

# Advanced Biofuels

## Activity 3C - Algae chromatography

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

- Extract pigment from algae.
- Separate and compare the pigments in red and green algae.
- Analyse the distance of pigment migration.

**Keywords** Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, yield, waste, photosynthesis, algae, varieties, chromatography.

The green algae contain chlorophyll a and chlorophyll b (green pigments). The red algae contain chlorophyll a and phycobilin (a red pigment). Although chlorophyll is the major pigment for photosynthesis, the other pigments help algae harvest light of different wavelengths, which is useful when they are deeper in water. You should point out to the students that algae are simple (lower) plants. It is also worth mentioning that all land plants originated from one group of green algae.

The aim of this activity is to show the diversity of algae, and to demonstrate that red algae also contain green pigments (the green chlorophyll) and photosynthesis. By extracting the pigments, and then analysing them using chromatography, it is possible to demonstrate that the red algae also contain green pigment, which is usually masked by their red pigment.

This activity was developed by the Department of Plant Sciences at Cambridge University for the Royal Society Summer Science Exhibition at the Royal Festival Hall in London, entitled 'Meet the Algae: diversity, biology and energy'.

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

**Duration:** Activity takes about 30 minutes. Preparation will need to be done in advance to culture the algae and collect a concentrated pellet.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, photosynthesis, plants, variation in organisms and techniques for separation including chromatography and solvents. Knowledge of photosynthesis and the properties of light will help students interpret the results of their investigations.

### What you will need:

- Green algae culture (*Chlorella*, *Euglena*)
- Red algae culture (*Porphyridium*)
- Small mortar and pestle
- 1 ml plastic pipettes
- Centrifuge or test tubes
- Strips of blotting paper (Whatman 3MM)
- Ethanol
- 50 cm<sup>3</sup> beaker
- Fine paint brushes

#### Optional

- Centrifuge

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## Health and Safety

Wear eye protection. Ethanol is highly flammable, therefore there must be no naked flames and you must wash your hands afterwards. If using a centrifuge ensure that the centrifuge tubes are balanced and that the tubes used for centrifugation are sealed.

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® 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® Recipe book RB26 (Chromatography solvents and locating agents)

CLEAPSS® Hazcards 40A (Ethanol)

CLEAPSS® Guidance PS 04 (COSHH: risk assessments in situations where microorganisms might be involved), PS 67-14 (Chromatography)

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)

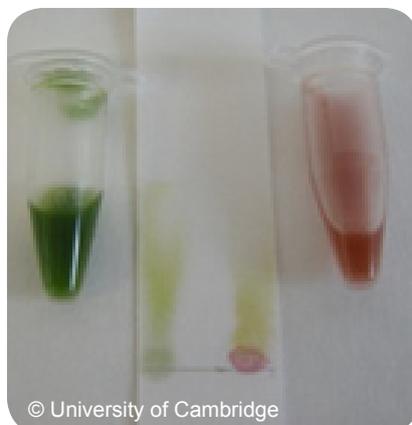
Further advice can also be sought from the Society for General Microbiology [www.microbiologyonline.org.uk/teachers/safety-information](http://www.microbiologyonline.org.uk/teachers/safety-information) and the Microbiology in Schools Advisory Committee.

## Method

1. Prepare two stocks of algae: red algae (*Porphyridium*) and green algae (*Chlorella*). *Euglena* is another green-coloured alga you can use.
2. Centrifuge 10 ml of culture in a centrifuge tube to form an algae pellet. If you do not have a centrifuge, put the algae mixture in a small test tube in the dark and allow to settle. This should take around one hour.
3. Carefully pour off the liquid, trying to avoid disturbing the pellet of algae cells at the bottom. Then place the tube containing the algae pellet in a freezer overnight.
4. Grind the pellet in a small mortar and pestle and resuspend the ground pellet in a small amount of water using a 1 ml plastic pipette. Start off with 1-2 drops and add more if necessary, but try to use the minimum so that the solution is as concentrated as possible.
5. Draw a line in pencil about 2 cm from the bottom of the paper you've been given. Use a fine paintbrush to place small dots of samples of green *Chlorella* and red *Porphyridium* next to each other. Add the samples a little at a time leaving them to dry in between.
6. Place the paper in 5 ml of ethanol (**Note:** make sure the solvent level is not above the level of the pencil line) in a 50 ml beaker and cover with a watch glass. Leave for around 10 minutes to allow the solvent to move up the paper. **What do you observe?**

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The green spot moves up the paper, displaying no further colours. The red spot becomes bright red and green pigments move up the paper.



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Algae chromatography

## Suppliers

Red algae *Porphyridium purpureum*, other red algae, and the green *Chlorella* and *Euglena* can be obtained from Sciento, [www.sciento.co.uk](http://www.sciento.co.uk)\_61 Bury Old Road, Whitefield, Manchester M45 6TB tel: 0161 773 6338 fax: 0161 773 6338

A microcentrifuge suitable for school use 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

## Further reading

*Meet the algae: diversity, biology and energy* [www.bbsrc.ac.uk/news/policy/2010/100625-n-royal-society-summer-science.aspx](http://www.bbsrc.ac.uk/news/policy/2010/100625-n-royal-society-summer-science.aspx)

Read more: [Which variety of algae has the highest oil content | Answerbag www.answerbag.com/question/view/778159#ixzz1Emw0gMdk](http://www.answerbag.com/question/view/778159#ixzz1Emw0gMdk)

Algal Research in the UK: A Scoping study for BBSRC, July 2011. <http://bbsrc.ac.uk/news/industrial-biotechnology/2011/110922-n-algal-research.aspx>

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 Alison Smith, Department of Plant Sciences, University of Cambridge [www.plantsci.cam.ac.uk/MeetThealgae](http://www.plantsci.cam.ac.uk/MeetThealgae)