

# Advanced Biofuels

## Activity 2D - Bacterial cellulase

**Learning outcomes:** By the end of the session students should be able to:

- Describe the use of cellulose in paper and sources of naturally produced cellulases.
- Carry out an experiment to investigate the presence of cellulase producing bacteria in soil.
- Assess the pros and cons of the method for identifying cellulase producing bacteria.

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, cellulose, cellulase, lignocellulose, microbes, yeast, enzyme, fermentation, varieties, bioprospecting.

## Background

The production of cellulases by bacteria can be investigated by sourcing bacteria such as *Cellulomonas sp.* which produces extracellular cellulose or *Pseudomonas fluorescens*, or testing samples of soil, and incubating them in nutrient broth with paper as a source of cellulose. Bioprospecting involves searching in suitable environments for organisms that have beneficial features for producing biofuels. Researchers focus on agricultural and forestry microecosystems where fermentation is taking place such as manure, leaf litter, rotting wood and straw rich soil. Once researchers isolate yeast, bacteria or filamentous fungi, they can create a profile of the phenotype by testing their ability to ferment different biofuel feedstocks and resist pretreatment conditions.

In this activity students can carry out their own bioprospecting to see if they can discover cellulase producing bacteria and test their ability to breakdown the cellulose in paper. This activity is based on one from the Society of General Microbiology (SGM) publication Practical Microbiology for Secondary Schools, available from [www.microbiologyonline.org.uk/teachers/resources](http://www.microbiologyonline.org.uk/teachers/resources).

**Age Range:** This experiment is suitable for primary and secondary students.

**Duration:** set up 30 minutes, incubation 1-2 weeks.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, soil constituents, enzymes, the carbon cycle, decomposition and ecosystems.

## What you will need

- Conical Flasks
- Test tubes
- Test tube rack
- Pipettes
- Nutrient broth
- Paper samples cut into strips
- Cotton wool
- Soil
- Nitrile gloves
- Eye protection

### Optional

- *Cellulomonas sp.*

# Advanced Biofuels

## Health and Safety

Use disposable pipettes or autoclave the pipettes afterwards. Ensure hands are washed following this activity. Be aware of the risk of inadvertently culturing pathogenic microorganisms and treat this activity as if potentially harmful microorganisms could be cultured from the soil samples. Seal the test tubes and do not allow students to open the test tubes once they have been incubated. Paper samples should only be observed inside the test tubes while recording results. The samples should be disposed of appropriately using disinfectant or autoclaving and glassware should be decontaminated.

The following factors should be considered when planning to carry out any investigations involving microorganisms: nature of the organism used, source of the organism, temperature of incubation, culture medium used, type of investigation and the facilities available, chance of contamination, expertise of people involved. If necessary change the conditions or limit the involvement of students perhaps by carrying out the experiment as a demonstration. CLEAPSS® handbook - “perfectly safe if the organisms studied are known to be non-pathogenic, such as brewer’s and baker’s yeast, the bacteria in yoghurt or edible mushrooms”.

CLEAPSS® laboratory handbook – section 15.2 Microbiology (COSHH, good practice and safety precautions, levels of practical work, using microorganisms in practical work, equipment and materials, sterilisation and disinfection) page 1505.

CLEAPSS® Guidance PS 04 (COSHH: risk assessments in situations where microorganisms might be involved).

CLEAPSS® guides R101 (Steam sterilisation: Autoclaves & pressure cookers).

CLEAPSS® Model risk assessment 3.026 (Microorganisms used in food production).

Further advice can also be sought from the Society for General Microbiology [www.microbiologyonline.org.uk/teachers/safety-information](http://www.microbiologyonline.org.uk/teachers/safety-information) and the Microbiology in Schools Advisory Committee.

VirKon is a suitable disinfectant for general surface cleaning and sterilisation as well as for discard pots (follow manufacturer’s instructions).

For Gram staining and preparation of slides see CLEAPSS® Guidance leaflet 95, Recipe sheet 90 and Hazcards 32, 36A, 40A and 85.

Methanal (CLEAPSS® Hazard 63) Toxic. Students should wear eye protection when observing test tubes with added methanol.

## Method

1. Make up nutrient broth in a conical flask and autoclave.
2. Collect soil samples or obtain a sample of *Cellulomonas*.
3. Set up test tubes as below and label with contents, name and date.

|                      |           |              |            |              | Control   |
|----------------------|-----------|--------------|------------|--------------|-----------|
| Nutrient             | ✓         | ✓            | ✓          | ✓            | ✓         |
| Soil or Cellulomonas | ✓         | ✓            | ✓          | ✓            | ✗         |
| Paper                | Newspaper | Filter paper | Rice paper | Glossy paper | Newspaper |

# Advanced Biofuels

4. Add 5 ml of nutrient broth to the control tube using a pipette, and seal.
5. Add the soil sample to 30 ml of nutrient broth in a conical flask. Swirl the flask to form an evenly distributed soil suspension and then allow the particulate debris to settle for 1-2 minutes.
6. Add 5 ml of the soil suspension to each test tube using a pipette.
7. Add the paper samples to the tubes and seal the tubes.
8. Incubate the test tubes for about 1 week at room temperature.
9. If the nutrient broth turns cloudy indicating bacterial growth add 1 drop of 40% methanal solution per 10 ml of broth to each test tube to kill the bacteria before allowing students to examine them.

## Extension activities

If you can source cellulase producing bacteria such as *Cellulomonas sp.* for the students, or students identify a particularly effective source of cellulase-producing microorganisms, their ability to resist lignocellulosic bioethanol pretreatment conditions can be investigated. Bacteria could be exposed to varying temperatures or alkali treatments prior to repeating the test with different types of paper. If exposing bacteria to varying temperatures, ensure that they are exposed for a short duration and that temperatures above 30°C are not used to avoid growing any potentially pathogenic microorganisms.

To demonstrate the presence of bacteria and microorganisms in the soil samples students could examine samples under the microscope. Students can create a soil suspension with water and then visualise the bacteria, by using a technique such as Gram staining, before placing on a slide.

## Suppliers

A variety of bacteria including cellulase producing *Cellulomonas* on agar slopes can be obtained from National Centre for Biotechnology Education (NCBE) [www.ncbe.reading.ac.uk/menu.html](http://www.ncbe.reading.ac.uk/menu.html) University of Reading, 2 Earley Gate, Whiteknights Road, Reading RG6 6AU tel: 0118 9873743, fax: 01189 750140 or *Pseudomonas fluorescens* on agar slopes can be obtained from Blades Biological Limited [www.blades-bio.co.uk](http://www.blades-bio.co.uk) Cowden, Edenbridge, Kent TN8 7DX tel:01342 850 242, fax: 01342 850 924

## Further reading and links

Ahmad, M. *et al*, 2011. Identification of DypB from *Rhodococcus jostii* RHA1 as a Lignin Peroxidase, *Biochemistry*, **50** (23), 5096–5107.

Alterthum, F., & Ingram, L.O., 1989. Efficient ethanol–production from glucose, lactose, and xylose by recombinant *Escherichia coli*. *Applied Environmental Microbiology*, **55**, 1943–1948.

Goncet, C., Messenger, S., 2010. Biofuels from waste. *Catalyst: Secondary Science Review*, **21**, Issue 1, pages 6-8. Practical Biology and SGM: Microbes ate my homework/Microbes and cellulose (2007), [www.practicalbiology.org](http://www.practicalbiology.org)

Burdass, D., Grainger, J.M. and Hurst, J.(editors) 2006, Basic Practical Microbiology – A Manual and Grainger, J. M. and Hurst, J. (editors) 2007, Practical Microbiology for Secondary schools available free from the Society for General Microbiology (SGM) [www.microbiologyonline.org.uk/teachers/resources](http://www.microbiologyonline.org.uk/teachers/resources)

# Advanced Biofuels

First wood-digesting enzyme found in bacteria could boost biofuel production [www.bbsrc.ac.uk/news/industrial-biotechnology/2011/110609-pr-wood-digesting-enzyme.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2011/110609-pr-wood-digesting-enzyme.aspx)

Cows' stomachs could hold key to green fuels [www.roslin.ed.ac.uk/news/2011/07/29/cows%27-stomachs-could-hold-key-to-green-fuels/](http://www.roslin.ed.ac.uk/news/2011/07/29/cows%27-stomachs-could-hold-key-to-green-fuels/)

The Royal Society, January 2008. *Sustainable biofuels: prospects and challenges*, ISBN 978 0 85403 662 2. <http://royalsociety.org/Sustainable-biofuels-prospects-and-challenges/>

Nuffield Council on Bioethics, April 2011, *Biofuels: ethical issues* [www.nuffieldbioethics.org/biofuels-0](http://www.nuffieldbioethics.org/biofuels-0)

## Research groups

Professor Nigel Peter Minton, BSBE Second Generation, Sustainable, Bacterial Biofuels Programme, School of Molecular Medical Sciences, The University of Nottingham, Nottingham, NG7 2RD [www.bsbec.bbsrc.ac.uk/programmes/second-generation-sustainable-bacterial-biofuels.html](http://www.bsbec.bbsrc.ac.uk/programmes/second-generation-sustainable-bacterial-biofuels.html) [www.nottingham.ac.uk/bioenergy/index.aspx](http://www.nottingham.ac.uk/bioenergy/index.aspx)

Professor Katherine Smart, BSBE LACE Programme, School of Biosciences, University of Nottingham, Sutton Bonington Campus [www.bsbec.bbsrc.ac.uk/programmes/index.html](http://www.bsbec.bbsrc.ac.uk/programmes/index.html) [www.nottingham.ac.uk/bioenergy/index.aspx](http://www.nottingham.ac.uk/bioenergy/index.aspx) [www.nottingham.ac.uk/bioenergy/lace/](http://www.nottingham.ac.uk/bioenergy/lace/)

Biofuel Research Centre, Edinburgh Napier University [www.napier.ac.uk/bfrc](http://www.napier.ac.uk/bfrc)

Professor Timothy Bugg, Department of Chemistry, University of Warwick [www2.warwick.ac.uk/fac/sci/chemistry/research/bugg/bugggroup/research/](http://www2.warwick.ac.uk/fac/sci/chemistry/research/bugg/bugggroup/research/)

Professor Frank Sargent, Molecular Microbiology, College of Life Sciences, University of Dundee [www.lifesci.dundee.ac.uk/groups/frank\\_sargent/](http://www.lifesci.dundee.ac.uk/groups/frank_sargent/)

Professor David Archer, BSBE LACE programme Strand 2, University of Nottingham, [Regulation of cellulase enzyme expression in Trichoderma](http://www.nottingham.ac.uk/bioenergy) [www.nottingham.ac.uk/bioenergy](http://www.nottingham.ac.uk/bioenergy) [www.nottingham.ac.uk/bioenergy/lace](http://www.nottingham.ac.uk/bioenergy/lace)