

## 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

#### Procedure

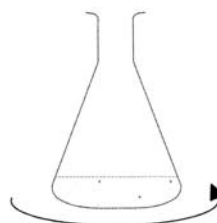
1. Label your tubes. You are going to set them up as follows:

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<sup>3</sup> nutrient broth in tube A.

3. Place the soil and 30 cm<sup>3</sup> 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<sup>3</sup> 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 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
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### 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
- ▷ 120 cm<sup>3</sup> sterile nutrient broth
- ▷ 5 g soil
- ▷ 250 cm<sup>3</sup> conical flask
- ▷ sterile 5 cm<sup>3</sup> graduated pipette and filler
- ▷ marker pen
- ▷ 1 × 2 cm<sup>2</sup> strips of different types of paper:
  - filter paper (× 2)
  - unprinted newspaper
  - heavily printed newspaper
  - glossy magazine cover
  - thin cardboard

### 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?

## 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.