MICROBIOLOGY SOCIETY

Practical Microbiology for Secondary Schools

A RESOURCE FOR KEY STAGES 3, 4 & POST-16
AND THE EQUIVALENT SCOTTISH QUALIFICATIONS

Microbiology Society
Microbiology in Schools Advisory Committee (MiSAC)

About this resource

Microbiology is a popular option for practical work in schools. Micro-organisms are ideal for use in the school laboratory as they are relatively easy to maintain, and they can be used to demonstrate a wide range of biological processes and applications, as well as directly reflecting the microbiology content of national curricula.

The text provides a series of safe, tried and tested practical investigations suitable for Key Stages 3 and 4/GCSE science and equivalent Scottish qualifications. Many of the experiments also meet the needs of the AS/A2 specifications and can be adapted for project work. It is based on an earlier, out-of-print publication *Practical Microbiology & Biotechnology for Schools* (Macdonald, 1987) but has been significantly modified to match current practice and recent curriculum changes. The book incorporates new material, including some open-ended investigations.

Credits & Acknowledgements

This resource has been funded by the Microbiology Society.

It has been compiled by a working party of the Microbiology in Schools Advisory Committee (MiSAC) comprising: Peter Fry, John Grainger, Janet Hurst, John Tranter and Paul Wymer, with invaluable input from Dariel Burdass of the Microbiology Society.

The team would like to thank other members of MiSAC for their help and advice. They are also grateful for the errors pointed out and suggestions for improvement made by Helen Nankervis and Marilyn Whitworth of the University of Nottingham, who trialled the investigations in the laboratory.

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Contents

Using this resource

Photocopiable double-page spreads are provided for each investigation. The right-hand page of each spread is the student worksheet, illustrated with simple line drawings, whilst the left-hand page provides notes for teachers and technicians to assist with preparation and risk assessment, including a list of materials required and suggestions for student questions. Both pages provide planning support. It should be noted that many investigations require up to a week for microbial growth to occur before usable results are available.

The worksheets are arranged in four main themes which relate to areas of the National Curriculum for Science for England and Wales and the Scottish specifications, supporting, in particular, the Key Stage 3 Schemes of Work. Investigations can be selected to fit individual teaching programmes; they are not intended to be used in sequence. Each activity is stand-alone and offers students the opportunity to develop the investigative skills required for National Curriculum Key Stages 3 and 4. To help students experience the whole process of investigating selected problems from start to finish, two suggestions for open-ended explorations using micro-organisms are provided on pp. 42 and 43.

Whenever working with micro-organisms there are practical procedures to ensure safe transfer and avoid contamination which are common to most activities. An outline of these appears on p. 3, together with some other useful tips relevant to many of the investigations.

Basic Practical Microbiology: A Manual, published by the Microbiology Society, is recommended. Cross references to specific techniques in this work are listed on the student worksheets where appropriate.

The publications normally used in schools are:

- *Topics in Safety*, 3rd edition (ASE, 2001)
- *Safeguards in the School Laboratory*, 11th edition (ASE, 2006)
- CLEAPSS Science Publications, CD-ROM (latest edition)
- *Safetynet* (SSERC)
- *Safety in Science Education* (DfEE, available from the ASE website)

Good microbiological laboratory practice

Just like any other practical activity in school science, all microbiology investigations require the user to adopt good microbiological laboratory practice (GMLP). The techniques and activities included in this book, and the micro-organisms suggested, present minimum risk given good practice. Here is a brief overview to help those involved in preparing for, or using, the various activities to carry them out safely. Further detailed information is available in the resources listed on p. 44.

Risk assessment

School microbiology will generally be safe, but before any practical activity is undertaken, risks must be assessed. Each individual (be they students, technicians or teachers) embarking on a practical activity is responsible for his or her own health and safety and also for that of others affected by the work. Risk assessment will involve comparing the steps involved in an intended activity with procedures suggested in model risk assessments.* This will identify the safety precautions that need to be taken in the context of the level of work and, possibly, the need to amend the procedure so that risks to health and safety from any hazard(s) are minimised. Local rules must also be complied with. Of greatest importance in risk assessment is a consideration of the skills and behaviour of the students about to tackle a practical activity; a procedure that is safe for one group of individuals may need to be modified with a different class. Also important is ensuring that a procedure is safe for pupils, but also does not endanger the health and safety of technicians or teachers during preparation or disposal. In deciding on the appropriate precautions to adopt, it is prudent that all cultures are treated as potentially pathogenic (for example, because of possible contamination). Emergency procedures, such as dealing with spills, should also be considered.

No one should perform a microbiological procedure without receiving appropriate training from, or supervision by, a competent person.

Microbiology safety

There are five areas for consideration when embarking on practical microbiology investigations, which make planning ahead essential.

- Preparation and sterilisation of equipment and culture media.
- Storage and maintenance of stocks of microbial cultures for future investigations and preparation of an inoculum for the current investigation.
- Inoculation of the media with the prepared culture.
- Incubation of cultures and sampling during growth, if required.
- Sterilisation and safe disposal of all cultures and decontamination of equipment.

Good organisational skills and a disciplined approach ensure that every activity is performed both safely and successfully.

Personal protection

Food or drink should not be stored or consumed in a laboratory or prep room that is used for microbiology. No one should lick labels, apply cosmetics, chew gum, suck pens or pencils or smoke in a laboratory or prep room. Facilities should be provided within the laboratory or prep room for hands to be washed with soap and warm water after handling microbial cultures and whenever leaving the laboratory. Paper towels, or some other hygienic method, should be used for drying hands. If contamination of the hands is suspected, then they should be washed immediately with soap. Cuts or abrasions should be protected by the use of waterproof dressings or disposable gloves. Safety glasses may be worn according to local requirements. Appropriate protective clothing may be required.

Practical techniques and tips

Aseptic technique

Sterile equipment and media should be used in the transfer and culture of micro-organisms. Aseptic technique should be observed whenever micro-organisms are transferred from one container to another. This involves working in an area in which draughts are controlled, for example close to a lit Bunsen burner so that there is an updraught of air away from the bench, thereby minimising the risk of contaminating open cultures.

Flaming loops and bottles

Only sterile instruments should be used to transfer cultures of micro-organisms. With pipettes or syringes, these will have been pre-sterilised but, for most transfers, an inoculation loop will be used which is sterilised by heating along its length until it glows red. This is done both before and after each transfer. When the loop is loaded, beware of 'splutter' when the culture is rapidly heated. This is minimised if the loop is heated first near the handle and slowly drawn through the flame so that the tip of the loop is prewarmed before it reaches the flame. Formation of microbial aerosols is avoided by the careful use of loops and pipettes.

The necks of open bottles or tubes should also be flamed, both before and after transfers, by passing them briefly through a hot Bunsen burner flame. The aim is not to sterilise the glass (which may break if heating is prolonged), but to create a convection current of air away from the open mouth of the vessel, so helping to minimise the risk of contaminants entering.

Pipettes and syringes

A variety of pipettes is available for the aseptic transfer of cultures, suspensions of natural materials and sterile solutions. Usually it is adequate to control the volume to be transferred in terms of the number of drops delivered, e.g. for routine inoculation using a Pasteur (dropping) pipette to provide a culture. For measured volumes, e.g. when making dilutions, some form of calibrated pipette is required. Suitable types include a dropping pipette with volumes marked on the barrel and one that delivers drops of a known volume. The long calibrated pipettes that are used in chemistry are also suitable. Pipetting by mouth is forbidden. A teat should always be used. Relatively inexpensive calibrated pipette fillers (e.g. Bibbettes) are also available from suppliers. Syringes and micro-pipettes holding the appropriate volume provide a useful alternative.

A non-absorbent cotton wool plug about 1 cm long is placed in the wide end of the pipette before sterilisation. This prevents airborne microbes from contaminating the fluid being transferred and prevents the operator and environment from contamination by a culture. However, the cotton wool plug must remain dry because a wet plug does not act as a barrier to the passage of microbes. Glass pipettes should be sterilised in either an autoclave or hot air oven.

Handling and labelling agar plates

After inoculating an agar plate, it is customary to tape the lid to the base to prevent accidental opening. It is best to use clear adhesive tape since masking or biohazard warning tape may obscure the observation of cultures that grow. Only two or four small pieces of tape are required; covering the top and bottom of an agar plate with a 'cross' of tape merely wastes tape and may interfere with the viewing of the plate's contents. Before incubation is complete, an agar plate must never be sealed completely by taping around the circumference; this will create anaerobic conditions which may prevent the normal growth of microbes or isolate anaerobes which may be pathogenic. If there is reason to suspect that an incubated plate may contain unknown species (which may be pathogenic), for example when sampling from the environment, it is sensible to seal plates completely just before they are returned to students for observations.

Agar plates are normally inverted after inoculation to avoid any moisture forming on the lid during incubation and dripping down onto the agar. Since plates will normally be inspected with the agar base uppermost and the microbial growth observed through the agar in the base, it is also necessary to label the base, rather than the lid. Labelling should be small to prevent obscuring the view of microbes that develop. Using small adhesive labels may be more convenient than writing on the dish.

Incubation and storage

For the purposes of the investigations in this book, virtually all cultures can be incubated at room temperature. At the lower temperatures now recommended, an incubator may not therefore be needed but, if used, it should be set at around 20–25 °C and certainly no higher than 30 °C to avoid the inadvertent growth of pathogens. An exception to this rule is in the fermentation of milk to make yoghurt. If an incubator is not used, plates should be incubated (and subsequently stored) where they cannot be tampered with, for example in a cupboard rather than left on a shelf in a laboratory. It is not recommended that incubated plates are stored in a refrigerator for more than a few days; long-term storage will cause, or increase, problems of condensation.

Disposal and disinfection

Contaminated materials and equipment should preferably be heat-sterilised by autoclaving if incineration is not possible or appropriate. This may be time-consuming but it is considerably safer than the use of a disinfectant which may also not ensure complete sterilisation. A disinfectant will be needed for items such as pipettes and syringes that cannot be resterilised after use by flaming, for swabbing benches and for dealing with accidental spills. Time is needed if disinfection is to be achieved and this may take up to 30 minutes with some types, for example sodium chlorate(1) (hypochlorite) or domestic bleach. Not all disinfectants are stable when used (for example bleach is degraded by organic matter). Virkon and sodium chlorate(1) are likely to be of most use.

Life forms and processes

Finding and growing microbes

Microbes are found everywhere. A litre of pond water can contain millions of microbes. A similar number can be present in a gram of soil. Many microbes and their spores can be carried by winds, waters and animals across and between continents.

Learning objectives

To show:

- ▷ that microbes are everywhere
- ▷ the variety of microbes
- ▷ that the microbial population varies from place to place

Age range

Year 7 and above

Duration

Session 1	40 minutes
Session 2	20 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. To limit the number of agar plates required, each group should use either nutrient or malt agar plates. Nutrient agar supports the growth of a wide range of bacteria and fungi from soil and air. Malt extract agar supports better growth of fungi because the low pH and nutrient content reduce competition from bacteria.
2. The plates exposed to the air should be left in a variety of places both inside and outdoors. Do not place the dishes in toilets. Ensure that other students/teachers are aware of why the plates are there or the experiment may be disrupted.
3. The students should be told that one agar plate is being kept unopened in the classroom/laboratory as a control.
4. The plates should be kept at room temperature or incubated at 20–25 °C for 2–3 days. After incubation the plates should be taped around the circumference so that the lids cannot be removed.

Materials (each group)

- ▷ 3 nutrient agar plates
  or
  3 malt extract agar plates
- ▷ bottle containing 1 g soil suspended in 100 cm^3 tap water
- ▷ bottle containing 10 cm^3 pond water
- ▷ 2 sterile glass or plastic spreaders
- ▷ 2 sterile dropping pipettes
- ▷ beaker of disinfectant
- ▷ Bunsen burner
- ▷ adhesive tape
- ▷ marker pen

Notes

Exposure of the agar plates in a variety of places should, after incubation, produce growth of bacteria and fungi. The number of colonies may be a reflection of the disturbance of the air by convection currents or people. Microbes in disturbed air may not be detected as they are held in suspension. Soil and water will probably yield more microbial colonies than air.

Questions

Session 1

1. Where do you think microbes may be found?
2. Is a single microbial cell visible to the naked eye?
3. What conditions do you think would encourage the growth of microbes?
4. What different kinds of microbes exist?
5. Do you expect any difference between the malt agar plates and the nutrient agar plates? If so, why?
6. What is the purpose of the 'control' plate?

Session 2

7. Describe the appearance of each of your plates. Do they all look the same?
8. Which plate shows most microbial growth? Can you suggest any reasons for this?
9. Do your plates look the same as those from other groups, using a different kind of growth medium? If not, suggest a reason why.
10. Write down any ideas you have about how microbes might be important in the places you have investigated.

Life forms and processes

Finding and growing microbes

Microbes are found almost everywhere, but they are mostly far too small to be seen by the naked eye. When microbes grow, they multiply and form colonies that can be seen easily. You are going to grow microbes from different places by supplying them with suitable growth conditions.

Learning objectives

To show:

- ▷ that microbes are everywhere
- ▷ the variety of microbes
- ▷ that the microbial population varies from place to place

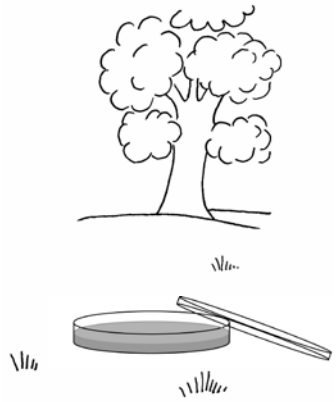
Techniques required

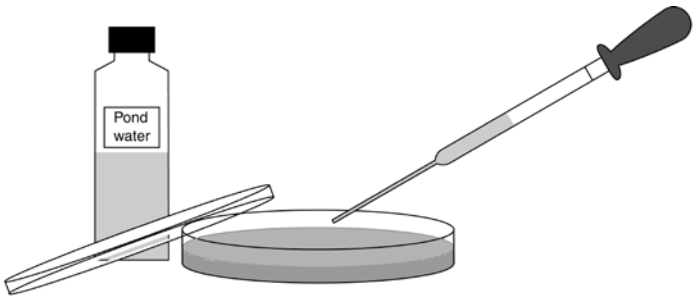
See *Basic Practical Microbiology*

- ▷ flaming the neck of a bottle (p. 10)
- ▷ using a pipette (p. 9)
- ▷ using a spreader (p. 13)

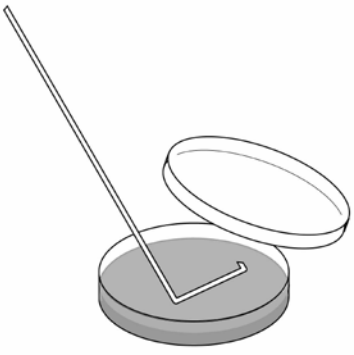
Procedure

1. Take one agar plate. Choose a suitable place to leave it open to the air. Label the base with your name, the date and the place. Take the lid off the dish and keep it open until the end of the lesson. Replace the lid.





2. Label another agar plate with your name, the date and 'pond water'. Shake your bottle of pond water to mix the contents. Remove the top, flame the neck in the Bunsen burner and draw up a small amount of water with a dropping pipette. Flame the neck again and replace the top. Lift the lid of the dish and dispense 2 or 3 drops on to the surface of the agar. Use a sterile glass spreader to spread the drops evenly over the agar. Discard pipette and spreader into the beaker of disinfectant.
   The spreader can also be sterilised by flaming with alcohol.



3. Follow the same procedure as (2), labelling the plate 'soil', using the bottle of soil suspension, a clean dropper and a fresh spreader.

4. Retrieve your plate from (1). Tape up all three agar plates and turn them upside down. They will be incubated until the next lesson.

Safety! *Do not open the plates.*

Next lesson...

5. Examine your agar plates and answer the questions.

Life forms and processes

Estimating the number of bacteria in water

Most methods of estimating numbers of viable bacteria involve the inoculation of a solid medium with the sample (or diluted sample). After incubation the number of cells in the inoculum can be estimated from the number of colonies which develop on or in the medium. It is usually assumed that each colony has arisen from a single cell; the number of cells that actually grow into colonies depends at least partly on the type of medium used and on the conditions of incubation.

Learning objectives

To show:

- the presence of bacteria in natural waters
- a realistic method of estimating bacterial numbers

Age range

Year 7 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. The microbial population of natural waters varies widely from place to place and time to time. It is essential to try this experiment before the lesson, to check that the dilution suggested produces the desired result, i.e. a countable number of colonies.
2. Care should be taken with the temperature of the nutrient agar at pouring. Below 45 °C the agar will start to set and be lumpy; at higher than 55 °C some of the microbes may be killed.
3. When students are mixing the agar and culture in the Petri dish, they should be instructed to swirl the contents gently in a figure of eight to ensure even distribution of the sample.
4. As an alternative to river or pond water, an overnight nutrient broth culture of *Bacillus subtilis* or *Micrococcus luteus* can be used to illustrate the colony counting technique. In this case it is essential to use more dilute samples (1/100, 1/1000) or the growth might be too heavy to count colonies.

Materials (each group)

- pond or river water in a beaker
- 2 Universal bottles each containing ca 15 cm^3 molten nutrient agar held at 45–50 °C
- 2 Petri dishes
- bottle containing sterile distilled water
- sterile clean test tube with cap
- 2 × 10 cm^3 sterile syringes or calibrated pipettes and fillers
- Bunsen burner
- beaker of disinfectant
- marker pen
- adhesive tape

Questions

Session 1

1. What was the point of diluting the original water sample?
2. By next lesson, any bacteria in the water will give rise to 'colonies' visible in and on the agar. What will these colonies consist of?
3. What is the difference between a 'viable' cell count and 'total' cell count?
4. What other kinds of microbes might be present in the water sample?
5. How would you expect your dishes to look next lesson?

Session 2

6. Describe the appearance of your Petri dish and count the number of colonies. Explain your results.
7. Calculate the number of bacteria per litre of your original water sample.
8. How accurate do you think this estimate of numbers is? Explain your answer.
9. If your results are not what you expected, suggest some reasons why not.
10. Do you think the colonies that you can see came from microbes in your original water sample? If not, where else might they have come from? How could you check this?
11. What steps are taken to ensure that no harmful microbes are found in mains water?

Life forms and processes

Estimating the number of bacteria in water

River and pond waters contain a wide variety of microbes. Most of these are entirely harmless but dangerous organisms may be present due to contamination from farm animals, etc. It is therefore necessary to treat domestic water supplies to reduce the number and type of microbes to levels considered safe for human consumption. This is known as sanitation. Counting the numbers of microbes in water is an important part of checking the effectiveness of treatment and you are going to use a method of doing this. Because there can be very high numbers of microbes in the water, it is necessary to dilute the samples to carry out an accurate count.

Learning objectives

To show:

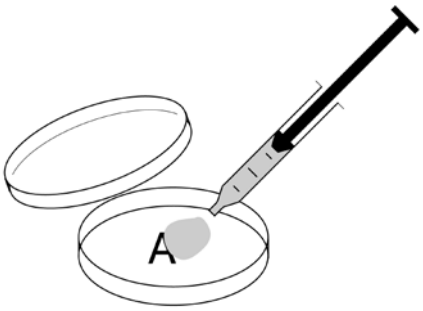
- the presence of bacteria in natural waters
- a realistic method of estimating bacterial numbers

Techniques required

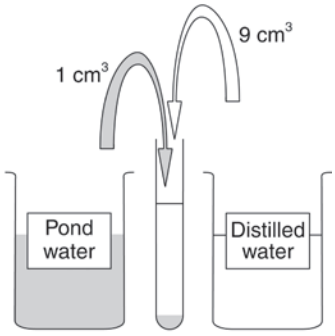
See *Basic Practical Microbiology*

- using a pipette (p. 9)
- flaming the neck of a bottle (p. 10)

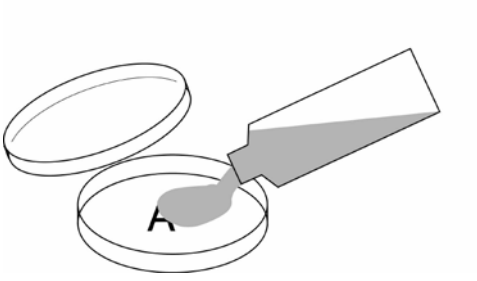
Procedure



1. Label the base of the Petri dishes A and B and with your name and the date. Use a sterile 10 cm^3 syringe or calibrated pipette and filler to remove 2 cm^3 of your pond/river water sample from the beaker. Remove the test tube cap, flame the neck in the Bunsen burner and put 1 cm^3 of the sample in to the tube. Flame the neck again and replace the cap. Lift the lid of Petri dish A and place the remaining 1 cm^3 of the sample into it. Replace the lid as soon as possible and discard the pipette or syringe into the disinfectant.



2. Use another sterile pipette and filler or syringe to add 9 cm^3 distilled water to the test tube, flaming the neck after removing the cap. Refill and empty the pipette two or three times to mix the contents. Finally, place 1 cm^3 of the diluted sample in Petri dish B. Discard the pipette or syringe into disinfectant.



3. Take a bottle of melted agar. Remove the top and pass the neck through the Bunsen flame two or three times. Pour the contents into Petri dish A. Mix the agar and water sample in the Petri dish thoroughly but gently, as directed by your teacher. Avoid splashing the agar on to the cover or over the edge of the dish. Repeat with the other bottle of agar and Petri dish B.

4. Allow the agar to set. Tape up the dishes and turn them upside down. They will be incubated until the next lesson.

Safety! *Do not open the plates.*

Next lesson...

5. Examine your agar plates. Count and record the number of colonies in each one.

Life forms and processes

Breakdown of starch by microbes

Amylase enzymes are widespread in many organisms including micro-organisms. Microbial amylases are secreted by cells and degrade starch molecules that are too large to pass through the cell wall. The amylases produced by microbes are often used in industry, particularly in starch processing, where the products of starch degradation (sugars, syrups) are used in the manufacture of foods and beverages.

Learning objectives

To show:

- ▷ that some microbes produce amylase
- ▷ a simple assay technique for amylase activity
- ▷ an aspect of the industrial importance of microbes

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. The cultures of *Bacillus subtilis* and *Escherichia coli* should be inoculated into nutrient broth at least 48 hours before the lesson. *B. subtilis* (catalogue no. B53) from Sciento produces amylase.
2. The paper discs can either be purchased (Whatman Antibiotic Assay discs) or punched from filter or chromatography paper with a cork borer (ca 6 mm diameter) or a hole punch.
3. After the first lesson and incubation at room temperature for a few days, the agar plates should be inverted and filter papers soaked in 40 % methanal (formaldehyde)* solution placed in the lids. These should be left overnight to kill the microbes and then removed, after which the students can irrigate the plates with iodine solution.

* *Safety! Methanal is toxic and corrosive. Avoid breathing in the vapour. Use eye protection, gloves and a fume cupboard.*

Notes

Some strains of *B. subtilis* produce amylases whereas *E. coli* does not. Other microbes suitable for school use, such as the fungus *Aspergillus oryzea*, also show marked amylase activity. This requires starch malt agar, which is made by adding 100 cm^3 4 % starch suspension to each 100 cm^3 malt agar (made with light malt). If using *A. oryzea*, a culture grown in malt extract broth for 7 days is required.

Materials (each group)

Session 1

- ▷ test tubes/bottles containing 2 cm^3 each of:
  Bacillus subtilis nutrient broth culture
  Escherichia coli nutrient broth culture
- ▷ starch nutrient agar plate
  (Heat 4 g soluble starch in 100 cm^3 distilled water to form a suspension. Allow to cool and mix with 100 cm^3 molten nutrient agar before sterilisation.)
- ▷ 0·1 % amylase solution
- ▷ 4 paper discs (see recommendations)
- ▷ forceps
- ▷ sterile distilled water
- ▷ Bunsen burner
- ▷ beaker of disinfectant
- ▷ marker pen
- ▷ adhesive tape

Session 2

- ▷ iodine solution and dropper
- ▷ graph paper and ruler

Questions

Session 1

1. What reaction is catalysed by amylase enzymes?
2. Why do microbes produce amylase enzymes?
3. What is the purpose of the control disc soaked in distilled water?

Session 2

4. Describe the appearance of your agar plate after it has been flooded with iodine.
5. Do all microbes produce amylases?
6. What physical factors might affect the amylase activity?
7. Suggest some reasons why microbes are used as a source of industrial amylases.
8. Suggest some uses of amylase enzymes in industry and the home.

Life forms and processes

Breakdown of starch by microbes

Amylase is an enzyme which breaks down starch molecules into sugars and is produced by our bodies to break down the starch we eat. Certain microbes also produce amylase. Some of these are used as a source of amylase for the starch processing industry. You are going to compare the activity of amylase from different sources.

Learning objectives

To show:

- ▷ that some microbes produce amylase
- ▷ a simple assay technique for amylase activity
- ▷ an aspect of the industrial importance of microbes

Techniques required

See *Basic Practical Microbiology*

- ▷ flaming the neck of a bottle (p. 10)

Procedure

1. Turn a starch agar plate upside down and divide the base into four sections by drawing on it with a marker pen. Label the sections A, B, C and D. Write your name and date on the plate. Turn the plate the correct way up. You may like to keep a key to the sections:

A	*Bacillus subtilis*
B	*Escherichia coli*
C	0·1 % amylase solution
D	Distilled water

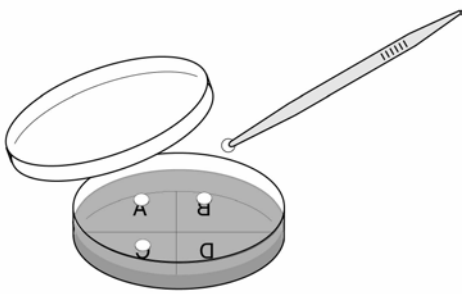
2. Pass the forceps through the Bunsen burner flame, allow them to cool and use them to pick up one of the paper discs. Open the culture of *Bacillus subtilis*. Flame the neck of the bottle and dip the disc into the broth. Allow any excess to drain off, reflame the neck and replace the top on the bottle. Transfer the disc to the middle of section A on the agar plate. Flame the forceps.

   Repeat using another disc, the culture of *Escherichia coli* and section B of the plate.

   Repeat again using another disc, the amylase solution and section C of the plate.

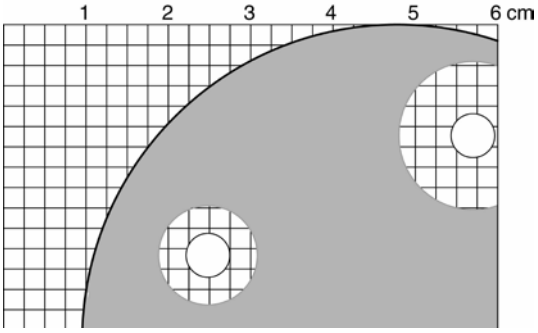
   Finally, use the same procedure to place a disc soaked in sterile distilled water on section D of the plate. Place the forceps in the beaker of disinfectant.

   Tape up the dish. The plates will be incubated until the next lesson.



Next lesson...

3. Lift the lid of your Petri dish. This is permitted because the microbes have previously been killed. Using a dropper, place just enough iodine solution to cover the surface of the agar. Replace the lid.



4. Measure the diameter of any clear zones around the discs, by placing the agar plate on the graph paper. Record your results.

Life forms and processes

Breakdown of protein by microbes

Protease enzymes are produced by several bacteria and fungi. They catalyse the hydrolysis of proteins to amino acids. Industrial applications of proteases include detergent manufacture, brewing and baking, meat tenderisation and leather preparation. Microbes, including fungi of the genus *Aspergillus*, are used to produce proteases on a commercial scale.

Learning objectives

To show:

- that microbes produce proteases
- the chemical nature of proteins
- the use of proteases in industry

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. The cultures of *Bacillus subtilis* and *Saccharomyces cerevisiae* should be inoculated at least 48 hours before the lesson.
2. Plates must be treated carefully after inoculation to prevent the spreading of the drops of culture. They should not be inverted until the drops have dried.
3. When the milk agar is made, it is assumed that the microbial population of the milk will not affect the outcome of the investigation. A control plate could be used to cover this possibility, although the uninoculated area of the plate really suffices.
   This aspect could form the basis for a question exploring students' understanding of the difference between pasteurised and sterilised milk.

Notes

1. The milk agar is opaque due to the milk protein casein. After inoculation and incubation, clear areas around microbial colonies indicate protease activity.
2. An alternative procedure using paper discs can be used in this experiment (see page 8).

Materials (each group)

- bottles containing ca 2 cm^3 each of:
  Bacillus subtilis nutrient broth culture
  Saccharomyces cerevisiae malt extract broth culture
- 2 milk agar* plates
- 2 sterile dropping pipettes
- Bunsen burner
- beaker of disinfectant
- marker pen
- adhesive tape

*Milk agar. Make up and sterilise nutrient agar. Allow to cool to 45–50 °C and add pasteurised milk (10 % by volume) aseptically and mix carefully. The milk should be freshly bought and pasteurised. Skimmed, semi-skimmed or full cream milk can be used.

Questions

Session 1

1. What do proteins consist of?
2. Why are proteins important in our bodies?
3. How are proteins broken down in our bodies?
4. What is causing the cloudiness of the agar in your Petri dishes?
5. If the microbes you are using break down proteins, what do you expect your agar plates to look like next lesson?

Session 2

6. Describe the appearance of your agar plates.
7. Is there any evidence of protease production by the microbes?
8. If so, how is this beneficial to the microbes?
9. Suggest some uses of protease enzymes in industry.
10. How are these enzymes produced on a large scale?
11. What factors might affect the activity of the enzymes?
12. Suggest a procedure for investigating the effects of one of these factors.

Life forms and processes

Breakdown of protein by microbes

Proteins are large molecules made up of amino acids. They have to be broken down by organisms before they can be used. Milk protein (casein) is white and when mixed with nutrient agar, makes it cloudy. You are going to use the disappearance of this cloudiness as an indicator of protein breakdown by microbes.

Learning objectives

To show:

- ▷ that microbes produce proteases
- ▷ the chemical nature of proteins
- ▷ the use of proteases in industry

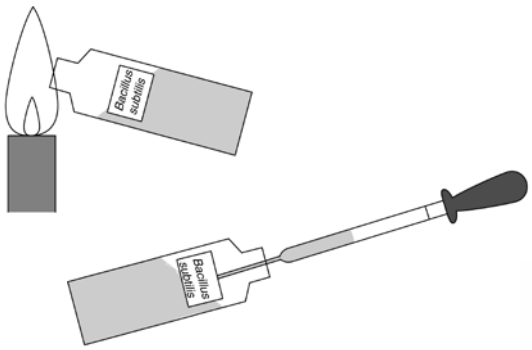
Techniques required

See *Basic Practical Microbiology*

- ▷ using a pipette (p. 9)
- ▷ flaming the neck of a bottle (p. 10)

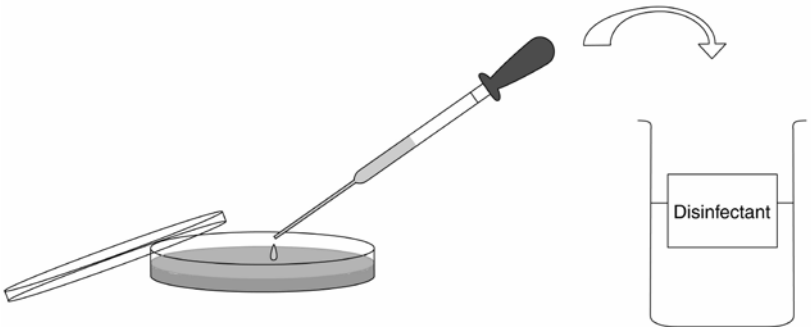
Procedure

1. Label the base of each agar plate with either *Bacillus subtilis* or *Saccharomyces cerevisiae* plus your name and the date.



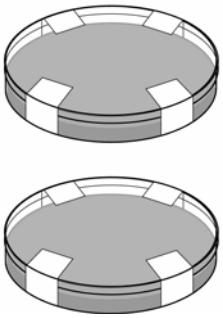
2. Open the culture of *Bacillus subtilis*. Flame the neck of the bottle in the Bunsen burner and use one of the dropping pipettes to remove a small amount of culture. Reflame the neck of the bottle and replace the top.

3. Lift the lid of the appropriately labelled plate and release one drop of culture on to the middle of the agar. Replace the lid. Place the dropping pipette into the beaker of disinfectant.



4. Repeat using the other culture, *Saccharomyces cerevisiae,* and other plate plus a fresh dropping pipette.

5. Tape up both of the dishes. Keep the plates upright until the drops have dried, then invert. The plates will be incubated until the next lesson.



Safety! *Do not open the plates.*

Next lesson...

5. Examine your agar plates. Answer the questions.

Life forms and processes

Alcohol production using immobilised yeast cells

In many industrial biotransformations, immobilised enzymes or cells are used. This is often advantageous for the production of high purity products free of residual protein. It also enables expensive enzymes to be recovered and used again. Immobilised yeast cells are not typically used in commercial alcohol production, but they are used here to demonstrate the technique.

Learning objectives

To show:

- the role of yeast in alcohol production
- the production of carbon dioxide by yeast cells
- the relative performance of free yeast cells and immobilised yeast cells

Age range

Year 7 and above

Duration

Session 1	40 minutes
Session 2	20 minutes
Incubation period:	min. 7 days between sessions

Recommendations

1. The yeast cultures should be inoculated in broth at least 48 hours before the lesson and incubated at 20–25 °C or room temperature.
2. To obtain distinct beads of immobilised cells, the syringe should be held 5 cm above the calcium chloride solution in the beaker. The latter should be swirled gently as the contents of the syringe are released drop by drop. If the syringe contents are released too quickly, separate beads will not be obtained. Although not difficult, the procedure should be demonstrated before students attempt it.
3. A few drops of Universal indicator may be added to the apple juice so that pH changes may be observed.

Notes

1. Cloudiness in the limewater should be apparent during the first week but after two weeks a crust will form and the solution will become clear.
2. Beads will rise during the incubation period because of carbon dioxide evolution by the entrapped yeast.
3. The investigation could be extended by exploring the continued re-use of the beads in either batch or continuous culture.

Materials (each group)

- ca 10 cm^3 *Saccharomyces cerevisiae* culture in malt extract broth in a small beaker
- 200 cm^3 apple juice
- 250 cm^3 2 % calcium chloride solution in 500 cm^3 beaker
- 10 cm^3 3 % sodium alginate solution made up in deionised water
- distilled water in a small beaker and in a wash bottle
- 100 cm^3 limewater
- 2 × 250 cm^3 conical flasks with bungs and connecting tubes
- 2 × 100 cm^3 conical flasks
- 250 cm^3 beaker
- 100 cm^3 measuring cylinder
- 10 cm^3 syringe
- beaker of disinfectant
- filter paper and filter funnel
- marker pen

Questions

Session 1

1. Describe what you see when the drops of yeast suspension/alginate are added to calcium chloride solution.
2. How do you explain this observation?
3. Describe what has happened to the yeast cells.
4. What is the purpose of the limewater in this investigation?
5. How will you know whether or not fermentation of sugar to alcohol has occurred in either flask?
6. Do you expect any difference in alcohol production between the two flasks? If so, explain why.

Session 2

7. Has the limewater gone cloudy in either case?
8. Describe the smell of the contents of either flask. Is this evidence enough to confirm alcohol production?
9. The immobilised cells are trapped in a jelly-like substance. How do they manage to ferment the apple juice?
10. How could you tell whether the immobilised cells are as good as the free yeast cells in fermenting the juice?
11. Which other factors may affect the rate of alcohol production? Outline some experiments to test your ideas.
12. What are the advantages of using immobilised cells in industrial processes?

Life forms and processes

Alcohol production using immobilised yeast cells

Yeasts are microbes which produce carbon dioxide and alcohol from sugars. This process is called fermentation. You are going to try two methods of making wine from fruit juice. One uses 'free' yeast cells, the other uses yeast cells immobilised in calcium alginate.

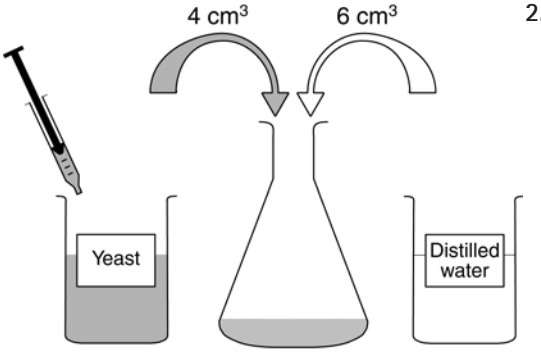
Learning objectives

To show:

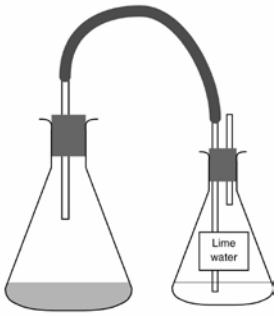
- the role of yeast in alcohol production
- the production of carbon dioxide by yeast cells
- the relative performance of free yeast cells and immobilised yeast cells

Procedure

1. Label the 250 cm^3 conical flasks with your name and the date, one with 'free cells', the other with 'immobilised cells'. Use the measuring cylinder to pour 100 cm^3 apple juice into each of the conical flasks.



2. Use the syringe to put 4 cm^3 yeast culture and 6 cm^3 distilled water into the flask marked 'free cells'. Replace the bung and connecting tube. The other end of the tube should be placed in a 100 cm^3 conical flask containing about 50 cm^3 lime-water. Ensure that the end is under the surface of the limewater.



3. Draw up 6 cm^3 sodium alginate solution into the syringe, followed by 4 cm^3 yeast culture. Thoroughly mix the contents of the syringe by turning it upside down several times. While swirling the beaker of calcium chloride gently to avoid spillages, release the contents of the syringe drop by drop, holding the nozzle about 5 cm above the solution. Discard the syringe into disinfectant.

4. Filter the contents of the beaker, using a filter paper in a funnel. Rinse the beads with distilled water.

5. Tip the beads into the other flask of apple juice. Fit the bung and connecting tube, placing the other end of the tube under the surface of the limewater in the other flask. Leave your flasks at room temperature.

Safety! *Discard your syringe into a beaker of disinfectant.*

Next lesson...

6. Examine your flasks regularly over the next 1–2 weeks. Answer the questions.

Life forms and processes

A microbe which moves towards light

Euglena, a single-celled organism, possesses chloroplasts and can photosynthesise like a plant. If cultured in the dark it tends to lose its chloroplasts. It moves by means of a flagellum. The organism contains a light-sensitive eyespot (or stigma) consisting of several pigment-containing lipid droplets.

Learning objectives

To show:

- some characteristics of *Euglena*
- the wavelengths of light that are useful in photosynthesis
- the importance of photosynthetic microbes in the carbon cycle

Age range

Year 9 and above

Duration

Session 1	60 minutes
Session 2	20 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. Considerable forward planning is required for this investigation because a relatively large volume of actively growing *Euglena* culture is needed. Cultures obtained from Sciento are usually dense and active. If necessary, grow up the *Euglena* in Biobred Algae Medium (also from Sciento), following the instructions provided.
2. Filters: sets of gelatin sheets of different colours can be purchased from school science suppliers (Philip Harris, Cinemoid colour filter sheets).
3. It is very important to ensure that no light enters the tube except via the 'windows'. The tubes should be left in natural light, but not direct sunlight (a north facing window ledge is ideal), for at least 2 days. If in a test tube rack, make sure that no windows are obscured by it.
4. The tubes should be removed gently from the cardboard sleeves, otherwise cells will become detached from the sides.
5. To aid microscopic examination, a drop of 10% methyl cellulose can be added to the drop of *Euglena* culture on the slide. This will slow down movement and help detailed observation. Focus on the culture using the low power lens first.

Materials (each group)

- bottle containing 10 cm^3 *Euglena* culture (available from Sciento and other suppliers)
- 20 cm^3 Biobred Algae Medium (available from Sciento)
- large boiling tube
- dropping pipette
- coloured filters (see recommendations)
- black paper
- sticky tape
- non-absorbent cotton wool
- aluminium foil
- scissors
- slide and cover slip
- microscope
- Bunsen burner
- marker pen
- beaker of disinfectant

Notes

The greatest accumulation of cells will occur at the colourless window. More cells will be concentrated at the red and blue windows than the green and yellow ones. The experiment is worthwhile even if microscopic examination is excluded.

Questions

Session 1

1. What enables *Euglena* to move?
2. Why is *Euglena* not a typical protozoan?
3. What is the name of the process by which *Euglena* makes its own food?
4. What is needed (besides light) for this process to occur?
5. What are the products of the process?
6. Which windows do you think *Euglena* will be most attracted to?

Session 2

7. Do your results confirm the prediction you made last lesson? If not, suggest some reasons why.
8. Which colours does *Euglena* absorb most?
9. How reliable do you consider your results to be?
10. Suggest further investigations which could be carried out to test your conclusions.

Life forms and processes

A microbe which moves towards light

Euglena is a microbe that makes its own food by photosynthesis. It is also able to move, by means of a flagellum. You are going to find out how *Euglena* responds to different colours in light.

Learning objectives

To show:

- ▷ some characteristics of *Euglena*
- ▷ the wavelengths of light that are useful in photosynthesis
- ▷ the importance of photosynthetic microbes in the carbon cycle

Techniques required

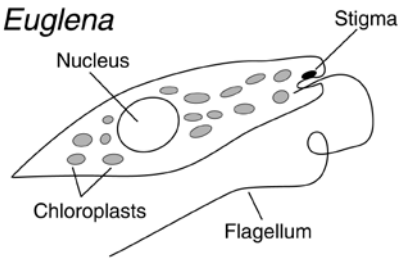
See *Basic Practical Microbiology*

- ▷ using a pipette (p. 9)
- ▷ flaming the neck of a bottle (p. 10)
- ▷ using a microscope (p. 22)

Procedure

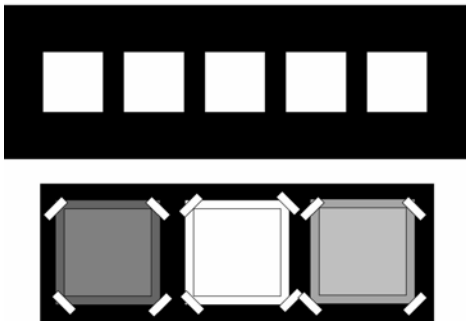
1. Gently swirl the contents of the bottle of *Euglena* culture. Take the top off and flame the neck. Draw a small amount of culture up into the dropping pipette. Flame the neck again and replace the bottle top.

2. Place one drop of culture on the microscope slide and put the cover slip over it carefully. Discard the pipette into a beaker of disinfectant. Examine the drop under first low (× 100) and then high power (× 400) with your microscope.



3. See how many of the labelled parts you can observe under the microscope. Draw one of the *Euglena* cells.

4. Cut a piece of black paper large enough to make a sleeve around your boiling tube. Cut five windows in the sleeve, each about 1 cm^2. Using small pieces of sticky tape, fix a different colour filter over each of the windows. Use red, yellow, green, blue and colourless filters.



5. Roll the sleeve around the boiling tube and stick it together with tape. Do not stick it to the tube as you will have to remove it later. Flame the neck of the culture bottle in the Bunsen burner and pour the remaining *Euglena* culture into your boiling tube. Fill the tube almost to the top with growth medium, leaving room for the bung. Make a cotton wool bung for your tube. Label the tube near the top with your name and the date. Finally, cover the top and bottom of the tube with foil and stand it in a well-lit place until the next lesson. Make sure none of the windows are obscured.

Next lesson...

6. Gently remove the foil and the sleeve from the boiling tube. The *Euglena* cells should have moved towards the light and become stuck to the sides of the tube in the places where the light shone through the windows. Record your results and answer the questions.

Microbes in the environment

Microbes and cellulose

Cellulose, a polymer of glucose, is used as a carbon source by a number of fungi and bacteria found in soil and compost heaps, etc., and by certain bacteria in the rumen (the grass-digesting part of the gut) in cows and other ruminants. These microbes produce cellulase enzymes that can degrade certain types of cellulose outside the cell into products which include glucose.

Learning objectives

To show:

- ▷ the role of microbes in decomposing organic waste
- ▷ the role of microbes in the carbon cycle
- ▷ the role of microbes in the production of industrial enzymes

Age range

Year 7 and above

Duration

Session 1	30 minutes
Session 2	20 minutes
Incubation period:	up to 3 wks between sessions

Notes

1. The types of paper indicated are only suggestions. Different types of paper have different physical and chemical properties that will make them more or less susceptible to cellulose degradation. Cardboard, for example, contains a lot of lignin which protects the cellulose from degradation, because of its branching structure. Ink on heavily printed paper is known to have a protective role against cellulose degradation because it forms a physical barrier to the enzyme.
2. An open-ended investigation to explore a cellulolytic activity is provided on p. 42.

Materials (each group)

- ▷ 6 test tubes with caps/cotton wool plugs
- ▷ 60 cm^3 sterile nutrient broth
- ▷ 5 g soil
- ▷ 250 cm^3 conical flask
- ▷ sterile 5 cm^3 graduated pipette and filler
- ▷ marker pen
- ▷ 1 × 2 cm^2 strips of different types of paper:
  - filter paper (× 2)
  - unprinted newspaper
  - heavily printed newspaper
  - glossy magazine cover
  - thin cardboard
- ▷ beaker of disinfectant

Questions

Session 1

1. What is the main component of paper?
2. What is produced when this substance is broken down?
3. What kind of animals have cellulase enzymes in their gut?
4. What is the main industrial source of cellulase enzymes?
5. How are microbes important in the carbon cycle?
6. Do you expect to see any differences between your tubes next lesson? Explain your answer.

Session 2

7. Which tubes show evidence of cellulase activity? Try to explain any differences.
8. What happens to domestic waste paper?
9. What are landfill sites? What are some of the major problems that they present?
10. What is compost? How are microbes important in its production?
11. Why is it important that microbes degrade cellulose?

Microbes in the environment

Microbes and cellulose

Cellulose is one of the main components of plant cell walls. It consists of long chains of glucose molecules. These can be broken down by cellulase enzymes produced by certain microbes in the soil and in the gut of certain animals, e.g. cattle. You are going to investigate the effectiveness of these enzymes in breaking down different types of paper.

Learning objectives

To show:

- ▷ the role of microbes in decomposing organic waste
- ▷ the role of microbes in the carbon cycle
- ▷ the role of microbes in the production of industrial enzymes

Techniques required

See *Basic Practical Microbiology*

- ▷ using a pipette (p. 9)

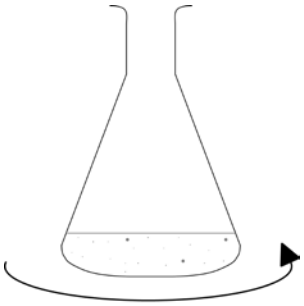
Procedure

1. Label your tubes. You are going to set them up as follows:

Tube	Content	Type of paper
A	Nutrient broth (sterile)	Filter paper
B	Nutrient broth + soil	Filter paper
C	Nutrient broth + soil	Newspaper (no print)
D	Nutrient broth + soil	Newspaper (heavily printed)
E	Nutrient broth + soil	Glossy magazine cover
F	Nutrient broth + soil	Thin cardboard

Also label with your name and the date.

2. Use the graduated pipette and filler to place 5 cm^3 nutrient broth in tube A.



3. Place the soil and 30 cm^3 nutrient broth in the conical flask. Swirl the contents to form a soil suspension. Allow this to settle for a minute to avoid blocking the pipette.

4. Pipette 5 cm^3 of the supernatant of the nutrient broth/soil suspension into each of the remaining tubes. Discard the pipette into a beaker of disinfectant.

5. Set up your tubes as shown, according to the table shown in (1). Leave them at room temperature for at least a week.

Next lesson...

6. Give each tube a tap with your finger. Carefully observe what happens to the paper strip. Record your results and answer the questions.

Microbes in the environment

Microbes and water pollution

Dissolving various minerals and other nutrients in pond or river water can simulate the effects of common sources of pollution. Different combinations and concentrations promote the growth of certain microbes selectively, leading to noticeable changes in the colour and opacity of the water.

Learning objectives

To show:

- changes in microbial populations of natural waters caused by pollution
- some of the major sources of water pollution
- some of the problems associated with eutrophication

Age range

Year 10 and above

Duration

Session 1	40 minutes
Subsequent sessions	10–15 minutes
Incubation period:	over 3–4 wks

Materials (each group)

- 600 cm^3 pond or river water
- hay (chopped)
- 1·5 g nutrient broth powder
- 1 g potassium nitrate
- 1 g potassium phosphate
- 6 × 250 cm^3 conical flasks
- 100 cm^3 measuring cylinder
- non-absorbent cotton wool
- access to a balance
- marker pen

Recommendations

1. Pond or river water samples should preferably be collected on the day of the lesson or the day before, ensuring that they come from a place where there is no risk from farm or sewage effluent.
2. Depending on facilities, it may be necessary to provide students with pre-weighed ingredients (especially 0·01 g nutrient broth powder).
3. If the number of conical flasks or storage space is limited, the experiment can be run as a demonstration.
4. Illumination by direct light (e.g. on a window sill) is adequate, but continuous artificial illumination, especially in winter, is more effective.
5. A shallow layer of water and use of cotton wool rather than rubber bungs helps aeration of the flask contents.

Notes

1. This is a long-term investigation which can be run for 4 weeks or more. Considerable fluctuations in the populations of different microbes are evident from week to week. This results in visible changes in colour and opacity.
2. Flasks B–D are likely to yield algae. Flask E will show cloudiness and a surface skin caused by the rapid growth of bacteria. Duplicates of these could be kept in the dark for comparison with those in the light.
3. Hay (flask F) provides microbes not naturally present in the water. Straw is less effective. Different substances can be added to the flasks to simulate industrial pollution, e.g. dehydrated potato or milk as examples from the food industry. These will also add different microbes.

Questions

Session 1

1. Why is it important for the flasks to be kept in the light?
2. How do you ensure that the air supply to the flasks is the same as that in the natural environment?
3. When you examine the flasks over the next few weeks, what would you expect the water in each of them to look like? Explain your answer.
4. What are the major sources of water pollution?
5. What is eutrophication?
6. Explain the sequence of events that might connect the death of fish in a river with the use of fertiliser on the land.

Session 2

7. Suggest possible reasons for differences between your own results and those of other groups.
8. How are the conditions in the experiment different from those in nature?
9. What conclusions, if any, can be drawn from your results? Describe further investigations that you could carry out to test your conclusions.

Microbes in the environment

Microbes and water pollution

Common sources of water pollution include 'run-off' of soluble materials from farmland, domestic sewage and industrial waste. When these reach natural waters, they encourage the growth of certain microbes that use them as a food supply. The microbes use up oxygen in the water, preventing the growth of larger organisms. You are going to investigate some microbes which might be found in water polluted in different ways.

Learning objectives

To show:

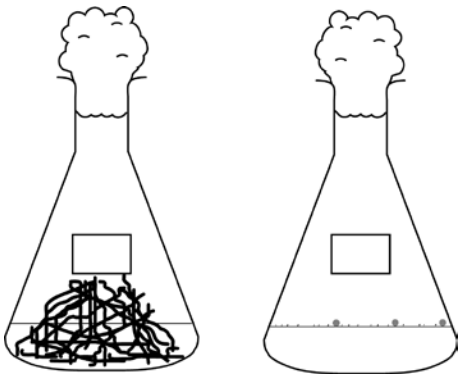
- ▷ changes in microbial populations of natural waters caused by pollution
- ▷ some of the major sources of water pollution
- ▷ some of the problems associated with eutrophication

Procedure

1. Label the flasks A to F. Add your name and the date. Weigh or place the appropriate materials into the flasks as follows:

Flask	Content
A	Nil
B	0·1 g potassium nitrate
C	0·1 g potassium nitrate + 0·1 g potassium phosphate
D	0·01 g nutrient broth powder
E	1 g nutrient broth powder
F	Chopped hay (enough to cover the palm of your hand)

2. Using the measuring cylinder, place 100 cm^3 river or pond water into each of flasks A–F. Plug the neck of each flask with cotton wool. Leave the flasks in a well-lit place at room temperature.



Safety! *Do not open the flasks.*

Next lesson...

3. Over the next few weeks, at regular intervals, examine the general appearance of each flask. Note the colour of the water and whether it is clear or cloudy. Record your results in a table and answer the questions.

Microbes in the environment

Nitrogen-fixing bacteria

The reduction of nitrogen to ammonia by nitrogen-fixing organisms is catalysed by a nitrogenous enzyme complex. As nitrogenase activity is very sensitive to the presence of oxygen, in aerobic bacteria inactivation by oxygen is prevented in several ways. *Azotobacter*, for example, has the highest respiratory rate of any organism, leading to rapid removal of oxygen by respiration. It also produces a protective slime layer which facilitates its identification in this investigation. *Azotobacter* is able to fix nitrogen when free-living in the soil (compare *Rhizobium*, pp. 22–23).

Learning objectives

To show:

- ▷ that free-living nitrogen-fixing bacteria occur in the soil
- ▷ one role of microbes in the nitrogen cycle
- ▷ how bacteria may be isolated from soil

Age range

Year 9 and above

Duration

Session 1	20 minutes
Subsequent sessions	20 minutes
Incubation period:	min. 48 h between sessions

*Nitrogen-free mineral salts agar

For 500 cm^3, first dissolve 0·05 g $FeCl_3.6H_2O$ in 500 cm^3 distilled water. Add 2 g K_2HPO_4, 0.25 g $MgSO_4.7H_2O$ and 10 g glucose. Dissolve and check pH, adjust to 8·3 if necessary using 0·1 M NaOH. Pour into a bottle containing 1 g $CaCO_3$ and 7·5 g agar. Autoclave at 121 °C for 20 minutes. When pouring into plates, mix to disperse the $CaCO_3$ first.

Nitrogen-free mineral salts agar can also be purchased from Philip Harris. Agar powder can be added to this at the rate of 2 g per 100 cm^3 before sterilisation.

Materials (each group)

- ▷ nutrient agar plate
- ▷ nitrogen-free mineral salts agar plate (*for recipe, see left*)*
- ▷ soil
- ▷ forceps or spatula
- ▷ marker pen
- ▷ adhesive tape

Recommendations

The plates containing soil should be kept at room temperature or incubated at 20–25 °C for 2–3 days.

Questions

Session 1

1. What is the difference between the two growth media?
2. What sources of nitrogen are available to microbes in the soil?
3. What sources of nitrogen are available to microbes in the two dishes?
4. Do you expect to see any difference between your dishes next lesson? Explain your answer.
5. Why is nitrogen essential for living organisms?

Session 2

6. Are there any similarities or differences between the plates? If so, do these confirm your predictions?
7. How can you identify any colonies of *Azotobacter* on either of the plates?
8. If you can, does this confirm that these bacteria are able to 'fix' nitrogen from the atmosphere?
9. Where else do nitrogen-fixing bacteria occur?
10. What would happen to the nitrogen cycle if nitrogen-fixing bacteria did not exist?

Microbes in the environment

Nitrogen-fixing bacteria

Microbes that can 'fix' nitrogen from the air occur freely in soil as well in the root nodules of certain plants. Despite their importance, they only occur in small numbers compared with other soil bacteria. Therefore, special methods which prevent or discourage the growth of other organisms have to be used in the laboratory to grow them from natural sources. In this investigation you will attempt to grow the nitrogen-fixing bacterium *Azotobacter* from the soil using media with and without nitrogen.

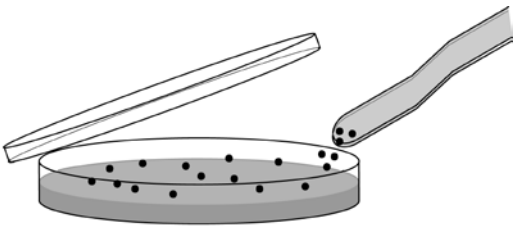
Learning objectives

To show:

- ▷ that free-living nitrogen-fixing bacteria occur in the soil
- ▷ one role of microbes in the nitrogen cycle
- ▷ how bacteria may be isolated from soil

Procedure

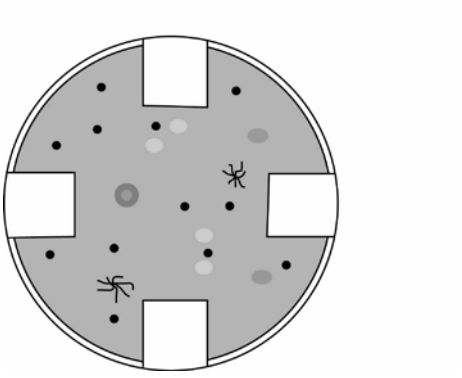
1. Take the Petri dish containing the nitrogen-fixing mineral salts agar medium and, using the spatula or forceps, place 10–20 crumbs of soil evenly over the surface. Replace the lid and tape and label it with your name, the date and 'N-free'.



2. Repeat step 1 using the nutrient agar plate. Label it with your name, the date and 'NA'.

3. The plates will be incubated until the next lesson.

Safety! *Do not open the plates.*



Next lesson...

4. Examine the plates for microbial growth. Colonies of *Azotobacter* look mucoid (slimy) and are often colourless. They will be found around the soil particles. Compare the growth on the dishes and answer the questions.

Microbes in the environment

Isolating microbes from root nodules

The roots of leguminous plants have small swellings (nodules) containing bacteria of the genus *Rhizobium*. This is a symbiotic relationship, with the plant providing nutrients and protection while the *Rhizobium* supplies the plant with 'fixed' nitrogen from the atmosphere. Plant growth is therefore enhanced even in nitrogen-poor soils. A nitrogen-containing medium is used here because *Rhizobium* is able to fix nitrogen only when inside the nodule.

Learning objectives

To show:

- the role of microbes in the nitrogen cycle
- how microbes can be grown from root nodules
- an example of symbiosis

Age range

Year 11 and above

Duration

Session 1	30 minutes
Session 2	20 minutes
Incubation period:	min. 48 h between sessions

*Mannitol yeast extract agar (MYEA)

Suspend 10 g agar in 1 l water. Heat to dissolve. Add 0·5 g K_2HPO_4, 0·2 g $MgSO_4.7H_2O$, 0·2 g NaCl, 0·2 g $CaCl_2.6H_2O$, 10 g mannitol and 0·4 g yeast extract. Dispense and sterilise by autoclaving.

Recommendations

1. Advance planning is necessary to obtain suitable plant material. Clover (obtain seeds from school science suppliers or plants from a field or lawn) is recommended as the nodules are relatively soft.
2. Potato dextrose agar supplemented with 0·25 g yeast extract per litre may be used instead of mannitol yeast extract agar.
3. The plates should be incubated preferably at 20–25 °C for 2–3 days or at room temperature.
4. If the nodules are cleaned well in the alcohol, a population of predominantly *Rhizobium* should result. Students should be informed that they are using sterile apparatus so that any bacteria that do grow on their plates are likely to have come from the root nodules.
5. 1% bleach solution can be used instead of alcohol to clean the nodules.
6. The colonies of *Rhizobium* are off-white with a sticky appearance. Colonies of other colours are NOT *Rhizobium*; they may be either intracellular contaminants from the nodule or soil microbes that have survived the washing and alcohol treatment.

Materials (each group)

- plant with root nodules e.g. clover, peas
- mannitol yeast extract agar plate (*for recipe, see left*)*
- sterile distilled water in beaker (covered)
- 70 % (v/v) industrial denatured alcohol in a small beaker or glass Petri dish covered in foil (*CAUTION: flammable, keep covered away from lit Bunsen burner*)
- 5 sterile Petri dishes
- Bunsen burner
- dropping pipettes: 1 sterile, 1 non-sterile
- sterile glass rod
- scalpel
- metal forceps (can be pre-sterilized by autoclaving)
- wire loop
- beaker of disinfectant
- marker pen
- adhesive tape

Questions

Session 1

1. What is the alcohol treatment for?
2. What kinds of plants have root nodules? What are they?
3. How do root nodules help the plant?
4. Does the plant help the microbes?
5. What name is given to this type of relationship?
6. What do you expect to see growing on your agar plate next lesson?

Session 2

7. Is there just one type of microbe growing, or more than one?
8. Where do you think most of the microbes growing on the dish have come from?
9. Where might any other microbes have come from?
10. Are the microbes fixing nitrogen while they are growing on the agar plate?
11. Explain the importance of nitrogen-fixing microbes in the nitrogen cycle.

Microbes in the environment

Isolating microbes from root nodules

Leguminous plants such as peas and beans usually have lumps or nodules on their roots. A bacterium called *Rhizobium* grows within these nodules which is able to change nitrogen gas from the air into a compound that the plant can use to help it grow. This process is called nitrogen fixation and it occurs only inside the nodules. Green plants use this supply of fixed nitrogen to make proteins that enter and pass through the food chain. You are going to isolate some of these microbes and grow them.

Learning objectives

To show:

- ▷ the role of microbes in the nitrogen cycle
- ▷ how microbes can be grown from root nodules
- ▷ an example of symbiosis

Techniques required

See *Basic Practical Microbiology*

- ▷ using a wire loop (p. 8)
- ▷ making a streak plate (p. 11)
- ▷ sterilising forceps (p. 7)

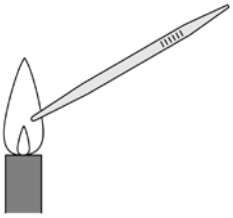
Procedure

1. Choose a length of root that has nodules and cut off a portion about 1 cm long using a scalpel. Hold the portion of root by forceps and wash free of soil using tap water.

2. Transfer several drops of 70% (v/v) industrial denatured alcohol by Pasteur (dropping) pipette fitted with a teat to a sterile Petri dish. The pipette need not be sterile for this operation; put the pipette into a discard pot. Transfer the washed portion of the root to the alcohol in the Petri dish with forceps and leave immersed for 1–2 minutes to sterilise it.

   Use aseptic technique from this stage forward.

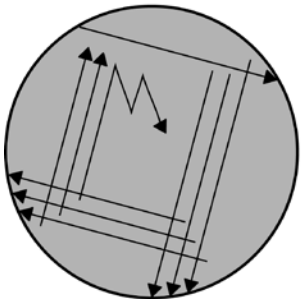
3. Transfer sufficient sterile water to cover the base of another Petri dish using a sterile Pasteur pipette fitted with a teat. If it is necessary to re-use the pipette, keep it sterile, e.g. by resting under the lid of a sterile Petri dish base.



4. Use sterile forceps or sterilise them by dipping in alcohol (keeping the points facing downward) and passing quickly through the Bunsen burner flame, allow to cool and use to transfer the portion of root to the sterile water in the Petri dish to rinse off the alcohol. Repeat this operation at least twice more with fresh sterile water. If using alcohol take care to keep the pot well away from the Bunsen burner flame.

5. Transfer a few drops of sterile water to a sterile Petri dish and add the portion of root using flamed metal forceps. Macerate the nodules using a sterile glass rod (or forceps) to produce a milky fluid.

6. Label the base of a mannitol-yeast extract agar plate with your name, the date and MYEA. Sterilise a wire loop by flaming, cool it, take a loopful of the nodule macerate and streak it out on the plate as shown here. Reflame the loop. Tape the plate, invert it and incubate for 3–4 days. Dispose of contaminated materials appropriately.



Next lesson...

7. Examine the MYEA plate without removing the lid, noting the appearance of any colonies growing on the agar. Answer the questions.

Microbes in the environment

Effects of chemical elements on microbial growth

In this investigation, the green alga *Scenedesmus* provides an alternative to traditional water culture experiments with higher plants to demonstrate the effects of mineral deficiency on growth. *Scenedesmus* is one of the commonest bloom-forming freshwater algae and it can therefore provide a useful introduction to the effects of eutrophication in natural waters.

Learning objectives

To show:

- the relative importance of selected chemical elements on plant growth
- the uses of different elements by plants
- the process of eutrophication

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	2–3 wks between sessions

Recommendations

1. This investigation requires a large number of conical flasks per group.
2. A large quantity of an actively growing culture of *Scenedesmus* (available from Sciento) is required. Add the stock liquid culture to, e.g. 100 cm^3 complete Sach's medium in a 500 cm^3 flask. Add more growth medium at intervals until the required quantity is achieved. *Scenedesmus* should be kept in bright light (not direct sunlight). If daylight is inadequate (e.g. in winter) a bank of two or three 'warm light' fluorescent tubes can be used. The cultures for the investigation should be bright green in colour. Bulking up inevitably depends on the condition of the original culture and the time of year.

Eutrophication

In water enriched with inorganic ions beneficial to plant growth, algae may undergo a population explosion (an 'algal bloom'), particularly in the summer. Degradation of their dead remains eventually leads to the production of hydrogen sulphide and this, in addition to the deoxygenation of the water by saprophytic bacteria, causes the death of other organisms such as fish.

Materials (each group)

- bottle containing 20 cm^3 culture of *Scenedesmus* in complete Sach's medium (Mineral Deficiency Sach's Water Culture Set is available from Philip Harris. Make up stock solutions as required.)
- 5 × 250 cm^3 labelled conical flasks with non-absorbent cotton wool plugs containing:
  - A 100 cm^3 complete medium
  - B 100 cm^3 medium without K
  - C 100 cm^3 medium without P
  - D 100 cm^3 medium without N
  - E 100 cm^3 distilled water
- 5 cm^3 sterile syringe/calibrated pipette and filler
- sterile dropping pipette
- beaker of disinfectant
- slides and coverslips
- mounted needle
- microscope
- marker pen
- Bunsen burner

Questions

Session 1

1. What elements are needed to make proteins?
2. Why are fertilisers used by farmers?
3. What elements are included in the fertilisers?
4. What do you expect the appearance of your flasks to have in 2–3 weeks' time?

Session 2

5. In which flask has most growth taken place and in which flask has least growth taken place?
6. Which element(s) are most important for growth of the algae?
7. What do you think would happen if the flasks were kept in the dark?
8. When fertiliser runs off from farm land into waterways, what effect do you think this may have on: (a) microscopic plants; (b) larger water plants; (c) animals; (d) bacteria?

Microbes in the environment

Effects of chemical elements on microbial growth

Green plants need a variety of chemical elements, such as nitrogen, potassium and phosphorus to grow well. The roots take in these elements. Farmers often use fertilisers to increase the amounts of these elements in the soil. You are going to use a green alga called *Scenedesmus* to investigate the effects of some elements on plant growth.

Learning objectives

To show:

- ▷ the relative importance of selected chemical elements on plant growth
- ▷ the uses of different elements by plants
- ▷ the process of eutrophication

Techniques required

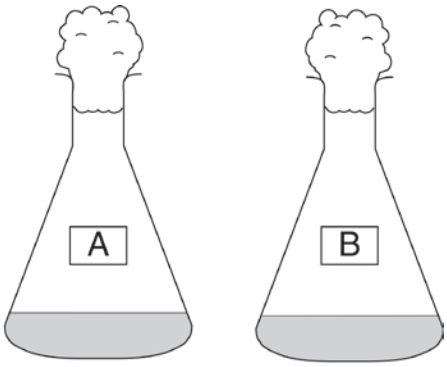
See *Basic Practical Microbiology*

- ▷ flaming the neck of a bottle (p. 10)
- ▷ using a microscope (p. 22)

Procedure

1. The conical flasks marked A–E contain different growth media as below:

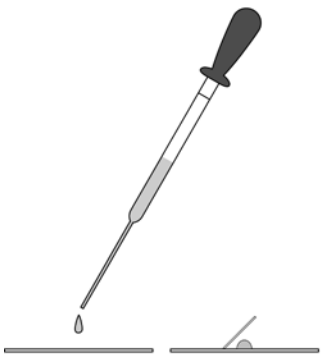
Flask	Content
A	Complete medium
B	Medium without potassium
C	Medium without phosphorus
D	Medium without nitrogen
E	Distilled water only



Write your name and the date on each flask.

2. Light the Bunsen burner. Gently shake the bottle of algal culture to mix the contents. Remove the cap, flame the neck of the bottle, draw up 1 cm^3 of culture with the pipette or syringe, flame the neck again and replace the cap. Take the cotton wool plug from flask A, flame the neck and empty the culture from the pipette/syringe into it. Flame the neck and replace the plug. Repeat for flasks B–E. Discard the pipette/syringe into the beaker of disinfectant. Gently swirl the contents of each flask to mix the contents. The flasks will be left at room temperature in the light for a few weeks.

3. Draw up a small amount of the remaining algal culture with the dropping pipette. Place a drop on a microscope slide and carefully place a coverslip over it using a mounted needle. Discard the pipette into disinfectant. Using first low power and then high power magnification (e.g. ×400), look at some of the cells. Try to identify features such as the cell wall, nucleus, chloroplasts, etc., and draw a typical algal cell.



Safety! *Do not break the coverslip.*

After 2–3 weeks...

4. Examine the flasks and record the appearance of each. Answer the questions.

Microbes and food

Breakdown of pectin by microbes

The ability of some microbes to degrade complex polymers into fermentable substances is fundamental to the carbon cycle. Pectin is a polymer in plant cell walls where it acts as a kind of glue, holding the walls together. Microbial breakdown of pectin leads to soft-rot in fruit and vegetables. Pectinase, the enzyme responsible, is produced commercially using micro-organisms such as *Aspergillus niger* and *Bacillus subtilis* for use in wine and fruit juice production. Some pectin passes into the juice during fruit pressing, reducing juice yields and increasing viscosity. This can be overcome by adding pectinases to fruit pulp prior to pressing.

Learning objectives

To show:

- ▷ that microbes produce pectinase
- ▷ that microbes are important in decomposition and nutrient recycling
- ▷ how pectinase is useful in the fruit juice industry

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	72 h between sessions

Recommendations

1. An actively growing culture of *Erwinia carotovora* is required. This should be grown up in nutrient broth incubated at 20–25 °C for at least 48 hours before the lesson.
2. The potato slices must be cut thinly so that they do not touch the lid of the Petri dish.
3. Carrots can be used as an alternative to potato, or as a comparison. Fruits could also be tested.

Notes

1. The effects of 'soft rot' should be visible in the inoculated potato slices after about 3 days.
2. Pectinases degrade pectin ultimately to simple sugars. Pectin molecules are too large to enter microbial cells and are degraded outside the cell by pectinases secreted by the micro-organism.

Materials (each group)

- ▷ bottle containing ca 2 cm^3 culture of *Erwinia carotovora*
- ▷ 2 Petri dishes each containing a slice of potato
- ▷ distilled water
- ▷ beaker of disinfectant
- ▷ Bunsen burner
- ▷ 2 sterile dropping pipettes
- ▷ marker pen
- ▷ adhesive tape

Questions

Session 1

1. Where is pectin found in plants?
2. What effect would the breakdown of pectin have on plant cells?
3. What source of pectinase are you using in this investigation?
4. Do you expect to see any difference in the potato slices next lesson?

Session 2

5. Describe the appearance of each vegetable slice. Explain any differences. Do your results support your predictions?
6. Do you think pectinase made by microbes is stored in the microbial cells or released outside them? Explain your answer.
7. How might pectinase be of use to a company that produces apple juice?

Microbes and food

Breakdown of pectin by microbes

Microbes help to decompose organic matter. This releases chemicals that can be used again for building cells in plants or animals. Microbes produce enzymes which cause decomposition. Pectinase is an enzyme which breaks down pectin, a substance in cell walls, into sugars. As well as being important in nature, this enzyme is used widely in the manufacture of fruit juice. You are going to test the effect of *Erwinia carotovora*, a bacterium which produces pectinase, on vegetable matter.

Learning objectives

To show:

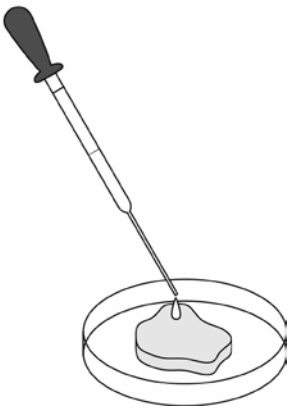
- ▷ that microbes produce pectinase
- ▷ that microbes are important in decomposition and nutrient recycling
- ▷ how pectinase is useful in the fruit juice industry

Techniques required

See *Basic Practical Microbiology*

- ▷ using a pipette (p. 9)
- ▷ flaming the neck of a bottle (p. 10)

Procedure



1. Take a Petri dish containing a slice of potato or carrot. Use a dropping pipette to place 2–3 drops of distilled water in the centre of one slice. Discard the pipette into the beaker of disinfectant. Put the lid on the Petri dish, tape it and label with your name, the date and 'control'.

2. Take the culture of microbes. Remove the top from the bottle and pass the neck through a Bunsen burner flame two or three times. Take up a small amount of culture with a sterile dropping pipette. Flame the neck again and replace the top on the culture bottle. Use the pipette to place 2–3 drops of culture on the other slice of potato. Discard the pipette into the beaker of disinfectant. Replace the lid on the Petri dish, tape it and label with your name, the date and '*Erwinia*'. Wash your hands. The dishes will be incubated with the lids uppermost until the next lesson.

Safety! *Do not open the plates.*

Next lesson...

3. Examine the potato slices, without removing the lids of the plates, and record your observations. Answer the questions.

Microbes and food

Microbes and breadmaking

Bread and related bakery products are normally produced from wheat flour, water or milk, salt, fat, sugar and the yeast *Saccharomyces cerevisiae*. When the ingredients are mixed and the sugars fermented, the dough rises due to the formation of carbon dioxide. The alcohol produced by the fermentation is lost during baking. The fermentation achieves three primary objectives: leavening (carbon dioxide production), flavour development and texture changes in the dough.

Learning objectives

To show:

- the importance of yeast in breadmaking
- the effect of temperature on yeast activity
- the relationship between yeast activity and enzyme activity

Age range

Year 7 and above

Duration

50 minutes

Recommendations

1. *The yeast suspension should be prepared in a beaker before the lesson. Add a 15 g sachet of dried bakers yeast and a teaspoon of sugar to each 150 cm^3 water.
2. The use of a paste as opposed to a proper dough ensures useful results in a limited period of time. It is important that the paste does not touch the sides of the measuring cylinder when being transferred from the beaker.
3. Wide-necked funnels, e.g. powder funnels, should be used if available.
4. Each group should be allocated to a waterbath at a particular temperature. Glass measuring cylinders are preferable to plastic ones which may float.
5. After 35–45 minutes, the protein (gluten) skin of the paste will break and carbon dioxide will escape. Readings need only be taken up to this point.

Materials (each group)

- 30 cm^3 yeast suspension (*see left*)*
- 1 g sugar
- 25 g plain or wholemeal flour
- 250 cm^3 beaker
- funnel
- 50 cm^3 measuring cylinder
- 250 cm^3 measuring cylinder
- spatula
- stopclock
- access to a balance
- 3 waterbaths: 20 °C, 30 °C, 37 °C
- graph paper
- marker pen

Notes

In addition to illustrating the effects of temperature on yeast (enzyme) activity, the experiment can be extended to investigate the effects of substrate concentration, quantity of yeast and pH.

Questions

1. What makes the dough paste rise?
2. Did it start to rise immediately? If not, after how long?
3. Why was sugar added to the mixture?
4. Describe the effect of temperature on the rate of rising.
5. Do you think the paste would have risen quickly or slowly or not at all at the following temperatures: 0 °C, 10 °C, 40 °C, 60 °C? Explain your answers.
6. What is yeast?
7. What do yeast cells produce that enables them to use the sugar?
8. How could you prove that the activity of the yeast cells was responsible for making the dough rise?

Microbes and food

Microbes and breadmaking

Yeast is a type of microbe used in breadmaking. It ferments the sugar, releasing carbon dioxide gas which makes the dough rise. You are going to investigate the effect of temperature on the activity of the yeast.

Learning objectives

To show:

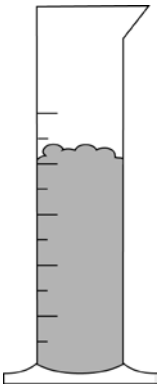
- ▷ the importance of yeast in breadmaking
- ▷ the effect of temperature on yeast activity
- ▷ the relationship between yeast activity and enzyme activity

Procedure

1. Weigh 25 g flour into the beaker and then add 1 g sugar.

2. Measure 30 cm^3 yeast suspension in the small measuring cylinder. Add it to the flour and sugar in the beaker. Stir with the spatula until you have a smooth paste.

3. Carefully pour the paste into the large measuring cylinder. Use a funnel and do not allow the paste to touch the side of the cylinder. You may need to push the paste down the funnel with the spatula. Label the cylinder near the top with your name.



4. Record the volume of the paste in the measuring cylinder. Place the cylinder in one of the three water baths as directed by your teacher. Note the temperature. Start the clock and record the volume of the paste every 5 minutes for about half an hour. Make a table as shown and copy the results for other temperatures from two other groups.

Time (minutes)	Volume		
	20 °C	30 °C	37 °C

5. Plot a graph showing how the volume of paste increased with time. On the same graph, plot the results of other students who used waterbaths at the other temperatures, so that you have three graphs on the same sheet of paper. Answer the questions.

Microbes and food

Preserving food

The various methods of food preservation aim to prevent or delay microbial and other forms of spoilage, and to guard against food poisoning. Such methods therefore help the product to retain its nutritional value, extend its shelf-life and keep it safe for consumption. Preservation techniques include refrigeration, packaging, acidity, chemicals such as nitrite or metabisulphate and heat treatment.

Learning objectives

To show:

- ▷ that microbes can cause food spoilage
- ▷ that controlling microbial growth aids food preservation
- ▷ different methods of food preservation

Age range

Year 7 and above

Duration

Session 1	30 minutes
Session 2	20 minutes
Incubation period:	48 h between sessions

Recommendations

Tube A should be kept in a refrigerator. Tubes B–H should be incubated at room temperature or at 20–25 °C and for at least 48 hours.

Notes

1. The turbidity produced in the tubes is caused by microbes (mainly bacteria) already present on the peas, growing on the nutrients of the vegetable. Very dense turbidity indicates around 10^9 microbes per cm^3 and turbidity just visible to the naked eye about 10^6 per cm^3.
2. When considering the preservatives used, it is interesting to compare the peas with bacon. The latter contains 3–6 % NaCl and small quantities (parts per million) of nitrite. Nitrite content is limited by law because of possible health dangers, but its antimicrobial action is enhanced in the presence of NaCl, in heat-treated foods and at acid pH values of 5–6. As an extension activity, students could experiment with combinations of preservatives in the tubes.

Materials (each group)

- ▷ 24 frozen peas
- ▷ stock flasks containing:
  - dilute (1 %, w/v) sodium chloride solution
  - concentrated (20 %, w/v) sodium chloride solution
  - sodium nitrite (5 %, w/v) solution
  - sucrose (20 %, w/v) solution
  - clear white vinegar
- ▷ 8 test tubes
- ▷ forceps
- ▷ non-absorbent cotton wool
- ▷ marker pen

Questions

Session 1

1. Why is food in shops marked with a 'use-by' date?
2. What causes food to go off?
3. How does vinegar act as a preservative?
4. What other methods of preservation are being used in this investigation? How do they work?
5. Some foods are preserved by vacuum-packing. How does this affect the growth of microbes?
6. How are packet soup and instant potato preserved?
7. How was meat preserved before refrigerators were invented?
8. What methods are used to preserve fish?
9. How do you keep food fresh at home?
10. How do you expect the liquid and/or peas in each tube to appear next lesson? Explain your answers.

Session 2

11. Describe the appearance of the peas and liquid in each tube. Why has the liquid sometimes gone cloudy?
12. Do your results confirm your predictions? If not, suggest reasons for any differences.
13. From your results, describe the effect of temperature on the growth of microbes.

Microbes and food

Preserving food

Most of the food we buy contains small numbers of microbes. These are usually harmless and do not spoil the food. If they multiply, however, they may cause the food to 'go off'. You are going to investigate various methods of preserving food.

Learning objectives

To show:

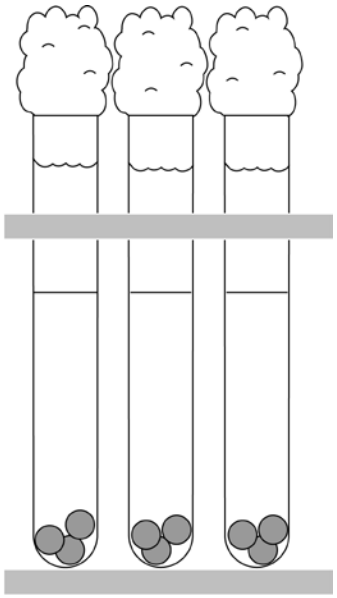
- ▷ that microbes can cause food spoilage
- ▷ that controlling microbial growth aids food preservation
- ▷ different methods of food preservation

Procedure

1. Label the test tubes A to H. Write your name and the date on each one. Using the forceps, put three peas in each tube.

2. Add nothing to tubes A and B. Partly fill tubes C–H as follows:

Tube	Content
A	None
B	None
C	Distilled water
D	Dilute salt solution
E	Concentrated salt solution
F	Sugar solution
G	Vinegar
H	Sodium nitrite solution



3. Plug each tube with cotton wool. Tube A will be kept in a refrigerator until the next lesson. Tubes B–H will be incubated at room temperature.

Safety! *Do not open the tubes.*

Next lesson...

4. Examine your tubes and record the appearance of the peas and the solutions. Answer the questions.

Microbes and food

Microbes and yoghurt making

Yoghurt is usually made from pasteurised low-fat milk that is high in milk solids. The milk is inoculated with a mixed culture of lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and incubated at 35–45 °C for several hours; the pH falls to ca 4·3, coagulating the milk proteins. The bacteria act co-operatively: *L. bulgaricus* breaks down proteins to amino acids and peptides, which stimulate the growth of *S. thermophilus*; formic acid produced by *S. thermophilus* in turn stimulates growth of the lactobacilli which convert lactose in the milk to lactic acid, producing the characteristic flavour.

Learning objectives

To show:

- the importance of microbes in yoghurt making
- that the process involves the production of acid
- the effect of heat on microbes

Age range

Year 7 and above

Duration

Session 1	40 minutes
Session 2	20 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. Add 0·5 % (w/v) glucose to nutrient agar before dispensing in bottles and sterilising. The glucose provides a fermentable substrate for the lactic acid bacteria.
2. Live yoghurt must be used (i.e. not pasteurised and containing no preservatives) or the investigation will not work. The product label often includes the names of the bacteria in the yoghurt.
3. *Resazurin tablets are available from school science suppliers. Dissolve 1 tablet in 50 cm^3 distilled water to give a 0·005 % (w/v) solution. Resazurin is a blue redox indicator dye which changes colour in response to microbial growth. It is pink when oxidised and colourless when reduced. The test gives an indication of the microbial activity of the sample.
4. The beakers of yoghurt need to be removed from the waterbath/incubator after 24 hours and refrigerated.

Notes

This investigation should be carried out in a laboratory. If students wish to make yoghurt which they can taste, then this should be done in a food technology room using a method suitable for kitchen conditions. One is available from the Microbiology Society on request (or see **www.microbiologyonline.org.uk**).

Materials (each group)

- live yoghurt in a 50 cm^3 beaker
- 250 cm^3 UHT milk in a beaker
- resazurin dye (*see left*)*
- Universal indicator
- 2 glucose nutrient agar plates
- distilled water in a wash bottle
- 3 test tubes labelled A, B, C, with bungs
- 2 × 50 cm^3 beakers labelled HY and UY
- 1 × 10 cm^3 syringe
- 1 × 1 cm^3 syringe
- 1 teaspoon
- 2 pipettes and fillers
- wire loop
- waterbaths/incubators: 37 °C, 43 °C
- stopclock
- Bunsen burner, tripod and gauze
- adhesive tape
- clingfilm
- marker pen
- eye protection

Questions

Session 1

1. What is UHT milk? How does it differ from pasteurised milk?
2. Resazurin becomes decolourised by microbial activity. What changes do you expect to see in tubes A, B and C?
3. Describe the effect of heat on the microbes in the yoghurt.
4. What differences do you expect to see in the contents of beakers HY and UY next lesson?
5. What differences do you expect to see between the agar plates next lesson?

Session 2

6. Describe the appearance of the agar plates and the milk in the beakers. Were your predictions correct?
7. What difference, if any, is there in pH between the contents of the beakers?
8. Does this investigation prove that microbes are responsible for yoghurt production?
9. If not, suggest how it could be modified so that it did.
10. What is the name of the sugar present in milk? What happens to it during yoghurt making?
11. Why is it important to ensure that milk used for yoghurt making is free from antibiotics?

Microbes and food

Microbes and yoghurt making

Yoghurt is made by the action of certain microbes on milk. It was first made in hot countries where raw milk goes sour quickly because, in the warmth, microbial numbers increase rapidly. However 'yoghurt' microbes stop the ordinary 'souring' microbes from spoiling the milk. Before the days of pasteurisation, by making it into yoghurt, raw milk could be kept edible for longer. You are going to make some yoghurt and show the presence of microbes in the final product as indicated by the change in colour of a dye.

Learning objectives

To show:

- ▷ the importance of microbes in yoghurt making
- ▷ that the process involves the production of acid
- ▷ the effect of heat on microbes

Techniques required

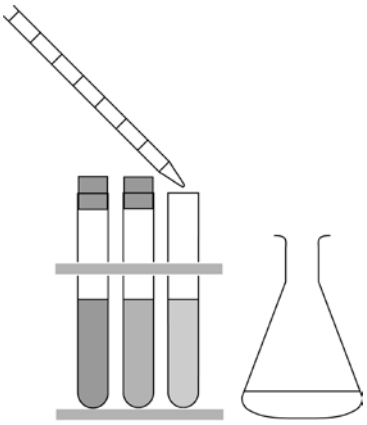
See *Basic Practical Microbiology*

- ▷ using a wire loop (p. 8)

Procedure

1. Put 3 teaspoonfuls of yoghurt into the beaker labelled HY. Wearing eye protection, stir it while heating gently on a tripod and gauze over a Bunsen burner. When it begins to bubble, turn off the gas and leave to cool. Wash the teaspoon.
2. Use the larger syringe to place 10 cm^3 UHT milk into test tube A and 5 cm^3 into each of test tubes B and C.
3. When the heated yoghurt has cooled, use a pipette and filler to put about 5 cm^3 into test tube B. Use a clean pipette and filler to put about 5 cm^3 unheated yoghurt into test tube C.
4. Use the smaller syringe to put 1 cm^3 resazurin dye into each of the three test tubes. Replace the bungs and invert each tube gently several times to mix the contents. Label the tubes with your name and place them in a 37 °C waterbath or incubator. Set the stopclock and leave them for 10 minutes.
5. Whilst the tubes are incubating, heat the wire loop in the Bunsen burner flame. Let it cool and dip it into the heated yoghurt. Lift the lid of one of the agar plates and gently spread the contents of the loop over the agar. Heat the loop again. Replace the lid, tape the plate and invert it. Label the bottom with 'HY', your name and the date.

   Repeat using the unheated yoghurt and the other agar plate, labelling it 'UY'. The plates will be incubated until the next lesson.



Safety! *Do not open the plates.*

6. After 10 minutes, remove test tubes A, B and C from the waterbath. Record the colour of each tube, using one of the shades from the list opposite.

Colour of sample	Quality of milk
Blue (no change)	Excellent
Lilac	Good
Deep pink/mauve	Fair
Pink	Poor
White	Bad

7. Use some of the remaining milk to half fill beaker HY containing the remains of the heated yoghurt. Into beaker UY put a teaspoon of the untreated yoghurt using a clean teaspoon, then half fill with the remaining milk. Cover each beaker with clingfilm. Label each beaker with your name. The beakers will be kept warm at 43 °C for 24 hours and then put in a refrigerator for you to see next lesson.

Next lesson...

8. Look at the beakers and record the appearance and smell of the contents. Test the pH of each with a few drops of Universal indicator. Answer the questions.

Microbes and food

Microbes and food spoilage

Almost any kind of food is a good medium for microbial growth. The process of 'going off' occurs when microbes grow on or in the food, altering its consistency, taste and smell. The processes used to preserve foods (see p. 30) are those that delay or prevent microbial growth. This investigation demonstrates the rate of bacterial growth in food, using a routine method of estimating microbial numbers.

Learning objectives

To show:

- ▷ the rapid increase in microbial numbers during storage of defrosted food
- ▷ the role of microbes in food spoilage
- ▷ the dangers of re-freezing thawed food

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Materials (each group)

- ▷ test tube A containing 3 freshly defrosted peas
- ▷ test tube B containing 3 peas defrosted and left at room temperature for 24 hours
- ▷ 2 nutrient agar plates
- ▷ 2 clean glass rods
- ▷ 2 × sterile 1 cm^3 syringes/pipettes and fillers
- ▷ 2 × sterile 10 cm^3 syringes/pipettes and fillers
- ▷ 150 cm^3 sterile distilled water
- ▷ adhesive tape
- ▷ marker pen
- ▷ 10 test tubes
- ▷ 2 calibrated dropping pipettes
- ▷ beaker of disinfectant

Recommendations

1. Ideally the apparatus should be sterile for best results; it should certainly be very clean.
2. The peas are in tubes to avoid contamination by the students.
3. The agar plates should be poured prior to the investigation to ensure that their surfaces are dry. After inoculation, they should not be disturbed until the drops have dried.
4. The plates should be incubated at 20–25 °C or at room temperature for 2–3 days and refrigerated until examined.
5. A Pasteur pipette can be calibrated by drawing into it a known volume, e.g. 1 cm^3 water, and counting the number of drops formed when this volume is discharged. Alternatively, plastic disposable Pasteur pipettes are available from school science suppliers. The drop size from these is ca 0·02 cm^3.

Notes

1. It is safe to use uncooked vegetables because of the types of microbe present, unlike raw meat which may contain pathogens and must not be used.
2. This is known as the Miles and Misra technique for estimating viable cell counts. Drops that contain large numbers of viable cells give rise to circular areas of confluent growth. Any drop containing less than about 15 viable cells will produce a small, countable number of colonies.

Questions

Session 1

1. Describe some types of food spoilage.
2. Which peas do you expect to have most microbes on them?
3. What is the purpose of making the two dilution series?
4. Why are the samples used in the order suggested?
5. What do you expect your agar plates to look like next lesson?

Session 2

6. Why do some of the six spots on each plate show more growth than others?
7. Do all the colonies look the same? If not, what does this suggest?
8. If one microbe gives rise to each colony, work out how many microbes were in each of the original pea suspensions.
9. Has storage for 24 hours after defrosting made any difference to the numbers of microbes?
10. Why is it unwise to refreeze frozen food that has thawed and been kept at room temperature?
11. Suggest the main sources of error in this investigation. How would you improve it to obtain reliable estimates of microbial numbers?

Microbes and food

Microbes and food spoilage

Although some microbes are deliberately used to make foods such as yoghurt and cheese, other microbes spoil food. Various methods are used to prevent foods from 'going off', such as preservatives, heat treatment, and drying and freezing. You are going to investigate what happens to microbes on frozen peas after the peas have been defrosted and stored.

Learning objectives

To show:

- ▷ the rapid increase in microbial numbers during storage of defrosted food
- ▷ the role of microbes in food spoilage
- ▷ the dangers of re-freezing thawed food

Techniques required

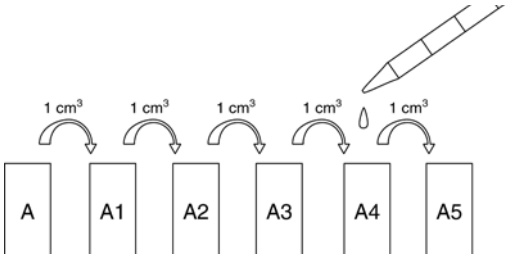
See *Basic Practical Microbiology*

- ▷ using a pipette (p. 9)

Procedure

1. Use the 10 cm^3 syringe or a pipette to add 5 cm^3 distilled water to test tube A containing freshly defrosted peas. Use a glass rod to gently crush the peas in the tube and mix them with the water as thoroughly as possible. Allow the mixture to settle.

2. Label five test tubes A1, A2, A3, A4, A5. Use the 10 cm^3 syringe or pipette to place 9 cm^3 distilled water in each one.



3. Using a 1 cm^3 syringe or pipette, mix the contents of tube A thoroughly by filling and emptying the syringe/pipette several times. Then transfer 1 cm^3 water from tube A to A1. Now transfer 1 cm^3 from A1 to A2. Mix thoroughly after each transfer. Continue to prepare the 'dilution series' as shown.

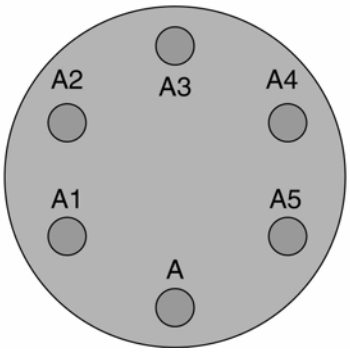
4. Mark an agar plate on the bottom as shown below. Label it with your name, the date and FP. Use the calibrated dropping pipette to draw up a small amount of sample from tube A5. Lift the lid of the Petri dish and release one drop on to the agar at position A5. The drop should be released as closely to the surface of the agar as possible to avoid splashing. Replace the lid. Return any excess sample from the dropping pipette to tube A5. Using the same pipette, repeat with the contents of tubes A4, A3, A2, A1 and A in that order. Discard the pipette into disinfectant. Allow the drops to soak into the agar. Tape the agar plate and invert it.

5. Label the other agar plate with '1DP', your name and the date. Repeat steps 1–4, using the 'day old' peas and clean test tubes and syringes/pipettes. Tape up and invert the plate as before. Both plates will be incubated until the next lesson.

Safety! *Do not open the plates.*

Next lesson...

6. Examine your agar plates and count the number of colonies visible at A, A1, A2, A3, A4, A5. Record your results in a table in two columns headed 'Fresh peas' and '1-day old peas'. Answer the questions.



Microbes and food

Microbes and milk quality

Milk contains water, protein, sugar, fat, vitamins and minerals. It is an excellent medium for growth of many bacteria. Pasteurisation kills pathogens which can be carried in milk, such as *Salmonella* and *Mycobacterium bovis*, as well as much of the natural milk flora. It also inactivates certain bacterial enzymes (e.g. lipases) which would otherwise cause spoilage. Milk is held at a minimum temperature of 72 °C for at least 15 seconds. Some harmless bacteria survive the process, multiplying as the milk ages and causing it to sour. UHT milk is heated to well above boiling point for about 4 seconds, killing virtually all bacteria and spores.

Learning objectives

To show:

- ▷ the presence of microbes in milk
- ▷ that microbial numbers increase in milk during storage
- ▷ the different effects of pasteurisation and ultra-high heat treatment on microbes in milk

Age range

Year 7 and above

Duration

50 minutes

Recommendations

1. The milk should be kept at room temperature for 24 or 48 hours as required.
2. Marking the tubes at 10 cm^3 levels precludes the need for several pipettes.
3. *Resazurin tablets are available from school science suppliers. Dissolve one table in 50 cm^3 distilled water to give a 0·005% (w/v) solution. Resazurin is a blue redox indicator dye which changes colour in response to microbial growth. It is pink when oxidised and colourless when reduced. The test gives an indication of the microbial activity of the sample.

Notes

During incubation of the milk samples, the metabolic activity of the microbes present causes the resazurin to change colour. The extent and range of colour change are a reflection of microbial activity in the sample. The colour code given on the worksheet is a simplification of that formerly used in the dairy industry for grading the keeping quality of milk. This is a simple investigation that gives visual evidence of the differences between samples within the duration of one lesson.

Materials (each group)

- ▷ 6 × 10 cm^3 labelled milk samples:
  - A fresh pasteurised
  - B 24 h pasteurised
  - C 48 h pasteurised
  - D fresh UHT
  - E 24 h UHT
  - F 48 h UHT
- ▷ 20 cm^3 resazurin dye solution (*see left*)*
- ▷ 6 test tubes marked at 10 cm^3, with bungs
- ▷ pipette and filler/syringe
- ▷ waterbath at 37 °C
- ▷ stopclock
- ▷ marker pen

Questions

1. What causes milk to go sour?
2. Does UHT milk go sour when kept? If not, why not?
3. What parameter could you use to assess the quality of milk?
4. How reliable do you think the resazurin test is? List the advantages and disadvantages of the test.
5. What is causing the dye to change colour?
6. Why are the tubes incubated at 37 °C?
7. Explain your results. What difference do they indicate between UHT and pasteurised milk?
8. How could you improve the reliability of your results?
9. What further investigations could you carry out to test the conclusions in your answer to question 7?

Microbes and food

Microbes and milk quality

Milk is a good source of food for microbes as well as for ourselves. Pasteurisation is a heating process which kills the harmful microbes that sometimes are found in raw milk. Some microbes survive this process and can cause the milk to 'go off'. Ultra-high temperature (UHT) milk is heated to a much higher temperature, killing virtually all bacteria. The number of microbes in milk is a measure of its quality. This can be estimated by finding the rate of change of colour of resazurin dye.

Learning objectives

To show:

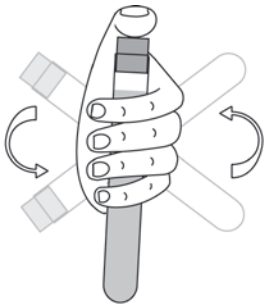
- ▷ the presence of microbes in milk
- ▷ that microbial numbers increase in milk during storage
- ▷ the different effects of pasteurisation and ultra-high heat treatment on microbes in milk

Procedure

1. Label your test tubes A–F, together with your name. Use a syringe or calibrated pipette and filler to place 2 cm^3 resazurin dye in the bottom of each tube.

2. The key to the milk samples is as follows:

A	fresh pasteurised
B	24h pasteurised
C	48h pasteurised
D	fresh UHT
E	24h UHT
F	48h UHT



Add milk sample A to tube A up to the 10 cm^3 mark. Replace the bung and turn the tube upside down three times. Repeat for the other five milk samples and tubes.

3. Record the colour of the contents of each tube, using the best match from the list opposite.

   Place the tubes in the waterbath and start the stopclock. Record the colours every 5 minutes for 30 minutes in a table as shown below. Answer the questions.

Colour of sample	Quality of milk
Blue (no change)	Excellent
Lilac	Good
Deep pink/mauve	Fair
Pink	Poor
White	Bad

Safety! *Do not open the tubes.*

Time (minutes)	Sample					
	A	B	C	D	E	F
0						
5						
10						
15						
20						
25						
30						

Health and hygiene

Effects of antiseptics on microbes

Antiseptics are used in the disinfection of living tissue. They may be used prophylactically (i.e. to prevent infection) or therapeutically (i.e. to treat infection). Any given antiseptic is usually more effective against some microbes than others and its activity may be greatly affected by factors such as dilution, temperature, pH or the presence of organic matter or detergent.

Learning objectives

To show:

- ▷ the effects of antiseptics on microbial growth
- ▷ the effects of dilution of antiseptics on microbial growth

Age range

Year 9 and above

Duration

Session 1	50 minutes
Session 2	20 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. The culture of *Bacillus subtilis* should be inoculated in nutrient broth and incubated at 25 °C at least 48 hours before the lesson.
2. Any proprietory antiseptic can be used.
3. The plates should be incubated preferably at 20–25 °C for 2–3 days, but can be kept at room temperature.
4. Filter paper discs (Whatman Antibiotic Assay discs) are available from school science suppliers or they can be cut from Whatman No. 1 filter paper with a cork borer (ca 6 mm diameter) or a hole punch.
5. When students are mixing the agar and the culture in the Petri dish, they should be instructed to swirl the contents gently a few times clockwise, anti-clockwise, forwards and sideways.

Notes

1. Other suitable bacteria include e.g. *Micrococcus luteus*, *Escherichia coli* and *Bacillus subtilis*.
2. Inhibition of growth is shown by a clear area around the disc. Growth appears either as many small colonies visible to the naked eye or, if a large volume of cells was added, a confluent area of turbidity.
3. The same approach can be used to investigate the effects of other antimicrobial compounds – see p. 43.

Materials (each group)

- ▷ *Bacillus subtilis* culture in nutrient broth
- ▷ Universal bottle containing ca 20 cm^3 molten nutrient agar held at ca 45–50 °C
- ▷ 70 % (v/v) industrial denatured alcohol in a small beaker or glass Petri dish covered in foil (*CAUTION: flammable*)
- ▷ Petri dish
- ▷ sterile dropping pipette
- ▷ ca 10 cm^3 antiseptic, e.g. TCP, Dettol, in Universal bottle labelled A
- ▷ 2 Universal bottles labelled B and C
- ▷ distilled water in a beaker
- ▷ beaker of disinfectant
- ▷ 10 cm^3 syringe or pipette and filler
- ▷ metal forceps (can be pre-sterilised by autoclaving)
- ▷ 4 sterile paper discs
- ▷ Bunsen burner
- ▷ adhesive tape
- ▷ marker pen
- ▷ graph paper

Questions

Session 1

1. What are antiseptics?
2. What is the difference between antiseptics and antibiotics?
3. What might affect the growth of microbes between now and the next lesson?
4. What is the concentration of antiseptic in bottles B and C?
5. Why are the discs added in the order suggested?
6. What do you expect the appearance of your agar plates to be next lesson?

Session 2

7. Do your results support the predictions you made last lesson?
8. Describe the range of results obtained by the class for each dilution. Suggest possible reasons for any differences between the groups.
9. Do the class results all indicate the same overall pattern? If so, what conclusions can you draw from them?
10. What further experiments could you do to confirm these conclusions?

Health and hygiene

Effects of antiseptics on microbes

Antiseptics are used to reduce the numbers of microbes in living tissue, e.g. from a cut or graze. They are milder than disinfectants that are used to clean objects and surfaces. You are going to investigate the effect of different dilutions of an antiseptic on microbes.

Learning objectives

To show:

- ▷ the effects of antiseptics on microbial growth
- ▷ the effects of dilution of antiseptics on microbial growth

Techniques required

See *Basic Practical Microbiology*

- ▷ using a pipette (p. 9)
- ▷ flaming the neck of a bottle (p. 10)
- ▷ sterilizing forceps (p. 7)

Procedure

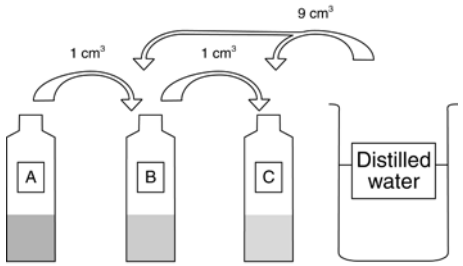
1. Light your Bunsen burner. Remove the cap from the bottle containing the culture of *Bacillus subtilis*. Pass the neck through the Bunsen burner flame 2–3 times. Remove a few drops of culture with the sterile dropping pipette. Flame the bottle neck again and replace the cap.

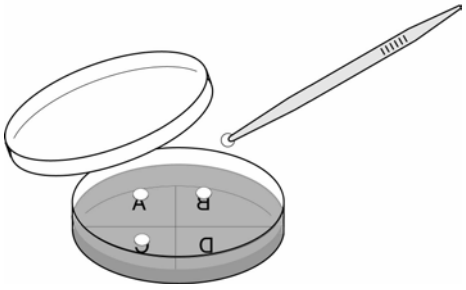
2. Lift the lid of the Petri dish. Place 2–5 drops of culture in the centre of the dish. Replace the lid as quickly as possible. Discard the dropping pipette into the beaker of disinfectant.

3. Take the bottle of melted agar. Remove the cap and pass the neck through the Bunsen burner flame 2–3 times. Pour the contents into the Petri dish carefully. Replace the lid as quickly as possible. Flame the neck again and replace the cap on the bottle.

4. Keeping the Petri dish flat on the bench, mix the agar thoroughly by gently swirling the plate as directed by your teacher. Avoid splashing the agar on to the lid or over the edge of the dish. Allow the agar to set. This is known as a pour plate.

5. Use a pipette and filler (or syringe) to place 9 cm^3 distilled water into empty bottles B and C. Using the same pipette, transfer 1 cm^3 antiseptic from bottle A to bottle B. Gently shake B to mix the contents, then transfer 1 cm^3 from B into C.





Safety! *Do not open the plates.*

6. When the agar has set, turn the dish upside down. Divide the base into four sections by drawing a cross on it with a marker pen. Label the sections A, B, C and D. Turn the dish over again.

7. Take sterile forceps, or sterilise them by dipping in alcohol (keeping the points facing downwards) and passing quickly through the Bunsen burner flame.

8. Using the sterile forceps, dip a paper disc into the remaining distilled water. Drain excess liquid from it, then place it on section D of the agar. Replace the lid as soon as possible.

9. Repeat for the other three sections using the samples in the order C, B, A. Tape and label the dish with your name and the date. It will be incubated until the next lesson.

Next lesson...

10. Examine your agar plate. Place it on a sheet of graph paper and record the diameter of any clear areas around the discs. Answer the questions.

Health and hygiene

Microbes and personal hygiene

This investigation uses the harmless yeast *Saccharomyces cerevisiae* to simulate the contamination of hands with faecal microbes and the effectiveness of handwashing in removing them. Different brands and thicknesses of toilet paper and different types of soap may be evaluated.

Learning objectives

To show:

- the permeability of toilet paper to microbes
- the importance of handwashing after visiting the lavatory
- how pathogens can be transmitted

Age range

Year 7 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. To prepare lawn cultures of *S. cerevisiae* inoculate malt extract agar plates with a few drops of the culture in malt extract broth. Spread the liquid evenly over the surface of the agar using a sterile glass rod spreader and incubate for 48 hours at 20–25 °C.
2. Even though all plates will be sterilised, it is important that students wash their hands thoroughly as directed to reduce the risk of inadvertently culturing organisms already present on the skin surface. Sterile gloves could also be worn.
3. All toilet paper should be placed in the autoclave bag immediately after use.
4. A combination of the traditional, smooth-style toilet paper and soft paper should be provided for comparison. Different thicknesses of paper could also be tested with different groups.

Materials (each group)

- 3 lawn cultures of *Saccharomyces cerevisiae* on malt extract agar*
- 3 malt extract agar plates
- different brands of toilet paper
- different types of soap
- autoclave bag
- adhesive tape
- marker pen

Malt extract agar. Dissolve 15 g malt extract and 18 g bacteriological agar in 1 l distilled water. Dispense into bottles and sterilise by autoclaving. Malt extract agar is also available from school science suppliers.

Questions

Session 1

1. What kinds of microbes occur in the human intestine?
2. Why is it important to wash your hands (a) before meals, (b) after using the lavatory?
3. What is an infectious disease?
4. What are the main defence mechanisms of the body against microbial infection?
5. What do you expect the appearance of dishes A, B and C to be next lesson?

Session 2

6. Does the appearance of your dishes match your predictions?
7. Are the class results consistent? If not, suggest reasons for any differences.
8. Do the class results show any evidence of differences in effectiveness of (a) toilet papers, (b) soaps?
9. What do the results suggest about personal hygiene procedures?
10. Suggest as many methods as you can to prevent the spread of infectious disease.

Health and hygiene

Microbes and personal hygiene

This investigation uses a harmless microbe, *Saccharomyces cerevisiae* (the bread-making yeast), to show that hands can be contaminated by microbes during use of the toilet. The permeability of different toilet papers and the effectiveness of handwashing in removing the microbes is also investigated.

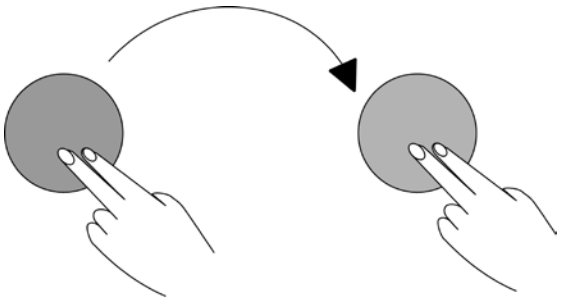
Learning objectives

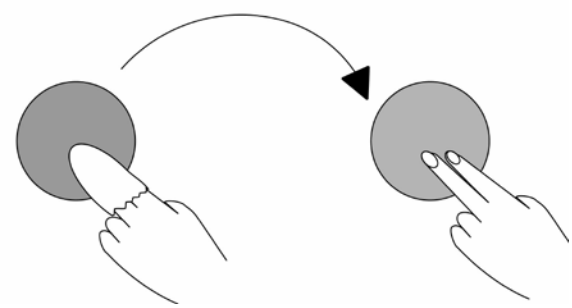
To show:

- the permeability of toilet paper to microbes
- the importance of handwashing after visiting the lavatory
- how pathogens can be transmitted

Procedure

1. Label the bases of three sterile malt agar plates A, B and C and with your name and the date. Wash your hands thoroughly using hot water and soap, then dry them on a clean paper towel. Open one lawn plate of *Saccharomyces cerevisiae* and wipe two fingers lightly over the surface. Next lift the lid of dish A, touch the agar surface lightly with the same two fingers and quickly replace the lid. WASH YOUR HANDS THOROUGHLY.





2. Wrap two fingers in a layer of the toilet paper provided. Open another plate of *Saccharomyces* and wipe the wrapped fingers lightly over the surface. Try to wipe them in as similar way as possible to step 1. Remove the toilet paper and place it in the bag provided. Then lift the lid of plate B, touch the agar surface lightly with the same two fingers and quickly replace the lid. WASH YOUR HANDS THOROUGHLY.

3. Repeat step 2 using a fresh dish of *Saccharomyces* and plate C. After removing the toilet paper and discarding it into the bag, wash your hands thoroughly with the type of soap provided and dry them on a clean paper towel. Touch the surface of plate C with your washed fingers and quickly replace the lid. WASH YOUR HANDS THOROUGHLY.

4. Tape all the dishes and turn them upside down. They will be incubated until the next lesson.

Safety! *Wash your hands before leaving the lab.*

Next lesson...

5. Examine your agar plates without opening them. Answer the questions.

Open-ended investigation

Investigating microbes and cellulose

Research brief

Using this information and other facts you have researched, plan an investigation into some aspect of the production of cellulase enzymes by microbes, or the effects of the enzymes. A basic procedure for testing the cellulolytic activity of microbes is described on p. 17. You may wish to use a different test according to the type of investigation you are planning.

Background

- Cellulose is a fibrous substance that helps to provide plants with a rigid structure. It is the most important plant polymer, making up some 40–50% of the mature plant cell wall. It is also the most abundant carbohydrate. The molecules are very large and long and contain carbon, hydrogen and oxygen. Cellulose is a very stable substance at ordinary temperatures, and the types of microbe that can decompose and thus recycle it are extremely important in sustaining the turnover of organic matter for the rest of the living world. It often occurs in a complex mixture with lignin (another plant polymer) called lignocellulose, in wood, forest and agricultural wastes, and in waste paper.
- Cellulose is not soluble in water, so microbes cannot absorb it into their cells. Instead, once close to it, they secrete cellulase enzymes which partly digest the cellulose. It is broken down into soluble sugar molecules which the microbes can absorb and use. Higher organisms do not make cellulases, which means that herbivores cannot digest cellulose themselves. Instead they depend on cellulolytic bacteria in their intestinal tracts to do the job for them.
- On land the major decomposers of cellulose are fungi, aided by a few aerobic and anaerobic bacteria. In marine habitats bacteria are primarily responsible for breaking down cellulose, but in fresh water aquatic fungi carry out this function in well aerated zones, with bacteria playing an increasingly important role as the amount of oxygen diminishes. Cellulolytic bacteria include species of *Cellulomonas*, *Pseudomonas* and *Ruminococcus*. Cellulolytic fungi include *Chaetomium*, *Fusarium*, *Myrothecium* and *Trichoderma*.

Designing your investigation

In designing your investigation you should consider the experimental and investigative skills to be assessed. These are:

- planning
- implementing
- analysing evidence and drawing conclusions
- evaluating evidence and procedures

(*See your coursework guide for further details*).

Points to consider when planning your investigation:

- Any hazards involved in doing the experiment and how the risks of the procedure can be minimised. (see *Basic Practical Microbiology: A Manual*)
- Whether you can make a prediction that you can test (e.g. cellulolytic activity will increase with temperature).
- What variables need to be taken into account (e.g. the source of the soil samples, the mixing of the soil suspension).
- How many tests you need for reliability (e.g. how many replicates of each treatment).

Examples

Here are some ideas that could be explored:

- The effect of temperature on the activity of cellulolytic microbes or cellulases (from school science suppliers)
- The cellulolytic activity of microbes from different soils
- The effect on cellulolytic activity of adding different nutrients to the soil samples
- Whether fungi or bacteria are more important in terms of cellulolytic activity, in particular types of soil

Writing Up

Your report should include the following sections, in this order: Introduction/plan; Hypothesis; Prediction; Materials and Methods (including a risk assessment); Results; Discussion; Evaluation/Conclusion.

Safety!

Your plan MUST be checked by your teacher before starting your investigation.

Open-ended investigation

Investigating the effects of antimicrobials

Research brief

Using this information and other facts you have researched, plan an investigation into some aspect of antimicrobial substances. The basic procedure for testing the effects of antimicrobials is described on p. 39. You may wish to modify this according to the type of investigation you are planning. For example, antibiotic production by soil bacteria can be investigated by inoculating a nutrient agar plate with some soil, incubating it and looking for zones of inhibition.

Background

- Antimicrobial substances include disinfectants, antiseptics and antibiotics. Disinfection is a procedure that destroys or inactivates microbes. It usually involves the treatment of non-living objects such as surfaces or liquids with chemicals (disinfectants) e.g. chlorine, phenols and hypochlorites. Antisepsis is the disinfection of living tissues with chemicals (antiseptics) e.g. hydrogen peroxide, iodine and diluted alcohol. Antibiotics are chemicals that, even at very low concentrations, inhibit or kill certain microbes. Penicillins are a well-known group of antibiotics.
- Disinfectants and antiseptics that kill bacteria are said to be bactericidal. Others merely halt the growth of bacteria and if inactivated, e.g. by dilution, bacterial growth may be resumed. These are said to be bacteriostatic. A bactericidal disinfectant or antiseptic may become bacteriostatic when diluted.
- Antibiotics are produced by microbes as a natural defence against other microbes. Some are still produced commercially using micro-organisms, although a large number are manufactured chemically. Some antibiotics are active against a narrow range of species whilst others affect a broad spectrum of organisms. The ability to make antimicrobial substances is not limited to microbes; most animals have antibacterial substances in their bodily secretions, such as lysosyme in sweat and tears. Plant materials such as garlic (*Allium sativum*), tea tree oil (*Melaleuca alternifolia*) and oil of cloves (*Syzyium aromaticum*) also have antimicrobial properties.

Designing your investigation

In designing your investigation you should consider the experimental and investigative skills to be assessed. These are:

- planning
- implementing
- analysing evidence and drawing conclusions
- evaluating evidence and procedures

(*See your coursework guide for further details*).

Points to consider when planning your investigation:

- Any hazards involved in doing the experiment and how the risks of the procedure can be minimised. (see *Basic Practical Microbiology: A Manual*)
- Whether you can make a prediction that you can test (e.g. higher concentrations of antimicrobial substance will be more effective).
- What variables need to be taken into account (e.g. incubation temperature, pH).
- How many tests you need for reliability (e.g. how many replicates of each treatment).

Examples

Here are some ideas that could be explored:

- The antimicrobial effects of different plant extracts
- The effect of concentration on the inhibitory effects of different antimicrobial agents
- Antimicrobial effects of different toothpastes, mouthwashes or deodorants
- Effects of antimicrobials on different microbes

Writing Up

Your report should include the following sections, in this order: Introduction/plan; Hypothesis; Prediction; Materials and Methods (including a risk assessment); Results; Discussion; Evaluation/Conclusion.

Safety!

Your plan MUST be checked by your teacher before starting your investigation.

Further resources

Websites

A wide range of information on practical microbiology is posted on the Microbiology Society's dedicated education website at www.microbiologyonline.org.uk and on MiSAC's website at www.misac.org.uk.

Practical techniques

*Basic Practical Microbiology: A Manua*l (Microbiology Society) provides details of the techniques used in the present resource, with particular emphasis on health, safety, aseptic technique and GMLP. It also includes culture maintenance and preparation and microscopy. The manual can be downloaded from www.microbiologyonline.org.uk

Sources of cultures, equipment and consumables

The following companies supply cultures and/or equipment for practical microbiology in schools. Inclusion in the list implies no recommendation. Before buying a culture ensure that the organism is suitable for use in schools by referring to Topic 15 in *Topics in Safety*, 3rd edition (Association for Science Education, 2001) or seeking appropriate advice (see below).

Blades Biological	Cowden Edenbridge Kent TN8 7DX	Tel. 01342 850242 Email sales@blades-bio.co.uk www.blades-bio.co.uk	Algae, protozoa, fungi and bacteria; culture media and antibiotic discs
National Centre for Biotechnology Education (NCBE)	University of Reading 2 Earley Gate Whiteknights Reading RG6 6AU	Tel. 0118 987 3743 Email ncbe@reading.ac.uk www.ncbe.reading.ac.uk	Limited range of cultures of bacteria and fungi; items for microbiology and molecular biology; microbiology kits
Philip Harris	Hyde Buildings Ashton Road Hyde Cheshire SK14 4SH	Tel. 0845 120 4520 Email orders@philipharris.co.uk www.philipharris.co.uk	Full range of cultures, media and equipment for practical microbiology
Sciento	61 Bury Old Road Whitefield Manchester M45 6TB	Tel. 0161 773 6338 Email sales@sciento.co.uk www.sciento.co.uk	Algae, protozoa, fungi, and bacteria; culture media and antibiotic discs

Sources of advice, including health and safety

The sources listed above will provide most of the information and guidance necessary to carry out the investigations in this book. Where further advice is required, the following organisations will be pleased to help.

Microbiology Society – Email education@microbiologysociety.org ; www.microbiologysociety.org; www.microbiologyonline.org.uk

MiSAC – Email microbe@misac.co.uk; www.misac.org.uk

CLEAPSS – Tel. 01895 251496; Email science@cleapss.org.uk; www.cleapss.org.uk

SSERC – Tel. 01383 626070; Freephone 0800 840 6998; Email sts@sserc.org.uk; www.sserc.org.uk

NCBE – Email ncbe@reading.ac.uk; www.ncbe.reading.ac.uk

Microbiology Society

The Microbiology Society is a membership organisation for scientists who work in all areas of microbiology. It is the largest learned society in Europe with a membership based in universities, industry, hospitals, research institutes and schools. An important function of the Society is the promotion of the public understanding of microbiology. The Microbiology Society produces and distributes a wide range of resources to support microbiology teaching in schools and colleges across all Key Stages and post-16. The Society also offers an information service to teachers and participates in schools competitions and other activities. The Microbiology Society has charitable status.

School Membership of the Microbiology Society offers many benefits including:

- *Microbiology Today*, the award-winning magazine
- Grants to develop teaching resources and outreach activities
- Priority mailings of Society teaching resources

Microbiology Society, Charles Darwin House, 12 Roger Street, London WC1N 2JU, UK

Tel. +44 (0)20 7685 2682

Email education@microbiologysociety.org

Web www.microbiologyonline.org.uk

Microbiology in Schools Advisory Committee (MiSAC)

MiSAC exists to promote the teaching of microbiology in schools. Founded in 1969, it consists of representatives of a wide range of educational and scientific bodies who work to help teachers recognise the potential of micro-organisms as educational resources in a variety of ways. MiSAC is a recognised source of authoritative advice on the safe use of micro-organisms, develops practical protocols, produces a regular bulletin *MiSAC Matters*, *Briefings* and *Activities* sheets, responds to government reports and maintains contact with examining bodies and school science suppliers. The annual MiSAC competition for schools is a major activity supported by sponsorship from professional organisations and industry. Core activities depend on the voluntary work of its members and financial support from the organisations they represent. MiSAC has charitable status.

c/o NCBE, University of Reading, 2 Earley Gate, Whiteknights, Reading RG6 6AU

Email microbe@misac.co.uk

Web www.misac.org.uk

Micro-organisms, bacteria, archaea, viruses, fungi, protozoa and algae are everywhere, and their activities are vitally important to virtually all processes on Earth. They play key roles in nutrient cycling, biodegradation/biodeterioration, global warming, food spoilage, the cause and control of disease, and biotechnology. Thanks to their versatility, microbes can be put to work in many ways: making life-saving drugs; the manufacture of biofuels; cleaning up pollution; and producing/processing food and drink.

This resource offers practical activities which enable the student to explore the exciting world of microbes, set within the context of course specifications and teaching the investigative skills required by the National Curriculum and equivalent qualifications in schools and colleges. Each of the investigations is safe and straightforward but also rewarding for the student. A teacher guide and student worksheet clearly sets out the requirements for each activity and the procedure to be followed. The text is accompanied by helpful line drawings.

The resource has been compiled by members of the Microbiology in Schools Advisory Committee (MiSAC), who are all experts of longstanding in microbiology teaching.

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