising is around 200 bpm, whereas a 60 year old has a maximal heart rate of around 160 bpm. To work out your maximal heart rate you need to do a simple calculation. You subtract your age in years from 220 e.g. 220 - 20 = 200 bpm. During exercise, your heart rate should reach no more than 60 – 75% of the maximal heart rate that you have calculated. To ease yourself into and out of exercise, it is best to have substantial warm up and cool down sessions.

A trained athlete's heart can pump more blood than average with each beat, therefore, his or her heart rate is slower. Likewise, an athlete's recovery time is shorter.
Similar to the heart, the lungs have two ways to increase oxygen intake in response to a changing demand during exercise. One is to breathe more quickly (respiratory rate) and the other is to breathe more deeply (volume).

Advance preparation

- Check to see if any student should avoid this activity.

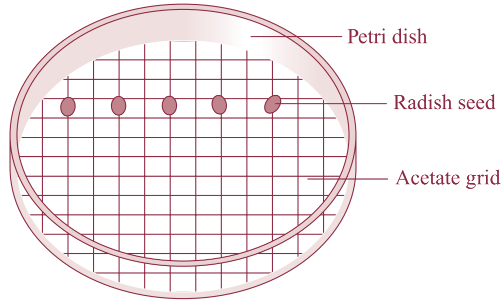
Helpful hints

- It is not essential to undergo each level of exercise for this activity e.g. a student may stop at brisk walking.
- The pulse can be felt at various points on the body where the arteries are just under the skin, such as the temples, neck, crook of the elbow, wrist, groin, back of the knee.
- The thumb should not be used when measuring the pulse. The thumb has its own pulse and may confuse your count.
- Do not press too hard when feeling for the pulse or you may block it.
- Heart recovery time can also be determined while doing this activity. After running, the pulse is measured and recorded every minute until it returns to resting heart rate. The time taken to return to resting heart rate is known as the heart recovery time.
- To measure breathing rate: breathing in and out once is counted as one breath, so just count the out breaths. Do keep in mind that it is difficult to accurately measure the effect of exercise on breathing as there is a certain degree of voluntary control involved.

TO INVESTIGATE THE EFFECT OF IAA GROWTH REGULATOR ON PLANT TISSUE

Materials/Equipment

Radish seeds
IAA solution (0.01% w/v)
Distilled water
2 Syringes (10 cm^3)
8 Graduated droppers
8 Small bottles
Thermometer
Beaker
8 Petri dishes
8 Circular acetate grids
8 Filter papers
Absorbent cotton wool
Disposable gloves
Adhesive tape
Incubator (25 °C)



Positioning of radish seeds

Procedure

1. Familiarise yourself with all procedures before starting.
2. Label the petri dishes as follows: 10^2 ppm, 10 ppm, 1 ppm, 10^{-1} ppm, 10^{-2} ppm, 10^{-3} ppm, 10^{-4} ppm, distilled water (control).
3. Label the bottles in the same way.
4. Using a syringe, add 10 cm^3 of the IAA solution to the first bottle (0.01% w/v or 10^2 ppm).
5. Using the other syringe add 9 cm^3 of distilled water to each of the next seven bottles.
6. Using a dropper, remove 1 cm^3 of the IAA solution from the first bottle and add it to the second bottle. Place the cap on the second bottle and mix.
7. Using a different dropper, remove 1 cm^3 of solution from the second bottle and add it to the third bottle. Place the cap on the third bottle and mix.
8. Using a different dropper each time, repeat this serial dilution procedure for the fourth, fifth, sixth and seventh bottles.
9. Discard 1 cm^3 of solution from the seventh bottle. Each bottle now contains 9 cm^3 of solution.
10. Fit a circular acetate grid inside the lid of each dish.
11. Place five radish seeds along a grid line in each dish as shown.
12. Place a filter paper on top of the seeds in each dish.
13. Using the appropriate droppers, add 2 cm^3 of each solution to its matching dish. Use the dropper bulb to press gently on the damp filter paper, to reduce the trapped air.
14. Spread a piece of cotton wool, about 0.5 cm thick and the approximate area of the dish, on top of the filter paper in each dish to absorb the excess solution.
15. Add the remaining 8 cm^3 of each solution to the cotton wool in the appropriate dish. Leave for a few minutes, until the cotton wool absorbs all the solution.
16. Put the base of each dish in place and secure with a small piece of adhesive tape on either side.
17. Stand the dishes vertically on their edge, to ensure the roots grow down. Leave in the incubator for 2 to 3 days.

18. Measure the length of the roots and shoots of the seedlings in each dish by using the acetate grids and record.
19. Calculate the total length and the average length of the roots and shoots in each dish and record.
20. Estimate the percentage stimulation or inhibition of root and shoot growth in each dish using the following formula:

Percentage stimulation/inhibition $= \dfrac{\text{(Average length – average length of control)}}{\text{Average length of control}} \times 100$

21. A graph should be drawn of percentage stimulation and inhibition of root and shoot growth against IAA concentration. Put IAA concentration on the horizontal axis.
22. Replicate the investigation or cross reference your results with other groups.

Result

Concentration of IAA (ppm)	**Length of roots (mm)**					**Total length (mm)**	**Average length (mm)**	**Percentage stimulation or inhibition**
	Seed 1	Seed 2	Seed 3	Seed 4	Seed 5			
0								
10^{-4}								
10^{-3}								
10^{-2}								
10^{-1}								
1								
10								
10^{2}								

Concentration of IAA (ppm)	**Length of shoots (mm)**					**Total length (mm)**	**Average length (mm)**	**Percentage stimulation or inhibition**
	Seed 1	Seed 2	Seed 3	Seed 4	Seed 5			
0								
10^{-4}								
10^{-3}								
10^{-2}								
10^{-1}								
1								
10								
10^{2}								

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF IAA GROWTH REGULATOR ON PLANT TISSUE

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the syringes
Use the droppers
Carry out a serial dilution
Place the seeds on the acetate grids in the dishes
Reduce the trapped air around the seeds
Spread the cotton wool to absorb the excess solution
Put the bases of the dishes in place
Secure the dishes with adhesive tape
Stand the dishes vertically
Use the thermometer

Observation
Observe the growth of the roots and the shoots
Note the differences in the length of the roots and the shoots in the different solutions

Recording
Write up the procedure
Record the length of the roots and the shoots
Calculate the average length of the roots and the shoots in each dish
Calculate the percentage stimulation or inhibition by each solution
Tabulate the results
Draw a graph with labelled axes

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organized and efficient manner
Work as part of a group or team
Label as appropriate
Clean up after the practical activity

Background information

In 1934 the important plant growth regulator, auxin, was identified as the compound indole-3-acetic acid or IAA. It had been recognised by early workers in the field that plant organs are relatively poor sources of this substance. It was found to be abundant, however, in malt extract made from germinating grains, in cultures of the bread mould *Rhizopus* and in human urine. In 1931, 40 milligrams of a substance called auxin A was isolated from 150 litres of human urine.

Other chemicals with similar structures and activity were soon isolated. Similar substances have also been synthesised, giving a whole class of plant growth substances called auxins (from the Greek *auxein,* to increase). Auxins are usually associated with cell enlargement and differentiation. During cell extension, the rigid cellulose framework of the cell wall must be loosened. ‘Wall loosening’ is induced by acid conditions and by IAA and other auxins. It seems that, in the presence of IAA, hydrogen ion secretion out of the cells and into the cell walls is stimulated. This causes a lowering of the pH outside the cell and hence wall loosening, possibly by an enzyme with a low pH optimum that breaks bonds in cell wall polysaccharides, thus allowing the walls to stretch more easily. Extension then occurs by a combination of osmotic swelling, as water enters the cell, and by the laying down of new cell wall material.

More recent evidence suggests that acidification of the walls may not be the first effect of IAA. Researchers are investigating the possibility that IAA binds to receptors on the surface membranes of epidermal cells and brings about changes in gene activity that result in the production of new enzymes or other proteins concerned with growth. For instance, in tissues where IAA is present, the activity of certain genes is increased leading to the production of enzymes which act to loosen the cellulose fibres in cell walls.

IAA is largely produced in the apical meristems of roots and shoots. Different concentrations of IAA have different effects on the roots and shoots of a plant. Very low concentrations of IAA stimulate root growth but have no effect on the shoot, whereas higher concentrations of IAA stimulate shoot growth but inhibit root growth. Very high concentrations of IAA inhibit both root and shoot growth.

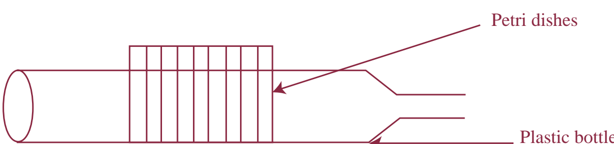
For most of the twentieth century it was thought that IAA was synthesised only from the amino acid tryptophan. In 1991, a new and tryptophan-independent mechanism of IAA synthesis was discovered.

Advance preparation

- Make the acetate grids.
- Make up IAA solution (0.01 % w/v).
- Set the incubator and check the temperature with a thermometer.

Helpful hints

- Indole acetic acid is insoluble in water. It is necessary to dissolve it in alcohol (see Section 5 on Stock solutions).
- Make acetate grids by photocopying graph paper onto two acetate sheets. Using a petri dish draw eight circles on the acetates and cut out.
- If acetate grids are not available students may use a ruler to measure the length of roots and shoots.
- Dishes may be left on a horizontal surface but it may be more difficult to measure the length of the roots and shoots than when they are placed vertically.
- All the dishes containing the same concentration of IAA from the class may be supported in separate one-litre plastic bottles as shown.



- If seeds slip from their original position it is still possible to measure the length of the roots and shoots.
- Seeds may not be available all the year round. March and April are good months in which to buy them.
- Small bottles, such as specimen bottles or culture bottles may be used to make the serial dilutions.

INVESTIGATE THE EFFECT OF WATER, OXYGEN, AND TEMPERATURE ON GERMINATION

Materials/Equipment

Seeds e.g. radish
Distilled water
Gas generating kit sachets
Anaerobic jar
4 Petri dishes
2 Thermometers
Incubator (25 °C)
Fridge (≤ 4 °C)
Absorbent cotton wool
Labels

Procedure

1. Familiarise yourself with all procedures before starting.
2. Set up the four petri dishes with a wad of absorbent cotton wool in each.
3. Label the dishes A, B, C, D.
4. In dish A, leave the cotton wool dry - seeds lacking water.
5. Wet the cotton wool in each of the other dishes.
6. Place 10 seeds in each dish.
7. Place dish B in the fridge - seeds lacking a suitable temperature.
8. Place dish C in the anaerobic jar, activate and seal - seeds lacking oxygen.
9. Place dishes A, C (in the anaerobic jar) and D, in the incubator at 25 °C.
10. Dish D has seeds with water, oxygen and a suitable temperature.
11. Check the dishes daily for 2 – 3 days.
12. Record the results.
13. Replicate the investigation or cross reference your results with other groups.

Result

Conclusion/Comment

Dish	Germination
A – with oxygen and suitable temperature (no water)	
B – with water and oxygen (unsuitable temperature)	
C – with water and suitable temperature (no oxygen)	
D – with water, oxygen, and a suitable temperature	

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF WATER, OXYGEN, AND TEMPERATURE ON GERMINATION

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Prepare petri dishes for seeds
Add seeds, suitably separated
Set the incubator

Observation
Observe the appearance of the seeds at the start of the activity
Check the seeds for germination
Observe the effect of suitable/unsuitable conditions

Recording
Write up the procedure
Record the development of the seeds

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

To provide anaerobic conditions, an anaerobic kit may be used. This kit consists of an airtight jar and an oxygen-removing system. When used as directed, the oxygen is rapidly absorbed and an indicator may be used to indicate when anaerobic conditions are reached.

Germination is the onset of growth of the embryo, usually after a period of dormancy. Three environmental factors, *water, a suitable temperature and oxygen,* are required for the germination of all seeds.

Water

Water is required to activate the biochemical reactions associated with germination, because these take place in aqueous solution. Water plays a part in the transport of substances into and within the seed e.g. the food reserves, hormones and oxygen. Water is also an important reagent at this stage in the hydrolysis (digestion) of food stores.

The initial uptake of water by a seed is by a process called imbibition. It takes place through the micropyle and testa and is purely a physical process caused by the adsorption of water by colloidal substances within the seed. The consequent swelling of the contents of the seed causes the testa to burst. Water subsequently moves from cell to cell by osmosis.

Some seeds have such a hard testa or some covering structure that prevents water uptake that they need to be scarified to physically break the testa to allow water uptake.

Temperature

Germination of seeds normally occurs within the range 5 °C – 40 °C and is related to the normal environment of the plant concerned. Temperature influences the rate of enzyme-controlled reactions.

It is the temperature of the soil (not the air temperature) that controls seed germination in nature. Seeds have a minimum temperature at which they will germinate but until the soil temperature increases to the optimum temperature range for that particular seed the rate of germination will be much reduced.

Oxygen

Oxygen is required for aerobic respiration, releasing energy for the growth of the embryo. Soils in which seeds are germinating need to be well aerated and should not be too compacted or waterlogged.

Advance preparation

- Set the incubator.
- Set the fridge.
- Check the temperatures of the incubator and the fridge with a thermometer.

Helpful hints

- Radish seeds are recommended, as they usually germinate after 24 hours in ideal conditions.
- Some seeds are not easily available all year round. March/April is a good time to buy them.
- The cotton wool only needs to be moistened, not saturated.
- Ensure that the cotton wool is absorbent.
- Anaerobic jars come in various sizes, but a jar that holds 12 petri dishes is adequate for a class activity.
- Anaerobic jars may be listed in the microbiology section of catalogues.
- If access to an incubator is a problem, heating mats, which are available from plant nurseries, can be used. In the event that neither is available, room temperature will suffice.

USE STARCH AGAR OR SKIMMED MILK PLATES TO SHOW DIGESTIVE ACTIVITY DURING GERMINATION

Materials/Equipment

4 Soaked broad bean seeds	Incubator (18 °C – 20 °C)
2 Sterile starch/skimmed milk agar plates	Forceps
Iodine solution/biuret reagent	Backed blade
Disinfectant	Marker/labels
Sterilised water	Chopping board
Mild disinfectant solution	Disposable gloves
2 Beakers	Paper towels
Boiling water bath	Timer
Thermometer	Bunsen burner

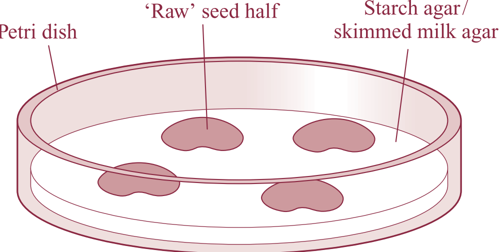


Fig. 1 'Raw' seed plate

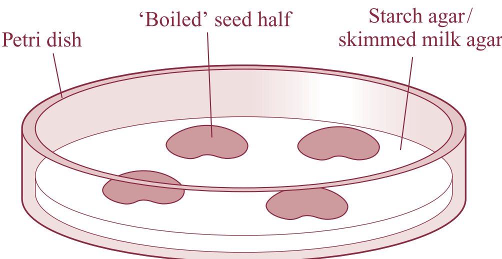


Fig. 2 'Boiled' seed plate (control)

Procedure

1. Familiarise yourself with all procedures before starting.
2. Swab the laboratory bench with disinfectant.
3. Label one of the sterile plates 'Raw'. Initial and date.
4. Label the other plate 'Boiled'. Initial and date.
5. Kill two of the seeds by boiling them for five minutes. These will act as controls.
6. Split each seed in half, to separate the cotyledons.
7. Sterilise all seeds by soaking them in the disinfectant solution for ten minutes.
8. Rinse the seeds twice using sterilised water.
9. Sterilise the forceps by flaming it in a Bunsen flame. Allow to cool.
10. With minimal opening, use the forceps to place all the seed halves facing down on the agar in the appropriate plates.
11. Re-flame the forceps and re-swab the bench.
12. Incubate the plates upright at 18 °C – 20 °C for 48 hours.
13. After 48 hours, remove the seeds from the plates.

14. If using
    (a) Starch agar plates:
        i. Flood the plates with the iodine solution and leave for two minutes.
        ii. Pour off the iodine solution.
        iii. Observe the pattern of starch digestion by holding the plate up to the light.
    (b) Skimmed milk plates:
        i. Flood the plates with the biuret reagent and leave for two minutes.
        ii. Pour off the biuret reagent.
        iii. Observe the pattern of protein digestion by holding the plate up to the light.
15. Record the result.
16. Replicate the investigation or cross reference your results with other groups.

SKILL ATTAINMENT

USE STARCH AGAR OR SKIMMED MILK PLATES TO SHOW DIGESTIVE ACTIVITY DURING GERMINATION

Following instructions

Familiarise yourself with all procedures before starting

Follow instructions step by step

Listen to the teacher's instructions

Correct manipulation of apparatus

Use the thermometer

Label the plates

Boil the seeds

Use the timer

Split the seeds

Sterilise the seeds

Sterilise the forceps

Transfer the seeds onto the agar

Remove the seeds after 48 hours

Flood the plates with iodine solution/biuret reagent

Pour off the excess iodine solution/biuret reagent

Observation

Observe the difference between the control and experiment plates

Observe the change in the starch/skimmed milk agar

Observe the effect of iodine solution/biuret reagent

Recognise the benefit of holding the plate up to the light

Recording

Write up the procedure

Draw labelled diagrams

Interpretation

Draw reasonable conclusions from your observations and results

Appreciate the importance of leaving the plates for the appropriate time

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others

Work in an organised and efficient manner

Work as part of a group or team

Use aseptic technique throughout

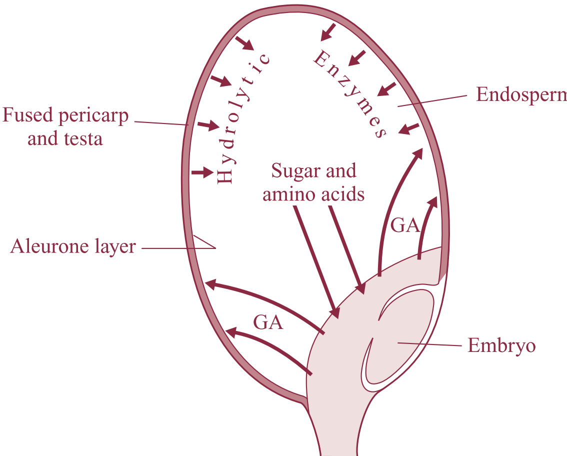
Label as appropriate

Clean up after the practical activity

Background Information

Changes in germinating seeds:

The structural changes that occur in a germinating seed are as a consequence of the physiological and metabolic changes that occur in the cells and tissues of the seed. The first changes are mainly the result of cell expansion following the uptake of water. Later changes involve the growth of new cells and tissues at the apices of the radicle and plumule. Growth requires raw materials and energy and initially it occurs at the expense of energy-rich molecules such as starch and lipids stored in the seed in the cotyledons or in the endosperm. The food reserves in the seed are insoluble in water and cannot be transported in the seedling. Gibberellic acid (GA), produced by the embryo, stimulates the production of hydrolytic enzymes by the aleurone layer of the seed. These enzymes convert the starch and lipids into simpler molecules which are soluble and can be transported to the growing tips where they are respired to release the energy necessary for growth.



Carbohydrates: The hydrolysis of starch into the soluble disaccharide maltose is catalysed by a set of complex enzymes called amylases. In a seed undergoing germination, the maltose is further digested to glucose by maltase for transport to the growing apices. At the tips, some of the glucose is used for the synthesis of cellulose, hemicelluloses and pectic compounds and some is respired for energy for growth.

In the starch agar plates which contained the raw seeds, starch in the agar is digested by the amylase produced in the seeds. The plates, when flooded with iodine solution will predominantly stain blue-black due to the presence of starch in the agar. However, where starch digestion has occurred (under the seeds) the areas will be clear.

Proteins: Proteins are hydrolysed to amino acids by peptidase enzymes. Some amino acids are moved to the embryo. Most are carried as amides. At the growing tips these amides are converted to amino acids, which are then used to manufacture structural and enzymatic proteins.

In the skimmed milk agar plates which contained the raw seeds, proteolytic/peptidase enzymes digest the milk protein in the agar. The plates, when flooded with biuret reagent will predominantly stain violet due to the presence of protein. However, where protein digestion has occurred (under the seeds) the areas will be clear.

Lipids: Lipids are first hydrolysed to glycerol and fatty acids. These are then oxidised to release energy or converted to glucose.

Advance preparation

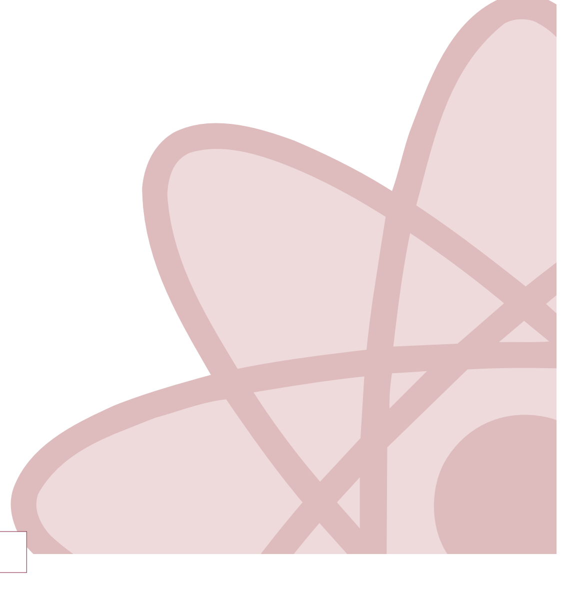
- Pre-soak seeds in distilled water for two days. Transfer seeds to a container with damp filter paper for one day to allow germination to begin.
- Prepare/purchase agar plates.
- Sterilise water.

Helpful Hints

- Take care in moving the agar plates with the seeds, to prevent the seeds from sliding.
- Have extra seeds boiled beforehand to allow for some being damaged during the procedure.
- Label the tops and bases of the plates and keep the labelling small.
- A blunt forceps is preferable to a sharp one as it is less likely to damage the seeds.
- A forceps can be used to hold the seed in place while splitting it with the backed blade.
- Have labelled petri dishes (‘Raw’ and ‘Boiled’) to hand when splitting the seeds.

Section Four
Materials and equipment

1.	Equipment	108
2.	Glassware	109
3.	Chemicals	109
4.	Biological materials	110
5.	Other resources	110
6.	Stock solutions and preparation of plates	111



1. EQUIPMENT

The following is a suggested list of equipment appropriate for Leaving Certificate Biology. It is neither prescriptive nor exhaustive. Valid alternatives may be available for some of the equipment listed.

Absorbent (blotting) paper

Adhesive tape

Anaerobic system

Animal rings or tag system

Animal traps

Aquarium

Autoclave

Autoclave bags

Backed blades

Baermann funnels

Basins

Bench Kote

Bijou bottles (plastic)

Binocular microscopes (4), (eyepiece lens: ×10, objective lenses: ×4, ×10, ×40)

Breathing rate apparatus

Brown paper

Bunsen burners

Chopping boards

Coffee filter paper

Colorimeter

Compasses

Cork borer set

Cotton wool (absorbent)

Counting instruments

Cylinder protectors(black neoprene)

Dialysis tubing (medium width)

Disposable gloves

Disposable inoculating loops

Disposal units (sharps, glassware, and biological)

Dissecting boards/trays

Dissecting kits (24): (awls, pins, fine and blunt forceps, scalpels and blades, fine and blunt scissors, mounted needles and seekers, safety backed blades)

Electric hotplates (6)

Electric magnetic hotplates (2)

Electronic mass balances (6) (to 0.1g reading)

Fermentation locks

Filter paper

First aid kit

Forceps

Growth tray and boxes

Hand/finger protectors, (silicone rubber)

Hand lenses

Hand microtome

Incubator

Inoculating loops

Jugs

Laboratory safety equipment

Lamps

Lens tissue

Light meters

Liquidisers

Metre sticks

Micropipettes

Monocular microscopes (20), (eyepiece lens: ×10, objective lenses: ×4, ×10, ×40)

Mounted needles

Mounted seekers

Mouth swabs

Nets: (insect, sweep, plankton and fish)

Paper towels

Parafilm

Pasteur pipettes/droppers

Pestle and mortar sets

Petri dishes

pH meter

Pipette fillers

Plastic syringes

Pooters

Pressure cooker

Pulse rate monitor

Quadrats

Retort stands

Ropes/line transects

Rubber bands

Rubber bungs

Rubber/plastic tubing

Rulers

Scalpel blade removers

Scissors

Secateurs

Sharp knives

Sieves

Small and large buckets

Small paintbrushes

Soil thermometers

Spades or trowels

Spatulas

Stopwatches

Tape measures

Tent pegs

Test-tube holders

Test-tube racks

Thistle funnels

Tullgren funnels

Wash bottles

Water baths (4)

Water deioniser

Weigh boats

2. GLASSWARE

The following is a suggested list of glassware appropriate for Leaving Certificate Biology. It is neither prescriptive nor exhaustive. Valid alternatives may be available for some of the glassware listed.

Beakers (various sizes)

Bijou bottles with wadless polypropylene cap

Boiling tubes (various sizes)

Clock glasses

Conical flasks

Cover slips

Droppers

Durham tubes

Funnels

Glass stirring rods

Graduated cylinders (various volumes)

Medical specimen bottles

Microscope slides

Petri dishes

Pipettes

Reagent bottles (various sizes, both amber and clear)

Separating funnels

Test tubes (Pyrex)

Thermometers

Tubing (capillary and solid)

Volumetric flasks (various volumes)

3. CHEMICALS

The following is a suggested list of chemicals appropriate for Leaving Certificate Biology. It is neither prescriptive nor exhaustive. Valid alternatives may be available for some of the chemicals listed.

Amylase

Agar plates (starch, malt, skimmed milk)

Agars

Albumen

Alcohol (methylated spirits or industrial spirits)

Aluminium foil

Aseptic wash solutions

Benedict's (qualitative) solution

Bicarbonate indicator (hydrogen carbonate indicator)

Biuret reagent

Buffer solutions (various pH values)

Calcium chloride

Calcium hydroxide

Cellulose paint marker

Chinagraph pencils

Copper sulfate

Diastase

Ethanol (95%)

Fehlings I and II solutions

Food dye

Glucose test strips

Hydrochloric acid (concentrated)

Hydrogen peroxide

Indole acetic acid (IAA)

Iodine

Malt powder

Methylene blue stain

Petroleum jelly

pH paper

Phloroglucin powder

Potassium iodide

Protease enzyme

Reducing sugar e.g. glucose

Salt

Skimmed milk powder

Soda lime

Sodium alginate

Sodium hydrogencarbonate

Sodium hydroxide or potassium hydroxide

Sodium hypochlorite

Soil test kit box for mineral content

Soil test kit box for pH

Starch powder (Analar)

Sucrose

Sudan III

Sulfuric acid (concentrated)

Trypsin

Universal indicator paper

Vegetable oil

Washing-up liquid

4. BIOLOGICAL MATERIALS

The following is a suggested list of biological materials appropriate for Leaving Certificate Biology. It is neither prescriptive nor exhaustive. Valid alternatives may be available for some of the biological materials listed

Cabomba
Elodea
Hearts
Milk
Onions
Radishes
Scenedesmus
Seeds
Variety of shoots
Yeast

5. OTHER RESOURCES

The following is a suggested list of other resources appropriate for Leaving Certificate Biology. It is neither prescriptive nor exhaustive. Valid alternatives may be available for some of the resources listed

Computer and software
CD, DVD player
Data-logging systems
Dishwasher
Fridge-freezer
Games
Guidebooks to identify fauna and flora, reference books, organism keys
Microscopic eye or visiview camera
Microwave oven
Models
Multimedia projector
Oven
Overhead projector
Printer
Scanner
Slide projector and slides
Television set
VCR and tapes
Video logging systems
Wall charts, posters

6. STOCK SOLUTIONS AND PREPARATION OF PLATES

Albumen solution (1% w/v): Place 1 g of albumen in water and make up to100 cm^3 with water. Prepare fresh solutions as required..

Copper sulfate solution (1% w/v): Dissolve 1 g of copper sulfate in 100 cm^3 of water.

Food dye: Add 3 – 5 drops of dark blue/green food colouring to 100 cm^3 of tap water.

Glucose solution (1% w/v): Dissolve 1 g of glucose in water and make up to 100 cm^3 with water. This solution keeps well.

Glucose solution (10% w/v): Dissolve 10 g glucose in distilled water and make up to 100 cm^3 with water.

HCl solution: Add 20 cm^3 of concentrated HCl to water. Cool. Make up to 100 cm^3. This provides the minimum strength (20%) HCl solution required. Stronger solutions, up to concentrated, may be used.

Indole acetic acid (0.01 % w/v): Wearing a face mask and following appropriate safety procedures, weigh out 0.1 g of indole acetic acid powder and place it in a large beaker.
Add 2 cm^3 of ethanol and stir to dissolve.
Add approximately 800 cm^3 of distilled water. Heat to 80 °C for 5 minutes to drive off the alcohol. Allow to cool to room temperature. Add to a volumetric flask and make up to 1 litre with distilled water. Mix well.

Iodine solution: Dissolve 2 g of potassium iodide crystals in 25 cm^3 of water. Add 1 g of iodine crystals. Make the solution up to 100 cm^3. Store in a dark glass bottle. Use 25 cm^3 bottles to distribute the solutions.

Phloroglucinol stain: Mix 5 g of phloroglucin powder with 100 cm^3 of 75 % alcohol or a 1 % solution may be made by dissolving 1 g of phloroglucin powder in distilled water and making up to 100 cm^3.
This preparation must be stored in a dark bottle as light causes the rapid breakdown of the phloroglucinol solution. The solution should be clear when being used. An orange colour indicates that the stain will not be effective.

Potassium iodide (10% w/v): Dissolve 10 g potassium iodide in distilled water and make up to 100 cm^3 with water.

Sodium hydrogencarbonate (1% w/v): Dissolve 1 g sodium hydrogencarbonate in distilled water and make up to 100 cm^3 with water. Dilute with distilled water as appropriate to get other concentrations.

Sodium hydroxide solution (10% w/v): Dissolve 10 g of sodium hydroxide in water and make up to 100 cm^3 with water. Stir to dissolve.

Starch solution (1% w/v): Shake 1 g of soluble starch (Analar) on to 100 cm^3 of water and bring to boiling point to obtain a clear solution. Cool. Make up to 100 cm^3 with water.

Preparation of starch agar plates (to make 4-5 plates):

1. Boil 50 cm^3 distilled water in a beaker.
2. Mix 1 g of soluble starch with a little cold distilled water.
3. Add the starch suspension to the boiling water, stirring continuously.
4. Mix 1 g of agar powder with a little cold distilled water.
5. Add this agar suspension to the boiling starch solution, stirring continuously.
6. Pour the starch agar solution into a conical flask and plug the top with cotton wool.
7. Sterilise the agar and the dishes at 121 °C for 15 minutes.
8. Allow all materials to cool a little.
9. Line the petri dishes up along the edge of the bench. Wearing heat-resistant gloves, remove the cotton wool plug from the flask and quickly flame the top of the flask.
10. With minimal opening, pour the starch agar into the sterile petri dishes.
11. Allow the starch agar to set.

Preparation of skimmed milk agar plates (to make 4-5 plates):

1. Mix 1 g of skimmed milk powder with 50 cm^3 of cold distilled water in a beaker.
2. Heat until dissolved but don't boil, stirring continuously.
3. Mix 1 g of agar powder with a little cold distilled water.
4. Add this agar suspension to the hot skimmed milk solution, stirring continuously.
5. Pour the skimmed milk agar solution into a conical flask and plug the top with cotton wool.
6. Sterilise the agar and the dishes at 121 °C for 15 minutes.
7. Allow all materials to cool a little.
8. Line petri dishes up along the edge of the bench. Wearing heat-resistant gloves, remove the cotton wool plug from the flask and quickly flame the top of the flask.
9. With minimal opening, pour the skimmed milk agar into the sterile petri dishes.
10. Allow the skimmed milk agar to set.

Preparation of malt agar plates (to make 30 plates):

1. Boil 500 cm^3 distilled water in a beaker.
2. Mix 2 g of malt extract powder with a little cold distilled water.
3. Add the malt suspension to the boiling water, stirring continuously.
4. Mix 7.5 g of agar powder with a little cold distilled water.
5. Add the agar suspension to the boiling malt solution, stirring continuously.
6. Pour the malt agar solution into a conical flask and plug the top with cotton wool.
7. Sterilise the agar and the dishes at 121 °C for 15 minutes.
8. Allow all materials to cool a little.
9. Line the petri dishes up along the edge of the bench. Wearing heat-resistant gloves, remove the cotton wool plug from the flask and quickly flame the top of the flask.
10. With minimal opening, pour the malt agar into the sterile petri dishes.
11. Allow the malt agar to set.

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