Activity 3A - Culturing algae

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

- Describe the requirements for algal growth.
- Culture algae in flasks or on agar.
- Compare the effects of growing conditions on algae and the growth of different species.
- Discuss the difficulties of growing algae in large quantities for biofuel production.

Keywords Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, yield, culture, photosynthesis, algae, varieties, photobioreactor.

Background

There are a wide range of bioenergy products that can be obtained from culturing algae including biomass for combustion to produce heat and electricity, fermentation to produce bioethanol, biobutanol or biogas, oil for conversion to biodiesel or even possibly algal synthesised biodiesel. The algae may be cultured in self-contained bioreactors, in open-air ponds or harvested from the environment. Microalgae can be grown in large bioreactors and continually harvested unlike crops or macroalgae. They could be grown using the waste carbon dioxide (CO_2) from industrial processes, power stations or waste treatment plants. Algae can grow in very nutrient-rich environments that are toxic to other plants so they could be used for treating 'waste waters', from a range of industrial sources. The ability of microalgae to grow using waste CO_2 emissions and sunlight, and not compete for land with crops, makes them an attractive proposition both economically and sustainably.

Photobioreactor

Unfortunately, the culture of algae on a large commercial scale has so far been restricted to sunny climates and produces either biomass for aquaculture or specialised products such as natural food colourants, omega-3 oils and antioxidants. The UK has a relatively cold climate, slowing growth of algae and reducing productivity, however, waste heat from industrial activity could be used to warm ponds and thereby increase growth rates.

The problems associated with culturing and harvesting algae differ in bioreactors, marine environments or large ponds that could cover hectares of otherwise unproductive land. In order to develop biofuels from algae, research is being conducted to find suitable strains that produce high levels of oils, can tolerate heat and high concentrations of carbon dioxide, and are easy to harvest. Some of these strains may well be grown using bubble columns and photobioreactors in conjunction with CO_2 from flue gas emissions. When growing algae in open systems it is inevitable that the ponds will get contaminated with algae from the environment. Therefore scientists are trying to identify natural strains that can compete in the environment whilst also considering the potential environmental impacts of growing large numbers of algae.

Algae can be cultured in solutions or on solid media such as agar. Algae require specific nutrients, just like plants and other organisms, to grow well. In this activity students can set up and grow algae on solid media or in culture. This activity may take up to 6 weeks and would be best set up at the start of a topic or term, alternatively cultures can be prepared in advance for students to compare. Depending on the facilities available, and the variables students investigate, a variety of approaches to culturing the algae may be appropriate. If investigating the nutrients and concentrations that are optimal for algal growth a recipe for

micronutrient solution is provided and can be adjusted by changing the amounts or omitting minerals. For simpler experiments a prepared nutrient mix containing the minerals required by algae is available from Sciento. Alternatively liquid plant food or fish fertiliser will do, though these will be lacking the trace elements needed by algae. The algae can be cultured in anything from sterile conical flasks to used drinks bottles, or even in a photobioreactor.

Suitable algae and microorganisms to culture include:

Scenedesmus quadricauda. These algae form colonies typical of four cells and have no means of propulsion. They are hardy and ideal for investigating photosynthesis.

Chlorella vulgaris. Single-celled spherical algae with no means of propulsion through the water. These tiny cells are some of the world's smallest plants at 2-12 µm in diameter. Often found in aged tap water.

Euglena gracilis. Single-celled microorganism capable of photosynthesis and featuring flagella to propel itself towards the light. Up to 60 µm in length and often found in ponds or farmyard puddles.

Pinnularia nobilis. Very small single celled alga about 3 µm in diameter. Part of the group of algae known as diatoms. They are nutrient rich and contain up to 11% oil that enters the food chain and is concentrated in fish livers (cod-liver oil).

The cultured algae can be displayed at science fairs and are most easily transported on agar plates. A large range of conditions can be demonstrated and colonies of algal growth can be observed.

Age Range: These experiments are suitable for primary and secondary students.

Duration: 60 minutes to set up, 2-5 weeks to culture.

Suggested prior knowledge: It is recommended that you elicit the existing student knowledge of microbes, photosynthesis and plants. An understanding of the nutrients and conditions required for plant growth and photosynthesis would help students plan investigations.

Algae on agar plates

What you will need

- Varieties of unicellular algae
- Petri dishes
- Agar
- BG11 solution (recipe below)
- A5 trace metal solution (recipe below)
- Distilled water
- Pipettes
- Measuring cylinder
- Conical flasks
- Bunsen burner
- Heatproof mat
- Streaking loops
- Light source or north facing window sill

Optional

- Prepared culture media
- Liquid plant food

Health and Safety

Take care with powdered nutrient algae medium (HARMFUL, IRRATATING, OXIDISING), avoiding contact with skin and eyes.

If carrying out culture of algae with primary pupils it is recommended that the following precautions are taken. Prepare culture media solutions or liquid plant food and dilute to the working concentration (according to the manufacturer's instructions) in advance. Use disposable Pasteur pipettes to add 1 ml of algal suspension to the plates and swirl the plate to distribute the algae evenly.

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 14.9 Fermenters (Safety, Practical considerations) page 1443-1451, 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, section 15.5 Plants and seeds (choosing suitable plant material, growing and cultivating plants, sources and suppliers of plants) pages 1540-1567

CLEAPSS® Recipe book RB93 (Stains for plant material).

CLEAPSS® Guidance G5p (Using chemicals safely), PS 04 (COSHH: risk assessments in situations where microorganisms might be involved).

Practical Fermentation – A guide for Schools and Colleges. 1999. National Centre for Biotechnology Education (NCBE) and Society of General Microbiology (SGM). www.ncbe.reading.ac.uk/ncbe/protocols/PDF/FermTG.pdf

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

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

Method

1. Wash some agar, then add the BG11 2x base (it is 2x, therefore dilute this 1:2) to conical flasks, stopper with foam bungs or non-absorbent cotton wool, foil and autoclave.
2. After autoclaving, while still hot, add A5 (1:1000).
3. Pour plates.
4. Using aseptic technique streak plates with algal suspension, seal the plates and place under a light source or on a north facing window sill. With primary pupils use disposable Pasteur pipettes and add 1 ml of suspended algae.
5. Algae will grow best at room temperature (18-22°C) under constant fluorescent illumination.
6. If adjusting the conditions, try different light sources and temperatures ensuring only one variable is changed at a time, e.g. consider that if growing algae in the fridge an equivalent light source will be needed to the algae grown outside the fridge.

For liquid cultures just omit the agar.

BG11 – 2x base:	
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
$NaCO_3$	0.02 g

Make up to 500 ml with distilled H_2O and autoclave. The pH should be 7.1 after sterilisation.

Trace metal A5_1000x mix:	
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg
Distilled water	1.0 L

Autoclave the final solution.

Algae in solution

What you will need

- Varieties of unicellular algae
- BG11 solution (recipe above)
- A5 trace metal solution (recipe above)
- Distilled water
- Pipettes
- Measuring cylinder
- Conical flasks
- Bunsen burner
- Light source or north-facing window sill

Optional

- Prepared culture media
- Liquid plant food
- Shaker
- Light bank
- Aquarium air pump
- Graticule
- Densitometer
- Cafetiére
- Coffee filter and filter papers

Method

1. Make up the nutrient solution in conical flasks If using the BG11 2x base dilute this 1:2, stopper with foam bungs or non-absorbent cotton wool, foil and autoclave.
2. After autoclaving, while still hot, add A5 (1:1000).
3. Using aseptic technique add equal inoculations of algae to each culture, 5-10 ml into 250 ml culture media should be sufficient. Swirling or inverting the algae prior to inoculation will distribute the algae equally.
4. Seal the flasks and place under a light source or on a north-facing window sill.
5. Algae will grow best at room temperature (18-22°C) under constant fluorescent illumination with agitation and added carbon dioxide. If a light bank and shaker are available these can provide the light and mixing required. Aeration can be easily achieved with an aquarium pump and is significantly safer and cheaper than a CO_2 canister.
6. Under constant illumination the density of algae in the culture should reach its maximum in 3-5 weeks.
7. If adjusting the conditions, try different light sources and temperatures ensuring only one variable is changed at a time, e.g. consider that if growing algae in the fridge an equivalent light source will be needed to the algae grown outside the fridge.

Extension activities

Post-16 students could monitor the growth of the algae by measuring algal density through cell counts in addition to recording the colour of the culture. Use microscopes, slides and cover slips or a counting chamber to do cell counts of the cultures over time. Ensure students record the data and create graphs of cell density over time.

Compare methods of cell number quantification using microscopes or densitometers.

Compare culture media using different concentrations of nutrients or omitting certain nutrients. Date can be recorded as cell density versus nutrient concentration for each sample time. Graphs can then be produced for each nutrient treatment.

As well as adjusting the light and temperatures the algae are cultured in, try replicating extreme environments and assessing the effect of salinity on algal growth. Create graphs of final cell density versus temperature or salinity.

The algal culture may also be set up as a bioreactor with recording of pH and temperature with data logging software. If setting the algal culture up this way try to ensure that the light source does not transfer too much heat to the culture. It may be worthwhile setting up a control culture without algae, if relying on sunlight, to monitor any difference in temperature.

Try different techniques of harvesting the biomass, dry it and measure the mass to assess the growth rates and yield. Cafetiéres and coffee filters can be easily obtained and used to separate the algae from the culture medium. The dried mass can then be investigated for oil or carbohydrate content and energy of combustion (using a fume hood). Ensure that the algae grown are not toxic before attempting this investigation.

Visualise algae under the microscope, staining for cellulose, lignin or lipid content see activity 2A - plant material testing-for more detail.

Suppliers

Algae and culture media can be obtained from Sciento, www.sciento.co.uk/ 61 Bury Old Road, Whitefield, Manchester, M45 6TB tel: 0161 773 6338 fax: 0161 773 6338

Algae can also be obtained from Blades Biological Limited www.blades-bio.co.uk Cowden, Edenbridge, Kent, TN8 7DX tel:01342 850 242 fax: 01342 850 924.

Bioreactors can be obtained from National Centre for Biotechnology Education (NCBE) 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 and links

Pittman, J., Dean, A. and Osundeko, O, 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour Technol*, **102**(1), 17-25.

Science and Plants for Schools (SAPS) www.saps.org.uk/ Cambridge University Botanic Garden 1 Brookside, Cambridge CB2 1JE tel: 01223 748455, saps@hermes.cam.ac.uk

Microbial Discovery Activity Effect of Nitrate and Phosphate levels on the Growth of Algae American Society for Microbiology, Education Department, 1752 N Street, NW, Washington, DC 20036 EducationResources@asmusa.org

Making Algae (Phytoplankton) Grow, The Feeding Frenzy: Seasonal Upwelling Teaching Box, Digital Library for Earth System Education (DLESE) www.teachingboxes.org/upwelling/lessons/lesson1_supplement/MakingAlgaeGrow.pdf

Towards the quintessential green technology, BBSRC business, Autumn 2010. www.bbsrc.ac.uk/news/industrial-biotechnology/2010/101013-f-towards-quintessential-green-technology.aspx

Scientists aim to improve photosynthesis to increase food and fuel production www.bbsrc.ac.uk/news/food-security/2011/110328-pr-photosynthesis-for-food-fuel-production.aspx

www.bioenergywiki.net/Algae_for_bioenergy
www.bioenergy.cam.ac.uk/abc.html
www.biofuelstp.eu/algae.html
www.biomara.org/
www.walesonline.co.uk/news/uk-news/2010/03/08/seaweed-the-green-fuel-of-the-future-for-our-cars-91466-25983211/

How to make an algae test photobioreactor www.instructables.com/id/How-To-Make-an-Algae-Photo-BioreactorPart-One/

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

Research groups

Professor Alison Smith, Department of Plant Sciences, University of Cambridge www.plantsci.cam.ac.uk/MeetThealgae

The Algal Bioenergy Consortium www.bioenergy.cam.ac.uk/abc.html

Professor Johnathan Napier, Rothamsted Research www.rothamsted.ac.uk

Dr Saul Purton, University College London www.ucl.ac.uk/biology/academic-staff/purton/purton.htm

Dr Sohail Ali, Plymouth Marine Laboratory www.pml.ac.uk/about_us/pml_people/sohail_ali.aspx

Carole Llewellyn, Plymouth Marine Laboratory http://marine-vectors.eu/about_us/the_pml_team/staff_directory/carole_llewellyn.aspx

Dr Jon Pittman, University of Manchester, Faculty of Life Sciences, Michael Smith Building, Oxford Road, Manchester, M13 9PT. Utilisation of microalgae for sustainable biotechnology. www.manchester.ac.uk/research/jon.pittman/research

Dr D Jim Gilmour, Microbial Physiology of Extremophiles, University of Sheffield www.sheffield.ac.uk/mbb/staff/gilmour

BioMara project www.biomara.com at The Scottish Association for Marine Science, Dunstaffnage marine laboratory, Oban www.sams.ac.uk

Culture Collection of Algae and Protozoa (CCAP) national algae collection www.ccap.ac.uk