

# Practical Biofuel Activities for School Engagement and Outreach



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# The Biofuel Researcher Practical Guide

## Who this is for:

**Researchers** - This guide will enable you to communicate and engage young people with the scientific principles and research in the fields of **bioenergy** and **biofuels** through outreach activities.

Bioenergy science and research are ideal topics to engage young people and support teachers in delivering the curriculum. Delivering a number of small public engagement activities across the UK to young people and families increases awareness of research and the issues that it raises whilst also providing opportunities for dialogue. Stimulating discussion within families raises the level of interest in science and provides an effective means of promoting further study in science subjects. By engaging with young people you will be able to consider the relevance of your research to the needs of future generations and potentially refine the direction of research you undertake to meet those needs.

## What is in the practical guide:

The background information on these pages will cover the basic science involved in the field of bioenergy and biofuels and links to the curriculum and further information on the research being conducted. There are also a range of practical activities with instructions. The activities can be carried out in universities and research institutes with visiting students as well as school science laboratories and classrooms, and with modifications most can be demonstrated at science fairs or other engagement activities. The topics cover plant science, microbiology, chemistry and a range of other areas of science and technology.

Each activity has background science, further reading and links to research groups to enable you to become familiar with the important developments that have occurred in the field of biofuel research. There are learning objectives, keywords, suitable age ranges, extension activities and curriculum links to help plan activities that will meet the needs of teachers and young people. Before carrying out activities, try to find out the

audience's existing knowledge or understanding of science concepts. You may want to consult teachers or parents prior to the activity. To help with this, suggested prior knowledge is included for each activity.

Activities take between 10 and 60 minutes depending on the age and ability of the participants. Some of the activities require incubation periods which can be carried out in advance or as part of a series of engagement activities with schools or young people. The time taken can be reduced if materials are prepared in advance or parts of the activities carried out as a demonstration. It is recommended that sufficient time before or after the activity is arranged and planned in advance so that the outcomes can be observed.

Many of the activities in this guide are suggested by exam boards to cover the knowledge, understanding or practical skills content required for GCSE, A-level or Higher examinations.

The activities can be carried out with equipment available in most school science laboratories.

Equipment and consumables can be obtained from Sigma-Aldrich, the National Centre for Biotechnology Education (NCBE), Philip Harris Education, Blades Biological Ltd, Mindsets, Sciento, Timstar Laboratory Suppliers Ltd, Bio-Rad and Edvotek.

**Key to abbreviations:** Association for Science Education (ASE), Biotechnology and Biological Sciences Research Council (BBSRC), Consortium of Local Education Authorities for the Provision of Science Services (CLEAPSS®), Control of Substances Hazardous to Health (COSHH), Department for Education and Employment (DfEE), National Centre for Biotechnology Education (NCBE), Royal Society of Chemistry (RSC), Science and Plants for Schools (SAPS), Society for General Microbiology (SGM), Scottish Schools Equipment Research Centre (SSERC), Scottish Qualifications Authority (SQA), Oxford, Cambridge and RSA Examinations (OCR), Assessment and Qualifications Alliance (AQA).

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These pages can be downloaded as pdf's from [www.bbsrc.ac.uk/biofuelsactivities](http://www.bbsrc.ac.uk/biofuelsactivities)



# About

## BBSRC



The Biotechnology and Biological Sciences Research Council (BBSRC) invests in world-class bioscience research and training on behalf of the UK public. BBSRC supports research and training in universities and strategically funded institutes to further scientific knowledge, to promote economic growth, wealth and job creation and to improve quality of life in the UK and beyond. BBSRC supports a total of around 1600 scientists and 2000 research students in universities and institutes in the UK. BBSRC research and the people we fund are helping society to meet major challenges, including food security, green energy and healthier, longer lives. Our investments underpin important UK economic sectors, such as farming, food, industrial biotechnology and pharmaceuticals.

BBSRC has a commitment to inspiring young people and provides a number of resources to support researchers, teachers and school pupils. This set of resources has been produced following consultation with a wide range of organisations and stakeholders to enable researchers to engage young people with the science and issues surrounding Biofuels and Bioenergy.

## BBSRC Sustainable Bioenergy Centre (BSBEC)



BSBEC was launched in 2009 to provide focus for research underpinning sustainable bioenergy and biofuels in the UK. It represents the largest UK public investment in bioenergy, with £20M from BBSRC and around £5M from industry. BSBEC is a virtual centre, integrating activities across six UK research hubs led by Rothamsted Research (RRes) and the Universities of Cambridge, Dundee, Nottingham (two hubs) and York.

BSBEC is part of the Research Councils UK Energy Programme, aimed at helping the UK move towards a low carbon future.

## Research Councils UK Energy Programme



The Research Councils UK Energy Programme aims to position the UK to meet its energy and environmental targets and policy goals through world-class research and training. The Energy Programme is investing more than £530 million in research and skills to pioneer a low carbon future. This builds on an investment of £360 million over the past 5 years.

Led by the Engineering and Physical Sciences Research Council (EPSRC), the Energy Programme brings together the work of EPSRC and that of the Biotechnology and Biological Sciences Research Council (BBSRC), the Economic and Social Research Council (ESRC), the Natural Environment Research Council (NERC), and the Science and Technology Facilities Council (STFC).

# Health and Safety

## The practical activities outlined here are for guidance only.

Provided appropriate equipment and facilities are used and safety precautions are made the experiments do not pose a health and safety risk to participants. It is essential that you check safety policies and procedures with your place of work, venues or school and follow the guidance provided before carrying out activities. Modifications to the procedures outlined here may be required to accommodate the circumstances under which the activity is conducted. When carrying out activities in laboratories ensure that good laboratory practice is followed by demonstrators and students. A copy of the Good Laboratory Practice with Young People (from CLEAPSS®) is provided.

Risk assessments should be carried out for all activities and it is recommended that CLEAPSS® guidelines are followed (or, in Scotland, SSERC). CLEAPSS® [www.cleapss.org.uk](http://www.cleapss.org.uk) is the school science and technology safety advisory service. BBSRC has membership of CLEAPSS® and further advice can be obtained from CLEAPSS® through BBSRC by contacting the Inspiring Young Scientists Coordinator on 01603 255017 or email [schools@bbsrc.ac.uk](mailto:schools@bbsrc.ac.uk). In Scotland, SSERC [www.sserc.org.uk](http://www.sserc.org.uk) has a similar role to CLEAPSS® and there are some reciprocal arrangements. Further advice is also available from the Association for Science Education (ASE) [www.ase.org.uk](http://www.ase.org.uk), which BBSRC is a member of, or consult the individual responsible for Health and Safety in your organisation.

COSHH Regulations (2002) and the Management of Health and Safety at Work Regulations (1999) require risk assessments for any hazardous procedure or activities with harmful microorganisms. Use of chemicals should adhere to COSHH regulations and some chemistry based activities should only be carried out in a suitable location such as a school science laboratory.

For activities involving microorganisms, suitable disposal and decontamination procedures should be planned prior to carrying out the activity. None of the practical activities suggested in this guide involve pathogenic microorganisms, however, they can be cultured by mistake and it is important to note that COSHH Regulations apply to activities involving pathogenic microorganisms. 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.

It is important to check for any relevant medical conditions, such as allergies, that young people or members of the public may have before carrying out these activities. It is recommended that participants are provided with nitrile gloves or made to wash their hands at the start and end of activities.

## Further reading

CLEAPSS® laboratory handbook

- section 13.2.1 Fossil Fuel Experiments (Distillation of coal, the distillation of crude oil, 'cracking' experiments) pages 1305-1309.
- section 14.9 Fermenters (Safety, Practical considerations) pages 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
- section 20.3.1 Carbohydrate tests page 2006.

# Health and Safety

CLEAPSS® Recipe book RB1 (Agar), RB3 (Alginate beads), RB11 (Benedict's qualitative reagent),

RB12 (Benedict's quantitative reagent), RB17 (Bromine water), RB19 (Calcium chloride and nitrate(V) solutions), RB21 (Carbon dioxide), RB26 (Chromatography solvents and locating agents), RB32 (Crude oil alternative), RB37 (Enzymes), RB40 (Fehling's solutions), RB48 (Indicators-carbon dioxide), RB50 (Iodine solution), RB71 (Potassium hydroxide), RB73 (Potassium manganate(VII)) RB93 (Stains for plant material), RB99 (Testing for gases), RB102 (Testing for organic functional groups).

CLEAPSS® Hazcard 12 (Benzene diols and triols), 15A and B (Bromine), 18 (Calcium oxide), 19A (Calcium salts), 20 (Carbon dioxide), 27C (Copper salts), 28 (Dichloromethane), 33 (Enzymes), 37 (Ethane-1,2-diol and other polyols), 40A (Ethanol), 40B (Methanol), 40C (Carbohydrates), 45 and 46 (Hydrocarbons), 54 (Iodine), Student Safety Sheet 48 and Hazcard 81 Potassium manganate(VII), 85 (Propanone), 91 (Sodium hydroxide), 92 (Sodium and potassium metabisulphate), 95C (Sodium and Potassium salts).

CLEAPSS® Guidance leaflets G5p (Using chemicals safely), PS 04 (COSHH: risk assessments in situations where microorganisms might be involved), PS 15 (Ventilation and levels of carbon dioxide and other gases in the laboratory & prep room), PS 67-01 (Testing for unsaturation), PS 67-05 (The viscosity of motor oils), PS 67-10 (Making bio-diesel), PS 67-14 (Chromatography), PS 89 (Measurement of anaerobic respiration in yeast).

CLEAPSS® Guides R57 (Colorimeters), R101 (Steam sterilisation: Autoclaves & pressure cookers).

CLEAPSS® Model Risk Assessment 3.002 (Chemical testing of food), 3.015 (Enzymes), 3.026 (Microorganisms used in food production).

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](http://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](http://www.microbiologyonline.org.uk/teachers/resources)

Royal Society of Chemistry (RSC) [Transporting chemicals for lecture demonstrations & similar purposes](#). January 2008.

Association for Science Education (ASE) Be Safe! 4th Edition, 2011, pages 16-17 (microorganisms), 18-19 (plants), 34-36 (chemicals). [www.ase.org.uk/resources/health-and-safety-resources/health-and-safety-primary-science/](http://www.ase.org.uk/resources/health-and-safety-resources/health-and-safety-primary-science/)

Association for Science Education (ASE), Safeguards in the school laboratory, 11th edition, 2006. [www.ase.org.uk/resources/health-and-safety-resources/](http://www.ase.org.uk/resources/health-and-safety-resources/)

Association for Science Education (ASE) Topics in Safety, Third edition, 2001, College Lane, Hatfield, Herts. AL10 9AA. ISBN: 0 86357 104 2.

Department For Education and Employment (DfEE) Safety in Science Education, 1996. HMSO, London. ISBN: 011 270915 X.

Scottish Schools Equipment Research Centre (SSERC) Limited, 1997, Hazardous Chemicals, A Manual for Science Education, ISBN 0 9531776 0 2.

## CLEAPSS guidance on Good Laboratory Practice with young people

It is expected that every university, research institute or school will have rules governing behaviour in the laboratory. No eating or drinking (or indeed smoking or the application of cosmetics) should be allowed in laboratories. Interference with mains services or equipment should be strictly forbidden, as should running or foolish behaviour generally.

Good hygiene is needed at all times, but especially when chemicals or living organisms are being used. Benches need to be wiped down after such activities and hands washed.

Suitable eye protection must be worn whenever the risk assessment requires it, ie, whenever there is a recognised risk to the eyes. This will certainly include activities in which chemicals are heated, heat is generated in a chemical reaction or any activities involving chemicals with a hazard classification. Eye protection is also necessary where there are mechanical hazards, eg when stretching wires to breaking point or evacuating vessels.

Many accidents occur during heating activities. Long hair should be tied back and ties, cardigans, scarves, baggy shirts, etc should not be allowed to hang freely. It is assumed that demonstrators (i.e. you) and teachers will show and remind students how to heat safely small quantities of solids in test tubes and liquids in boiling tubes (wide diameter test tubes), using small quantities so that the tube is not more than 1/5th full, and pointing the tube away from their own faces and other peoples' faces. The tube should be sloping so that the holder is not in a flame. For liquids, tubes should be gently shaken or a water bath used where appropriate. Students should stand, not sit, for most operations in which chemicals (and especially liquids) are handled.

Students need to be shown how to pour safely from bottles, pouring away from the label (so that it is not damaged by drips). Spills of chemicals should be wiped up at once. Some may require chemical treatment (eg, neutralisation) but, in the quantities normally handled by students, a damp cloth is usually sufficient. The cloth should then be rinsed. Students should be trained to use a spatula or similar device and never to handle chemicals with their fingers. Wherever possible, teat pipettes should be avoided. Even with well-behaved classes, too many accidents occur when liquids are squirted from them, eg, when clearing up at the end of a lesson. Except sometimes in the sixth form, work in schools rarely requires the use of protective gloves. However, when chemicals have been used or living organisms handled, students should be trained to wash their hands afterwards.

If the risk assessment requires the use of a fume cupboard, then this should meet the standard of *Building Bulletin 88, Fume Cupboards in Schools* (Architects and Buildings Branch, DfEE, 1998, HMSO) (previously *Design Note 29*).

If safety screens are required for a demonstration, then they should be sufficient in number to protect both the teacher and all the students. They should be sufficiently tall and sufficiently close to the apparatus to prevent objects going over the top. There should be a gap of 2 m or more between any demonstration and the students.

If microorganisms are in use, teachers unfamiliar with modern techniques may need training (see for example, *Topics in Safety, Safety in Science Education* or the *CLEAPSS Laboratory Handbook*). In any work in micro-biology, risks can be reduced to an acceptable level by observing good practice and following simple precautions. Sterile technique is needed to prevent cultures from becoming contaminated and to stop microorganisms escaping from cultures. This will involve ensuring that materials which will contact microbes are sterile before and afterwards; a pressure cooker or autoclave is essential, complemented by the use of appropriate chemical disinfectants to deal with spills and to clean working surfaces. By choosing appropriate organisms and growth media, avoiding the culture of microbes from dangerous sources and incubating at room temperature, together with the correct handling and sealing of cultures, exposure to pathogens can be minimised or eliminated. The culture of organisms that will be consumed, eg, yoghurt bacteria or baker's yeast, should not take place in a science laboratory.

# Introduction

Fossil fuels are dwindling and in order to maintain the current levels of energy use and the transport systems we rely on we need to find alternatives. There are also environmental concerns about the effects of using fossil fuels such as pollution and climate change. **Bioenergy** may be part of the solution to these problems

**Bioenergy** is the energy derived from harvesting biomass such as crops, trees or agricultural waste and using it to generate heat, electricity or transport fuels.

The benefits of bioenergy include, **sustainable** and **renewable** fuels, decreased carbon dioxide release into the atmosphere and turning the problem of waste into a source of energy. **Biofuels** can be 'effectively' carbon neutral and in some cases may use emissions from power plants as a carbon source.

**Biofuels** could power our cars, heat our homes and maybe fuel our planes. Liquid biofuels represent the only **sustainable** alternative to current transport fuels. BBSRC research is focusing on advanced biofuels from inedible and **non-food crops** as well as waste. Currently biofuels are combined with oil-based fuels so that typical UK petrol is composed of 3-4% biofuel. At present much of this biofuel comes from sources that directly or indirectly compete with land and resources that would otherwise be used to grow food.

**Biomass** can be burned directly to generate heat and/or power either on its own or 'co-fired' alongside conventional fuels such as coal. Alternatively, biomass can be treated to create gaseous or liquid biofuels which can be used on their own or in conjunction with conventional fuels such as coal or natural gas. The initial emphasis is on motor vehicle fuels, but the same principles apply to aviation fuels, where bio-products provide the only sustainable alternative to kerosene.

Biofuels, biofuel feedstocks and the technologies involved in producing them can be considered in terms of **current bioenergy** and **advanced bioenergy**. There are a wide range of sources of biomass used in producing biofuels commonly referred to as feedstocks. The procedures used to convert these feedstocks are equally varied as are the potential fuels produced. A number of terms have been used to describe this variety of approaches, the research being undertaken and developments that are taking place including 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> generation. These terms are in common use and have therefore been used on occasion in this document. However, this doesn't reflect the complex nature of the field and cannot completely describe the differences between biofuels.

**Current or conventional bioenergy** is often referred to as first generation whereas advanced bioenergy refers to second and third generation biofuels. First generation biofuel refers to established technologies used to produce biofuels, in particular the use of food crops such as sugar cane and maize, but also including biogas. Second and third generation biofuel refers to bioenergy solutions that either make use of waste or rely on non-food crops that can be grown on marginal land.

**Advanced bioenergy** solutions hold the unique promise of being able to provide a sustainable alternative to current oil-based liquid fuels, particularly for aviation, shipping and haulage.

While other technologies, such as electric or hydrogen vehicles, may someday replace the need for liquid fuels they are not a viable alternative at present. Electric vehicles may be excellent for short journeys, but the range provided by current battery technologies, and lack of infrastructure, make them impractical for longer journeys, haulage or aviation use, and hydrogen-based vehicles likewise still require technological and infrastructural developments.

Liquid biofuels, to replace petrol, diesel and aviation kerosene, can come from:

- (a) converting the large amounts of energy locked up in plant cell walls (lignocellulose)
- (b) harnessing the capabilities of algae and microbes



# Introduction

BBSRC bioenergy research focuses on **sustainable** energy (liquid fuels, heat and electricity) either from **non-food** feedstocks or from inedible elements and waste from agriculture, food crops or food processing. Research suggests that there is enough land in the UK to grow biomass for renewable electricity production without affecting food production. Changes in land use will inevitably affect the environment, for instance biodiversity, soil structure or water availability. Some changes may be positive while others may have negative impacts. The farmed and natural environment provide vital ecosystem services and it is important that proper assessments of environmental risks are carried out throughout the development of crops and new technologies before they make it to the farm.

New and better sources of bioenergy may come from: non-food crops, inedible parts of food crops, waste, microbial and algal metabolism, and biomass processing into biogas. Advanced bioenergy (rather than current or conventional bioenergy) solutions either make use of waste, agricultural residues or rely on non-food crops that can be grown on marginal land. For instance, research focuses on improving yields and conversion efficiencies for producing biofuels from miscanthus, willow and barley straw.

**Sustainably** produced biofuels offer the only mid-term option for replacing liquid transport fuels such as petrol. Crude oil production is expected to be in decline by the middle of the century. Even without the pressing environmental reasons for reducing the 'carbon footprint' of transport and manufacturing, the inevitable depletion of fossil fuel reserves means that we need to find alternative sources of energy and raw materials. Plant-based industrial biotechnology provides a viable route to achieve this. Industrial biotechnology can substitute for dwindling fossil fuel stocks by providing fuel and other high value products such as plastics, pigments and antioxidants.

Scientists are using biotechnologies to carry out their research and these include:

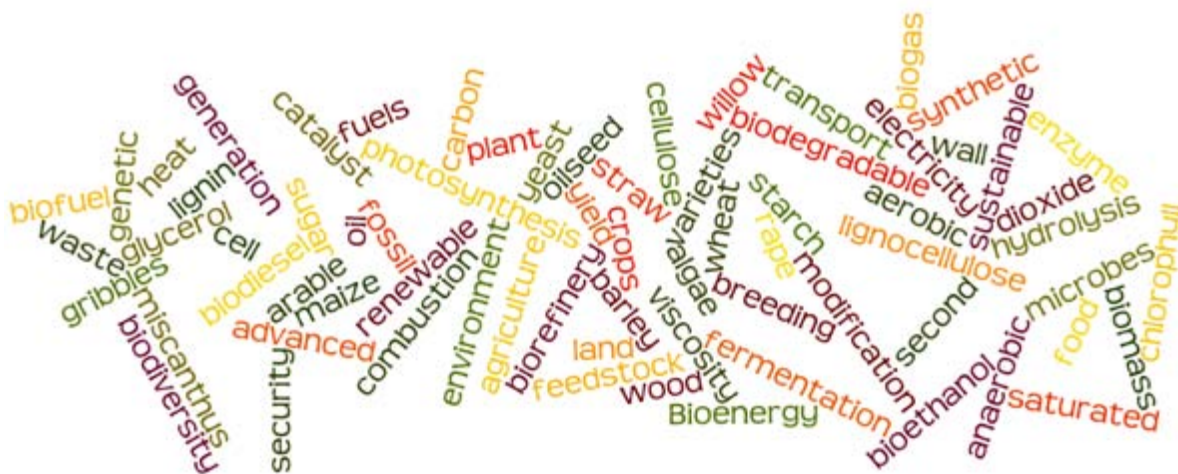
- Plant breeding
- Systems biology
- Genetic modification
- Metabolic engineering
- Directed evolution
- Anaerobic digestion
- Synthetic biology

It will take a decade or longer to develop new sources of biofuel from research being carried out today. Research is currently focused on developing biofuels from plant sugars while future options may include using synthetic biology techniques and algae to produce biodiesel directly.

# Introduction

## KEYWORDS

Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, biogas, yield, waste, food security, second generation, bioethanol, lignocellulose, lignin, cellulose, cell wall, microbes, yeast, enzyme, fermentation, gribbles, photosynthesis, algae, varieties, electricity, heat, transport, crops, straw, miscanthus, willow, barley, oilseed rape, wheat, maize, wood, sugar, starch, aerobic, anaerobic, oil, catalyst, carbon dioxide, combustion, chlorophyll, fossil fuels, glycerol, genetic modification, hydrolysis, synthetic, saturated, viscosity, arable land, biodiversity, advanced plant breeding, feedstock, environment, agriculture, biodegradable, biorefinery.



Keywords courtesy of <http://www.wordle.net/>

## FACTS and FIGURES

- To help combat climate change the UK has a target to reduce carbon emissions by 80% by 2050.
- 30% of the UK renewable energy could come from biomass heat and electricity by 2020.
- To meet the European Renewable Energy Directive, the UK's is aiming for 10% of transport energy to be from renewable sources by 2020.
- By 2020, 8% of our petrol and 5% of our diesel could come from crops grown in the UK.
- Photosynthesis is only 6% efficient and it may be possible to improve this to produce higher yielding plants or develop novel ways of capturing solar energy.

# Current Biofuels

## Biogas

**Keywords** Bioenergy, biofuel, biogas, sustainable, renewable, biomass, anaerobic, waste, bacteria, microbes, fermentation, methane.

## Background

Biogas feedstocks that have high water content, such as food wastes and livestock manure cannot be incinerated, but can produce biogas. Biogas can be burnt to produce heat for cooking, warming homes and producing electricity. It can also be compressed and used as a transport fuel in specially converted vehicle engines. The digested waste material is of use as fertiliser in agriculture.



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Biogas storage containers

**Biogas** is 60-80% methane and is created by a process termed anaerobic digestion, leaving behind a nutrient-rich substance termed digestate. Anaerobic digestion is carried out by a range of bacteria in the absence of oxygen. A number of bacteria and yeast have been identified in biogas production. Initially carbon dioxide is produced by the decomposing organic matter until an anaerobic environment is created. After the initial digestion a group of bacteria known as methanogens convert the products into methane and carbon dioxide.

Anaerobic digestion has a number of environmental benefits including production of 'green' energy and natural fertilisers. Biogas production reduces the need for fossil fuel mining and the use of fossil fuels in the production of artificial fertilisers, resulting in reduced greenhouse gases being released into the atmosphere. The problems associated with waste disposal are also alleviated by the generation of useful products and decreased release of the potent greenhouse gas, methane, from landfill sites.

Biogas is successfully generated in a number of developing countries and Europe. In the UK, research is being conducted in a number of areas of biogas production including:

- Assessment of how more automated production can be achieved and scaled up to make it efficient and cost effective.
- Assessment of how biogas production can be integrated into UK agriculture.
- Assessment of the environmental and economic benefits
- Assessment of the potential for generating fertiliser with the appropriate nutrients but with a lesser environmental impact from nitrogen and phosphorus pollutants released into the air and water.
- Use of novel yeast species to enhance the efficiency of anaerobic digestion.
- Use of agricultural crop waste consisting of carbon sources that require hydrolysis by chemical, physical and microbial enzymatic processes.

The capacity to utilise the carbon sources in cellulosic materials is recognised as the production of **advanced biofuels**, which can provide a much greater source of biomass for bioenergy.

# Current Biofuels

## Activity 1A - Biogas generator

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

- Describe the features of a biogas generator.
- Evaluate the pros and cons of biogas feedstocks.
- Create a biogas generator.

In this activity students design and construct a biogas generator from household materials, collect the gas produced over a number of weeks and test it. Biogas generators can be constructed from household materials such as fizzy drinks bottles, and the gas burnt using a Bunsen burner. A list of possible materials is provided below. The wider the range of materials you can provide, the more creative students can be.

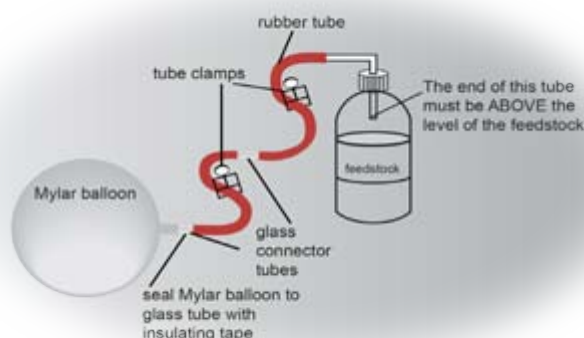


© Institute of Food Research  
Food waste

Designing and constructing a biogas generator makes an ideal project for students to express their creativity and problem-solving skills. The format of this exercise should be adjusted to suit the circumstances of the class and time available. It can be carried out in an hour-long session if sufficient materials are provided for the students to construct biogas generators and the gas from an already established generator is tested. If possible arrange time for students to design their own biogas generator and for the required materials to be collected. This activity works well as a long-term project that can be revisited periodically with a school or class. It can take up to six weeks to produce enough biogas to burn. This is also a good project for students to carry out towards British Science Association CREST awards or for a science fair.

First of all introduce the background to biogas production and explain the objective. Provide students with a schematic of an actual biogas generator and discuss the function of parts of the generator. You may want to show contrasting examples of biogas generators such as those used in developing countries for cooking and those used to generate electricity in power stations. Carry out a thought shower exercise in groups or with the whole class to decide on the materials required for the generator and to collect and burn the gas. Provide a list of organic material available for use in generating biogas and discuss the pros and cons before beginning construction of the generator. If students are designing their own biogas generator you will need to check their plans and ensure they have considered and can demonstrate how they will undertake the investigation safely.

An example of an effective set up is shown below:



# Current Biofuels

This activity is based on ones developed by the Department for Trade and Industry, [It's Only Natural: Science programme 2005](#) and the Gatsby Science Enhancement Programme (SEP): Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

**Age Range:** This activity is suitable for all secondary and post-16 students.

**Duration:** 20 minutes or longer depending on time and resources available.

**Suggested prior knowledge:** This activity does not require any specific prior knowledge but it is recommended that you elicit the existing student knowledge of fuels, microbes, properties of gases and health and safety.

## What you will need

- Water cooler bottle or fizzy drinks bottles
- Rubber tubing
- Clamps
- Bung or bottle top
- Measuring cylinder
- Tape
- Plastic tubes (a biro can be used so long as the hole in the tube is covered with tape)
- Mylar/foil balloon (rubber balloons are porous and allow the gas to escape)
- A variety of organic matter such as grass clippings, leaves, waste fruit and vegetables, tea bags
- Bunsen burner and heatproof mat
- Plasticine or blue-tack
- Disposable nitrile gloves

# Current Biofuels

## Health and Safety

Choose a suitable location to store and carry out the gas generation, bearing in mind the fire hazard.

CLEAPSS® Recipe book RB99 (Testing for gases).

Glass bottles should not be used to collect biogas due to the risk of explosion of a glass container.

The organic matter should be chosen bearing in mind microbial contamination, do not use any animal or human waste. Students should wear disposable gloves when handling organic matter and wash their hands at the end of the activity. Goggles and an apron are also recommended.

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. It is recommended that incubation is not carried out above 30°C to avoid the growth of potential human pathogens.

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.

## Extension activity

The amount of biogas produced by different feedstocks can be compared by the students. In order to do this, students will need to investigate and design a way of measuring the gas output of a biogas generator and compare the amount of gas produced by different feedstocks. Alternatively the same feedstock can be used and the effect of changing climatic conditions simulated by placing generators in different locations such as in front of a window, outside or in a dark room with relatively constant temperature.

The gas generated can be tested for the presence of saturated or unsaturated hydrocarbons by bubbling through bromine or iodine water.

## Suppliers

Standard laboratory equipment suitable for school use, including clamps, can be obtained through suppliers such as Rapid [www.rapidonline.com](http://www.rapidonline.com) Severalls Lane, Colchester, Essex, C04 5JS tel: 01206 751166 fax: 01206 751188, [Philip Harris Education](http://www.philipharriseducation.com), Hyde Buildings, Hyde, Cheshire, SK14 4SH, tel: 0845120 4520 fax: 0800 138 8881 and [Timstar Laboratory Suppliers Ltd](http://www.timstarlab.com), Timstar House, Marshfield Bank, Crewe, Cheshire, CW2 8UY, tel: 01270 250459, fax:01270 250601.

Mylar/foil balloons can be obtained from party shops as well as gift and card shops.

# Current Biofuels

## Further reading and links

Prochnow A, Heiermann M, Plochl M, Linke B, Idler C, Amon T, and Hobbs P J , 2009. "Bioenergy from permanent grassland--a review: 1. Biogas," *Bioresource Technology* **100**:4931-4944

Prakashama R.S. , Sathisha T, Brahmaiaha P, Subba Raoa Ch, Sreenivas Rao Ch, Hobbs P J.  
Biohydrogen production from renewable agri-waste blend: Optimization using mixer design Int. *Journal of Hydrogen energy*, **34**, 6143-6148.

Ravella SR, James SA, Bond CJ, Roberts IN, Cross K, Retter A, Hobbs PJ, 2010. *Cryptococcus shivajii* sp nov.: A Novel Basidiomycetous Yeast Isolated from Biogas Reactor. *Current Microbiology* **60**:12-16 [www.rothamsted.bbsrc.ac.uk/Research/Centres/ProjectDetails.php?Centre=NWR&ProjectID=5719](http://www.rothamsted.bbsrc.ac.uk/Research/Centres/ProjectDetails.php?Centre=NWR&ProjectID=5719)

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)

Gatsby Science Enhancement Programme (SEP): Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

Vicky Wong. Food Waste Recycling - Power from Potato Peelings, Catalyst: GCSE Science Review 2011, Volume 22, Issue 1 [www.nationalstemcentre.org.uk/elibrary/file/14517/catalyst\\_22\\_1\\_491.pdf](http://www.nationalstemcentre.org.uk/elibrary/file/14517/catalyst_22_1_491.pdf)

Biogas learning activities are also available from The PACE Virtual Explorer for Secondary Science [www.tusk.org/pace-biogas-project.asp](http://www.tusk.org/pace-biogas-project.asp)

Video animation of a biogas plant in operation [www.bioconstruct.com/technology/video-animation.html](http://www.bioconstruct.com/technology/video-animation.html)

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

Dr Phil Hobbs, Principal Research Scientist and Dr Sreenivas Rao Ravella, Fermentation Scientist, Bioenergy Group, Rothamsted Research, North Wyke [www.northwyke.bbsrc.ac.uk/pages/bioenergy.html](http://www.northwyke.bbsrc.ac.uk/pages/bioenergy.html)

# Current Biofuels

## Oil and Biodiesel

**Keywords** Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, oil, yield, food security, catalyst, methanol, glycerol, maize, oilseed rape, soya, potassium hydroxide, centrifuge.

## Background

Oil can be extracted from a variety of plants and converted into **biodiesel**. Most biodiesel is produced from soya, oilseed rape, maize (corn) and palm oils, though almost any vegetable oil can be used. The oil is concentrated in seeds, nuts or germ of the plant. In order to extract the oil the raw material is pressed and then a combination of solvents and steam distillation used to improve the quality of the final product. Biodiesel can replace diesel or be further refined to produce synthetic kerosene suitable for use in aviation fuel. Normal vehicles can function with fuel containing up to 5% biofuels without modification. Higher proportions of biofuel require increasing amounts of modification. In general biodiesel requires fewer modifications than bioethanol.

In the UK, 64% of biofuel is supplied as biodiesel and 36% as bioethanol. The major biodiesel feedstocks are soy (35%), oilseed rape (12%), palm (6%), tallow (9%) and used cooking oil (33%). Soy comes from the USA, Argentina and Brazil. Tallow comes from the USA and the UK, while used cooking oil comes from the UK.

Oilseed rape and sunflower seeds from Germany and the UK are used in European biodiesel production. Oilseed rape (*Brassica napus*) is the third most important crop in the UK after wheat and barley. In 2010 in the UK more

than 600,000 hectares (around 6% of the country's arable land) was devoted to growing oilseed rape which is used in a variety of vegetable and industrial oils and as a constituent of biofuel. Research is being carried out to improve the yields of oilseed rape by reducing the losses from seed pod shattering, and gaining a better understanding of the genome of the plant, as well as developing mathematical models to forecast pests in order to improve **food security**. It is therefore essential that there is appropriate development of technologies and policies to manage the conflicting demands of food security and bioenergy.

Palm oil comes from Malaysia and Indonesia. Oil palms are more productive than other oil crops but increasing demand for palm oil has resulted in deforestation and destruction of peatland and other ecosystems for plantations. This has caused the loss of habitat and impacts on biodiversity while the carbon release associated with these activities may outweigh any advantage as a renewable energy. There is also concern about the effects on the indigenous peoples of Malaysia and Indonesia and the treatment of workers on palm plantations.

Future sources of oil for biodiesel production may come from crops such as *Jatropha curcas*, which is a drought tolerant crop that grows on non-arable marginal land and produces inedible oil with a yield of up to 40% oil content. Alternatively, research into algal feedstocks may result in the production of sufficient quantities of oil to replace fossil fuel sources and meet demand for biodiesel.



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Field of Oilseed rape





# Current Biofuels

## Activity 1B - Oil extraction

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

- Describe the techniques used to extract oil from plant material.
- Carry out oil extraction from plant material.
- Discuss the ethical, economic and environmental issues associated with producing biofuels from plant material.

**Keywords** Bioenergy, biofuel, biodiesel, renewable, feedstock, yield, biomass, maize, oilseed rape, extraction, phase separation.



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Oil extraction with morta and pestle

## Background

Oil can be extracted by grinding vegetable matter in a mortar and pestle and separating out with water (phase separation). Suitable fruits, nuts or seeds include sweetcorn (where possible fresh) or sunflower seeds, though any plant matter can be used. Sweetcorn and sunflower seeds are recommended as they are easy to obtain, safe and are familiar to students. It should be explained to students that maize (*Zea mays*) is commonly known as corn, and includes popcorn and sweetcorn, as some may not be aware of this. Maize oil is produced in large quantities and is the primary feedstock for biodiesel in the USA. This activity provides opportunity for discussion about the issues raised regarding current biofuels conflicting with food security. The difference in difficulty of grinding the sunflower seeds and sweetcorn can be discussed in relation to energy usage and efficiency. Yield can be compared between sweetcorn and sunflower seeds and the issues involved in choosing suitable feedstocks discussed. Sunflower oil costs are not competitive enough in comparison to palm, coconut or soya oil for it to be a viable alternative. It is recommended that where possible feedstocks used to produce biofuels are used, though it should be noted some feedstocks such as rapeseed prove too difficult to grind.

This is a 'make and take' activity that can be used at public events and provides an ongoing experiment that young people can take away and then observe the separation of the oil over the coming days. Ensure that the oil is separated in a properly sealed Falcon tube (use tape or Parafilm to form a watertight seal).

This activity is based on one developed by the Gatsby Science Enhancement Programme (SEP): Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

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

**Duration:** 10-20 minutes.

**Suggested prior knowledge:** This activity does not require any specific prior knowledge but it is recommended that you elicit the existing student knowledge of fuels, properties of liquids - especially oil - and crops.

# Current Biofuels

## What you will need

- Mortars and pestles
- Spoons or spatulas
- Water wash bottles or disposable plastic pipettes
- Boiling tubes or skirted Falcon tubes
- Boiling tube rack or polystyrene tube holder
- Funnel
- Fruits, nuts or seed (preferably sweetcorn or sunflower seeds)

## Optional

- Centrifuge or hand centrifuge
- Centrifuge tubes or microcentrifuge tubes
- Disposable pipettes
- Balance

## Health and Safety

CAUTION: Be aware that some students may have allergies to foodstuffs and ascertain these prior to the activity.

Ensure that the centrifuge tubes are balanced and that the tubes used for centrifugation are sealed.

Follow CLEAPSS® guidance leaflet PS 67-03 and section 13.7 of the laboratory handbook.

## Method

1. Add a small spoonful of vegetable matter to the mortar (if calculating yield accurately, weigh the vegetable matter).
2. Grind the vegetable matter, adding a small amount of water if required.
3. Once completely ground, add more water using a wash bottle (about 20 ml).
4. Transfer the water and ground vegetable matter to a test tube or Falcon tube using a spatula or spoon (a funnel may be helpful if the vegetable matter is finely ground).
5. Wash the mortar and pestle to remove any remaining oils and add the extra water to the tube. Ensure it is about 2/3rds full.
6. Replace the lid, seal, label and leave on a flat surface for 30 minutes to 2 days to observe the oil separation.
7. Alternatively the oil can be separated using a centrifuge.

## Extension activities

The weight of the feedstock and oil could be measured and the yield calculated. Weigh the vegetable matter prior to the extraction. While the oil is separating weigh an empty container such as a microcentrifuge tube. Once the aqueous and oil layers are completely separated, the upper oil layer can be carefully removed with a disposable pipette and added to the weighed container. Calculate the yield:  $(\text{weight of oil} \div \text{weight of vegetable matter}) \times 100 = \% \text{ yield}$ .

# Current Biofuels

Biodiesel can be made from the vegetable oil extracted – see [activity 1D](#) Biodiesel production. The oil can be extracted with a disposable pipette from the surface and used to make biodiesel if there is a sufficient quantity of oil, and providing particulate matter is removed.

Tests for saturated or unsaturated oils can be carried out by GCSE or post-16 students. For further details see CLEAPSS® Guidance PS 67-01 (Testing for unsaturation), 'Unsaturation in fats and oils' from Practical Chemistry [www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html](http://www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html) or SEP Biofuels activity A5: Saturation of fuels.

If a large volume of oil can be produced prior to the lesson or in preparation for the next lesson, viscosity of the oil produced can be tested – see [activity 1C](#) Oil viscosity.

The resulting oil, biodiesel from [activity 1D](#), sugar from [activity 1E](#) and ethanol from [activity 1G](#) can be collected and tested for their combustion energy – see Gatsby SEP:Biofuels activity A7 'How much energy is released when a fuel burns?' or 'Energy values of food' from Practical chemistry [www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html](http://www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html)

Oilseed rape (*Brassica napus*) is a member of the *Brassicaceae* family and rapid-cycling *Brassicaceae* are especially amenable to experimentation. Rapid-cycling *Brassicaceae* are used as a model plant for a wide range of studies and can produce seeds in as little as 40 days. This makes the growth of rapid-cycling *Brassicaceae* ideal as a preparatory or follow-up activity with students.

## Suppliers

Sweetcorn, sunflower seeds and other plant material can be obtained from a local supermarket.

Mortars, pestles, test tubes and racks are standard equipment available in secondary school science laboratories otherwise they can be obtained from educational suppliers such as [Philip Harris Education](#), Hyde Buildings, Hyde, Cheshire, SK14 4SH, tel: 0845120 4520 fax: 0800 138 8881. and Rapid [www.rapidonline.com](http://www.rapidonline.com) Severalls Lane, Colchester, Essex, C04 5JS tel: 01206 751166 fax: 01206 751188.

Rapid-cycling *Brassica* kits and seeds are available from Philip Harris Education [www.philipharris.co.uk/secondary/biology/plants-as-organisms/rapid-cycling-brassica-basic-kit/?ev=search](http://www.philipharris.co.uk/secondary/biology/plants-as-organisms/rapid-cycling-brassica-basic-kit/?ev=search) or 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

Gatsby Science Enhancement Programme (SEP): Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

National Non-Food Crops Council (NNFCC), 2007, *Biorefineries: definitions, examples of current activities and suggestions for UK development*. National Non-Food Crops Council position paper. Available online at [www.nnfcc.co.uk/metadot/index.pl?id=3143:isa=DBRow:op=show:dbview\\_id=2457](http://www.nnfcc.co.uk/metadot/index.pl?id=3143:isa=DBRow:op=show:dbview_id=2457)

Exploring knowledge of gene function to combat pod shatter in oilseed rape [www.bbsrc.ac.uk/news/food-security/2011/110615-pr-improved-crops-food-security.aspx](http://www.bbsrc.ac.uk/news/food-security/2011/110615-pr-improved-crops-food-security.aspx) [www.jic.ac.uk/corporate/media-and-public/current-releases/110615CIRCProjects.html](http://www.jic.ac.uk/corporate/media-and-public/current-releases/110615CIRCProjects.html)

Information on the use of the rapid-cycling *Brassica* kits is available from Science and Plants for Schools (SAPS) [www.saps.org.uk/secondary/teaching-resources/126-rapid-cycling-brassica-kits-](http://www.saps.org.uk/secondary/teaching-resources/126-rapid-cycling-brassica-kits-)

Fast plants is a site dedicated to the educational use of rapid-cycling *Brassicaceae* [www.fastplants.org/#menu](http://www.fastplants.org/#menu)

Tompkins, S.P. and Williams, P.H., 1990, *Journal of Biological Education*, **24** (4), 239-250.

## Current Biofuels

Dissecting the genomes of crop plants to improve breeding potential [www.jic.ac.uk/corporate/media-and-public/current-releases/110731oilseedgenome.html](http://www.jic.ac.uk/corporate/media-and-public/current-releases/110731oilseedgenome.html)

Dissecting the genome of the polyploid crop oilseed rape by transcriptome sequencing [www.nature.com/nbt/journal/v29/n8/full/nbt.1926.html](http://www.nature.com/nbt/journal/v29/n8/full/nbt.1926.html).

Evans, N., Welham, S.J., Antoniw, J.F., Fitt, B.D.L., 2006. Development and Uptake of a Scheme for Predicting Risk of Severe Light Leaf Spot on Oilseed Rape. *Outlooks on Pest Management*, **17**(6), pp. 243-245.



© John Innes Centre

Shattering Rapeseed pods

The fungus forecast: protecting crops from attack.

Mathematical models help UK farmers counter threat to oilseed rape. [www.bbsrc.ac.uk/news/food-security/2010/101207-f-fungus-forecast.aspx](http://www.bbsrc.ac.uk/news/food-security/2010/101207-f-fungus-forecast.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

Undertaking research to improve oilseed rape yields to address **Food Security**. Professor Lars Ostergaard, Crop genetics, John Innes Centre [www.jic.ac.uk/profile/Lars-Ostergaard.asp](http://www.jic.ac.uk/profile/Lars-Ostergaard.asp)

The Bancroft Research Group, John Innes Centre. [www.jic.ac.uk/staff/ian-bancroft/](http://www.jic.ac.uk/staff/ian-bancroft/)

# Current Biofuels

## Activity 1C - Oil viscosity

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

- Explain the importance of identifying fuel viscosity.
- Carry out viscosity tests on a variety of different fuels.
- Evaluate the pros and cons of different transport fuels.



Oil

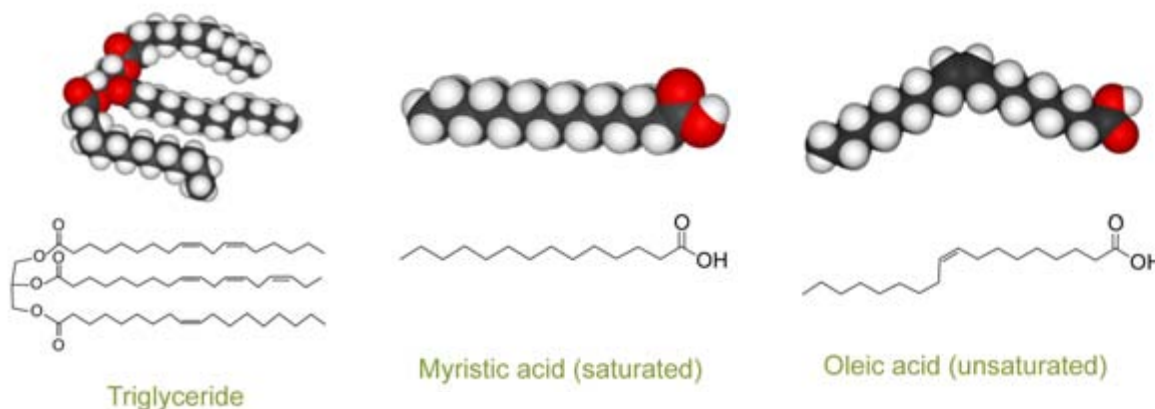
**Keywords** Bioenergy, biofuel, biodiesel, renewable, viscosity, waste, saturation, oil, density, saturation, unsaturated, double bond, Van der Waals.

## Background

The viscosity of fuels affects their melting points, ignition temperature, heat of combustion, the rate at which they burn, density, energy density and lubricity. The amount of energy stored in a defined volume of fuel (energy density) is important for identifying the best fuels. The higher the energy density the further a vehicle will be able to travel on the same amount of fuel and thus the smaller the fuel tank needs to be. The energy density of common fuels are listed in descending order: methane, natural gas, petrol, diesel, kerosene, biodiesel, ethanol.

Oils are appealing alternatives to petrol due to their greater density, safety and lower exhaust emissions. However, the viscosity of oils also affects the ease with which they can be converted to biodiesel and the engines they are suitable for. More viscous fuels leave more deposits in car engines and can cause problems with engine pressures and injection systems. The storage and 'shelf-life' of oil-based fuels is more limited due to the oxidation of polyunsaturated hydrocarbons.

Viscosity is influenced by the molecular properties of liquids such as vegetable oils. There is a direct relationship between the chain length and degree of saturation of the fatty acids that form triacylglycerols. Generally speaking saturated oils are more viscous than unsaturated oils. Unsaturated oils have double bonds in the hydrocarbon chains, whereas saturated oils feature more single bonds and attached hydrogen atoms. The linear "zigzag" structure of saturated fatty acid hydrocarbon chains enables the molecules to line up and form intermolecular Van der Waals interactions, reducing their viscosity. Unsaturated hydrocarbon chains have double bonds that produce "kinks" in the molecule. These "kinks" prevent the molecules getting as close to each other and forming as many Van der Waals interactions, thus increasing the viscosity of the oil.



Molecules (Jmol: an open-source Java viewer for chemical structures in 3D <http://www.jmol.org/>, CPK spacefill 50% vanderwaals)

# Current Biofuels

This activity involves testing the viscosities of a variety of vegetable oils based on the time a drop of oil takes to run down a sheet of plastic. This can be done using dropping pipettes or dropper bottles, alternatively you could also use a falling ball or cup viscometer.

Students can be introduced to the concept of differing viscosities by providing them with a selection of oils in sealed centrifuge or test tubes and asking them to arrange them in order according to how runny they are. The reason for these differences can be discussed and predictions made about the viscosity of the oils.

The experiment can be extended by repeating the investigations after warming the oils in warm water or a water bath. This will enable pupils to produce line graphs comparing the viscosity of the oils at different temperatures and establish if there is a linear relationship between viscosity and temperature.

The use of the falling ball or cup viscometer enables quantitative measurements to be made. Viscosity is measured in Pascal seconds (Pa.s) and dynamic viscosity can be calculated using the following equation:

$$\eta = \frac{2(\Delta\rho)ga^2}{9v}$$

$\eta$  = dynamic viscosity

$\Delta\rho$  = difference in density between the sphere and liquid

$g$  = acceleration of gravity

$a$  = radius of sphere

$v$  = velocity

**Age Range:** Measuring oil viscosity is suitable for secondary students and calculating viscosity is suitable for post-16 students.

**Duration:** 30-60 minutes.

**Suggested prior knowledge:** Secondary students should have a good understanding of the properties of liquids and how to carry out a fair test. Knowledge of molecules and the way their size or shape can affect their properties including the forces between molecules and the difference between saturated and unsaturated molecules will enable GCSE and post-16 students to form a better understanding of the results and evaluation of fuels.

## What you will need

- A selection of vegetable oils – sunflower, maize, olive, rapeseed
- A selection of fuels – ethanol (available from **activity 1G**), biodiesel (available from **activity 1D**), synthetic crude oil (CLEAPSS® recipe Book 32)
- Dropping pipettes or dropper bottles
- Plastic beakers
- Clamp stand, bosshead and clamp
- Timer or stopwatches
- Clean smooth polycarbonate (chemically resistant) plastic boards
- Shallow trays
- Cleaning materials especially blue roll

### Optional

- Falling ball or cup viscometer
- Water bath or warm water and thermometer

# Current Biofuels

## Health and Safety

Sesame and nut oils are not recommended due to the potential allergic reactions they can produce. Vegetable oils and fuels are flammable and should be kept away from naked flames. See also CLEAPSS® Hazcards 45 and 46 (Hydrocarbons), and guidance leaflets PS 67-01 (Testing for unsaturation) and PS 67-05 (The viscosity of motor oils).

## Method

1. The clamp stand and clamp should be arranged so that it is able to hold one end of the plastic board with the other resting in a shallow tray.
2. Mark a start and finish line on the plastic board approximately 20cm apart.
3. Decant the vegetable oils into dropper bottles or labelled containers.
4. Students drop the oil onto the plastic boards using dropping pipettes or dropper bottles.
5. They then use a timer to measure the time taken for the oil drops to cover the distance between the start and finish lines.

### *Optional*

6. Repeat with warmed oils and graph the results of time taken against temperature. Recommended temperatures are 20, 25, 30, 35 and 40°C. Do not use hot oils or fuels. Ensure that the plastic board is warmed to the corresponding temperature as the oils. Use pieces of plastic board that are short enough to fit in the water bath.

It is best to do some trial runs prior to starting this activity to choose a suitable angle for the plastic board and an appropriate distance between the start and finish lines. As the oil drops leave behind a residue they may not reach the finish line if the distance is too large. The plastic sheet will need to be wiped clean between each test so that residues do not affect the results.

This activity is based on one developed by the Gatsby SEP: Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

## Extension activities

As an extension you could give students the chemical formulae or molecular composition of a range of liquids including oils, biofuels, short and long chain, saturated and unsaturated fatty acids and triglycerides and ask them to predict their relative viscosities. These could be laid out on a line from least to most viscous.

They could then be provided with the corresponding viscosities and asked to draw conclusions on the molecular basis of viscosity based on this information, such as the longer the molecule the greater the viscosity, or the more saturated the molecule the more viscous.

# Current Biofuels

## Liquid viscosities and molecular structures

Substance	Dynamic Viscosity @ 25°C (mPa.s)	Molecular structure
Refined sunflower oil	48.98	10% (sat. f.a.s) 20% (monounsatur. f.a.s) 70% (polunsatur.f.a.s)
Refined maize oil	51.44	12% (sat. f.a.s) 31% (monounsatur. f.a.s) 57% polunsatur.f.a.s)
Olive oil	63.28	15% (sat. f.a.s) 75% (monounsatur. f.a.s) 10% (polunsatur.f.a.s)
Glycerol	1420 (20°C)	$C_3H_8O_3$
Biodiesel	5.75	$RCOOCH_3$
Bioethanol	1.1 (20°C)	$C_2H_5OH$
Water	1 (20°C)	$H_2O$

(Data from H. Abramovic and C. Klofutar, 1998, The temperature dependence of dynamic viscosity, some vegetable oils, *Acta Chim. Slov*, 45(1), 69-77, S. Kerschbaum and G. Rinke, 2004, Measurement of the temperature dependent viscosity of biodiesel fuels, *Fuel*, 83(3), 287-291 and <http://physics.info/viscosity/>)

If you would like to carry out further experimental work with post-16 students the iodine value of the vegetable oils can be calculated to determine the degree of saturation (CLEAPSS® Guidance PS 67-01). Alternatively an extension activity using bromine water to test for unsaturation can be carried out. This is a common practical activity carried out in school-see CLEAPSS® Guidance PS 67-01 (Testing for unsaturation), 'Unsaturation in fats and oils' from Practical Chemistry [www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html](http://www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html) or SEP Biofuels activity A5: Saturation of fuels.

## Suppliers

A selection of vegetable oils – sunflower, maize, olive and rapeseed - are recommended as they are easy to obtain in the majority of supermarkets.

Falling ball viscometers and cup viscometers are available from Mindsets (UK) Ltd [www.mindsetonline.co.uk/index.php](http://www.mindsetonline.co.uk/index.php)



# Current Biofuels

## Further reading and links

Abramovic, H. and Klofutar, C. 1998. The temperature dependence of dynamic viscosity for some vegetable oils, *Acta Chim. Slov.*, **45**(1), 69-77.

Kerschbaum, S. and Rinke, G. 2004. Measurement of the temperature dependent viscosity of biodiesel fuels, *Fuel*, **83**(3), 287-291.

Anand, K. Ranjan, A. and Mehta, P.S. 2010. Estimating the Viscosity of Vegetable Oil and Biodiesel Fuels, *Energy Fuels*, **24**, 664–672.

Unsaturation in fats and oils, Practical Chemistry [www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html](http://www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html)

CLEAPSS® Guidance PS 67-01 (Testing for unsaturation) Gatsby Science Enhancement Programme (SEP): Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

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 Jonathan Napier, Rothamsted Centre for Crop Genetic Improvement [www.rothamsted.bbsrc.ac.uk/Research/Centres/ProjectDetails.php?Centre=CGI&ProjectID=4950](http://www.rothamsted.bbsrc.ac.uk/Research/Centres/ProjectDetails.php?Centre=CGI&ProjectID=4950)

[www.rothamsted.bbsrc.ac.uk/Research/Centres/PressReleases.php?PRID=51](http://www.rothamsted.bbsrc.ac.uk/Research/Centres/PressReleases.php?PRID=51)

[www.rothamsted.bbsrc.ac.uk/Research/Centres/ProjectDetails.php?Centre=CGI&ProjectID=5015](http://www.rothamsted.bbsrc.ac.uk/Research/Centres/ProjectDetails.php?Centre=CGI&ProjectID=5015)

Dr Peter Eastmond, [www2.warwick.ac.uk/fac/sci/lifesci/people/peastmond/](http://www2.warwick.ac.uk/fac/sci/lifesci/people/peastmond/) Plant Lipid Metabolism Group, University of Warwick, [www2.warwick.ac.uk/fac/sci/lifesci/research/plantmetabolism/](http://www2.warwick.ac.uk/fac/sci/lifesci/research/plantmetabolism/)

# Current Biofuels

## Activity 1D – Biodiesel production

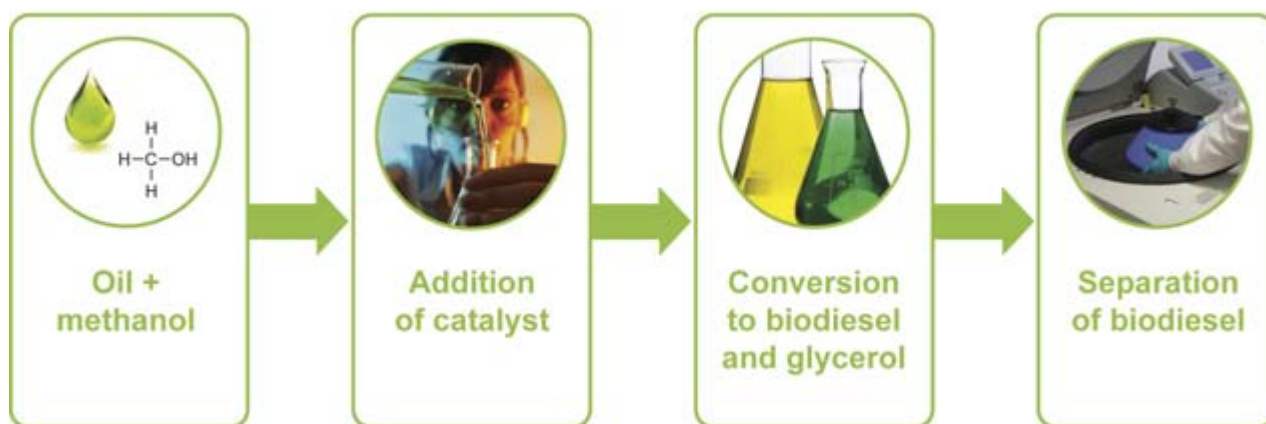
**Learning outcomes:** By the end of the session students should be able to:

- Describe the techniques used to produce biodiesel.
- Carry out the conversion of vegetable oil to biodiesel.
- Discuss the efficiency of biodiesel production and the uses of the by-products.

**Keywords** Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, yield, catalyst, methanol, glycerol, maize, oilseed rape, soya, potassium hydroxide, centrifuge, transesterification.

## Background

In order to convert oil to biodiesel it is mixed with methanol, or occasionally ethanol, and a catalyst (potassium hydroxide) added to speed up the reaction. This is a transesterification reaction that produces biodiesel and glycerol. Biodiesel is less dense than glycerol and the two products are separated by gravity or using centrifuges. The biodiesel can be used to replace regular diesel or mixed with regular diesel in varying concentrations, while glycerol can be used in soap and cosmetics. The potassium hydroxide (KOH) catalyst increases the rate of reaction but does not increase the yield. Most of the KOH separates out into the lower glycerol layer and any remaining in the biodiesel is removed by washing with water.



This activity is based on the ones published by CLEAPSS ©: Making Biodiesel. [www.cleapss.org.uk](http://www.cleapss.org.uk) and the Gatsby Science Enhancement Programme (SEP).

# Current Biofuels

**Age Range:** This activity is suitable for GCSE and A-level students.

**Duration:** 60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of fuels, properties of gases and liquids, catalysts and health and safety.

## What you will need

- Test tubes and stoppers or 15 ml centrifuge tubes
- Test tube racks
- Centrifuge (a hand centrifuge can be used effectively)
- Cooking oil or oil extracted and filtered from an oil extraction ([activity 1B](#))
- Methanol (VERY TOXIC and EXTREMELY FLAMMABLE)
- Potassium hydroxide (CORROSIVE)
- 5% w/w potassium hydroxide solution in methanol (5 g potassium hydroxide per 50 ml of methanol)
- Access to a fume cupboard
- Magnetic stirrer and stirring fleas
- Conical flask, 250 ml
- Conical flask, 100 ml
- Measuring cylinders 100 ml
- Disposable nitrile gloves
- Safety goggles

### Optional

- Balance
- Separating funnel
- Dropping pipettes
- Distilled water

## Health and Safety

It is essential that eye protection is worn, preferably goggles rather than safety spectacles. Nitrile gloves should be worn.

NOTE: when preparing the catalyst a fume cupboard is required in addition to goggles.

This experiment involves Methanol (VERY TOXIC and EXTREMELY FLAMMABLE) and potassium hydroxide (CORROSIVE). Methanol is the most effective solvent for use in this experiment but extra care needs to be taken to ensure that students wear Personal Protection Equipment (PPE) and act sensibly during this procedure. CLEAPSS® do not recommend using sodium hydroxide instead of potassium hydroxide because it has a lower solubility in ethanol. Consult technicians at a school prior to carrying out this activity as it is far safer to have the KOH methanol mixture made up in school in small aliquots already prepared in stoppered test tubes for students. If the reagents are taken into school or used at an event ensure that the correct risk assessments have been completed, the hosts have been informed of the planned activity and that the correct regulations concerning transport of chemicals are adhered to. The guide - Transporting chemicals for lecture demonstrations & similar purposes, Royal Society of Chemistry (RSC), January 2008-suggests that small volumes can be transported safely if clearly labelled with LQ (limited quantity) notices.

# Current Biofuels

NOTE: Provide clear instructions to the students NOT to shake the test tubes as the methanol may squirt out.

Any contact of the methanol solution or biodiesel mixtures with the skin should be washed off under a tap straight away.

The volume of methanol used in the preparation of the catalyst and the activity pose a low risk of exposure by vaporisation. Methanol and biodiesel mixtures are highly flammable and should be kept away from naked flames

For further information read the CLEAPSS® Guidance PS 67-10 (Making bio-diesel), Recipe book RB71 (Potassium hydroxide), RB102 (Testing for organic functional groups) and Hazcards 40B (Methanol), 40C (Carbohydrates), 45 and 46 (Hydrocarbons).

If using a centrifuge ensure that the centrifuge tubes are balanced and that the tubes used for centrifugation are sealed.

## Method

NOTE: Steps 1 and 2 should be carried out by a properly qualified adult in a fume cupboard. See the Health and Safety instructions for further information.

1. Prepare the catalyst. A 5% w/v potassium hydroxide (KOH) solution should be prepared with methanol by adding 5 g of KOH to 100 ml of methanol in a conical flask or beaker on a stirrer. Wait until all the potassium hydroxide has dissolved.
2. 1 ml or 1.5 g of the methanol KOH solution should be aliquoted into test tubes or centrifuge tubes and sealed prior to the activity. If any of the solution remains it should be stored in an airtight borosilicate glass bottle and clearly labelled, adding the hazard symbols CORROSIVE, TOXIC and EXTREMELY FLAMMABLE.
3. Students can add 10 ml or 10 g of vegetable oil to a test tube or 15 ml centrifuge tube.
4. Add the contents of the tube containing the methanol / potassium hydroxide catalyst to the tube containing the vegetable oil and ensure the tube is properly sealed.
5. In order to carry out the conversion reaction the tubes should be carefully and slowly inverted over 30 times to ensure adequate mixing.
6. Students should carefully observe the contents of the test tube. The biodiesel separates out in the top layer while a lower layer of glycerol gradually forms.
7. Students should label the tubes with the contents and their names.

Students will be able to observe a reaction almost immediately on mixing the oil and catalyst. The oil will quickly become less viscous. However, it is better to prepare biodiesel in one lesson, store it in labelled test tubes to allow the layers to separate fully, and perform any follow-up tests in the next lesson. If the products of the reaction are to be tested it is best to wait at least 24 hours for full separation or centrifuge the solutions.

Disposable pipettes can be used to separate the two products of the reaction by siphoning off the top biodiesel layer and transferring it to another test tube or centrifuge tube. Alternatively a separating funnel can be used to run out the lower layer of glycerol, leaving the layer of biodiesel behind. If a centrifuge is used to separate the products it is recommended that the mixture is transferred to microcentrifuge tubes using disposable pipettes and a small bench top centrifuge is used. Ensure that the centrifuge is balanced. Care is then needed to separate the two layers and a fine-tipped disposable pipette is recommended.

# Current Biofuels

## Extension activities

Improve the quality of the biodiesel by carrying out a further washing step to meet the standards required for use in vehicle engines. Impurities in biodiesel cause problems in modern and high-end car engines and it is important to 'wash' the biodiesel prior to use. This can be carried out by the students by adding an equal volume of distilled water to the biodiesel, mixing the solution and repeating the separation procedure.

Calculate the yield of biodiesel. Have students weigh the oil, methanol and resultant biodiesel. Weigh the test or centrifuge tube prior to adding the oil, after adding the oil and after adding the methanol. Once the biodiesel and glycerol layers are completely separated the upper biodiesel layer can be carefully removed with a disposable pipette and added to a weighed container. Calculate the yield:  $(\text{weight of biodiesel} \div \text{weight of oil and methanol}) \times 100 = \% \text{ yield}$ .

Compare the burning qualities of the biodiesel and vegetable oil. The biodiesel, oil from [activity 1B](#), sugar from [activity 1E](#) and ethanol from [activity 1G](#) can be collected and tested for their combustion energy – see Gatsby SEP: Biofuels activity A7 'How much energy is released when a fuel burns?' or 'Energy values of food' from Practical Chemistry [www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html](http://www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html). These activities should be carried out in a fume cupboard.

Compare the viscosity of the biodiesel and vegetable oil. See [activity 1C](#) Oil viscosity.

Test the biodiesel for saturation. For further details see CLEAPSS® Guidance PS 67-01 (Testing for unsaturation), 'Unsaturation in fats and oils' from Practical Chemistry [www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html](http://www.practicalchemistry.org/experiments/unsaturation-in-fats-and-oils.227.EX.html) or SEP Biofuels activity A5: Saturation of fuels.

Discussion activities about the practicalities of the technique used on an industrial scale, the sources of feedstocks and economic viability of producing biodiesel to replace fossil fuels.

Biodiesel UpD8 activity [www.upd8.org.uk/activity/256/Biodiesel.html](http://www.upd8.org.uk/activity/256/Biodiesel.html)

## Suppliers

Vegetable cooking oils can be obtained from supermarkets or local shops.

The laboratory equipment should be available in most secondary schools science departments. A small benchtop centrifuge can be used with smaller volumes.

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

Hand centrifuges can be obtained from Rapid [www.rapidonline.com](http://www.rapidonline.com) Severalls Lane, Colchester, Essex, C04 5JS tel: 01206 751166 fax: 01206 751188

A 'Green chemistry: production of biodiesel' kit containing the oil, alcohol and catalyst for this activity is available from Rapid (see above).

# Current Biofuels

## Further reading and links

CLEAPSS® Guidance PS 67-10 (Making bio-diesel).

Gatsby Science Enhancement Programme (SEP): Biofuels. 2009. [www.sep.org.uk](http://www.sep.org.uk)

Making Biodiesel pre-16, Royal Society of Chemistry <http://media.rsc.org/Learning%20about%20materials/Materials%20Biodiesel%20Part%201.pdf>

Making Biodiesel post-16, Royal Society of Chemistry <http://media.rsc.org/Learning%20about%20materials/Materials%20Biodiesel%20Part%202.pdf>

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 Gillian Stephens, University of Nottingham, [Process Intensification for Acceleration of Bio & Chemo Catalysis in Biorefining](http://www.nottingham.ac.uk/engineering/departments/chemenv/people/gill.stephens) [www.nottingham.ac.uk/engineering/departments/chemenv/people/gill.stephens](http://www.nottingham.ac.uk/engineering/departments/chemenv/people/gill.stephens)

Dr Sohail Ali, Plymouth Marine Laboratory, [Integrated approach to cost effective production of biodiesel from photosynthetic microbes](http://www.pml.ac.uk/about_us/pml_people/sohail_ali.aspx) [www.pml.ac.uk/about\\_us/pml\\_people/sohail\\_ali.aspx](http://www.pml.ac.uk/about_us/pml_people/sohail_ali.aspx)

Professor Antoni Slabas, Durham University, [An integrated approach to the cost effective production of biodiesel from photosynthetic microbes](http://www.dur.ac.uk/biosciences/about/schoolstaff/academicstaff/?id=40) [www.dur.ac.uk/biosciences/about/schoolstaff/academicstaff/?id=40](http://www.dur.ac.uk/biosciences/about/schoolstaff/academicstaff/?id=40)

Dr Sean Murphy, CAB International, [Impacts of tropical land use conversion to jatropha and oil palm on rural livelihoods and ecosystem services in India and Mexico](http://www.cabi.org/default.aspx?site=170&page=1019&siteid=1426) [www.cabi.org/default.aspx?site=170&page=1019&siteid=1426](http://www.cabi.org/default.aspx?site=170&page=1019&siteid=1426)

Professor Johnathan Napier, Rothamsted Research, [Rational metabolic engineering of oilseed fatty acid composition](http://www.rothamsted.ac.uk/Research/Centres/PersonDetails.php?Centre=CGI&PIID=137037) [www.rothamsted.ac.uk/Research/Centres/PersonDetails.php?Centre=CGI&PIID=137037](http://www.rothamsted.ac.uk/Research/Centres/PersonDetails.php?Centre=CGI&PIID=137037)

Dr Kerrie Farrar, Aberystwyth University, [W](http://www.aberystwyth.ac.uk/~k.farrar/)

Dr James Murray, Imperial College London, [Q](http://www.imperial.ac.uk/~j.murray/)

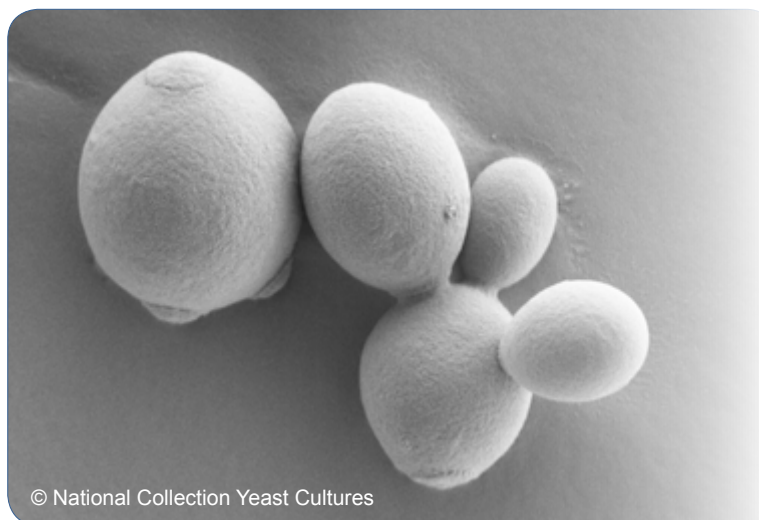
# Current Biofuels

## Bioethanol

**Keywords** Bioenergy, biofuel, bioethanol, sustainable, renewable, biomass, anaerobic, waste, bacteria, microbes, fermentation, yeast, methane, sugar beet, sugar cane, maize, wheat, yield, sucrose, glucose, polysaccharide, pentose.

## Background

Bioethanol is produced by fermentation of simple monosaccharide and disaccharide sugars by yeast such as *Saccharomyces cerevisiae* (seen on the right) or *Escherichia coli*. The bacterium *Zymomonas mobilis* is a promising alternative to yeast due to its greater sugar uptake, yields and resistance to ethanol concentrations. Currently sugar beet (15%), sugar cane (27%), maize (22%) and wheat (25%) are the main sources of sugar for bioethanol production using current methods, though rice is also a popular feedstock. Sugar cane is produced on a large scale in Brazil and sugar beet is cultivated in the UK. There are currently a number of bioethanol plants in the UK, using wheat or sugar beet as feedstocks.



© National Collection Yeast Cultures

SEM image Yeast cells budding

Bioethanol is currently produced and used more than any other biofuel. It is widely used in the USA and Brazil as a replacement for petrol. There are many advantages to the use of bioethanol as a transport fuel which has led to a rapid increase in production in the USA and Brazil. Currently common crops familiar to farmers can be grown on a large scale to produce bioethanol, with Brazil producing over half the ethanol traded globally. The processes involved in production, such as distillation, are well-established technologies with existing infrastructure for industrial levels of throughput and distribution. The final product is also compatible with existing vehicle engines and can be mixed with fossil fuels. Up to 10% blends with petrol can be used without modifying vehicle engines. In some parts of the USA this is now mandatory and in Brazil legally required blends are 25%. With further development, engines designed to accommodate greater proportions of bioethanol will be more efficient and produce less emissions. In comparison to petrol, life-cycle assessment of Brazilian bioethanol production leads to emission of 80% less greenhouse gases.

Bioethanol is not an ideal fuel as it is corrosive and attracts water (hygroscopic). This causes problems with existing vehicle engines and presents difficulties in storing and distributing the fuel through the current transport infrastructure. Vehicle engines require more modifications if using bioethanol than biodiesel. Bioethanol is also less efficient as it only yields about 70% of the energy content of petrol.

# Current Biofuels

## Activity 1E - Extracting sugar from sugar beet

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

- Describe the process of extracting sugar from sugar beet.
- Calculate the yield of sugar from sugar beet.
- Suggest ways of improving the process for more efficient extraction of sugar and commercialisation.

## Background

Sugar (in the form of sucrose) is the main feedstock used to produce bioethanol in Brazil. In countries that produce sufficient quantities of sugar cane it is an economically viable alternative to biodiesel production. In colder climates the majority of this sugar is obtained from sugar beet. This activity involves the extraction of sugar from sugar beet. The sugar is extracted from the beet by heating in water and then evaporating off the excess water. It is best to conduct this activity in the autumn term when the supply of sugar beet is most plentiful.

**Age range:** This activity is suitable for GCSE and A-level students.

**Duration:** 10-20 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of properties of compounds, methods of separation, states of matter and a basic understanding of the anatomy of plants.

## What you will need

- Sugar beet
- Peelers
- Knives
- Chopping board
- Beakers
- Bunsen burner
- Heatproof mat
- Tripod
- Gauze
- Evaporating basin
- Balance
- Water
- Timer
- Eye protection

## Health and Safety

Eye protection must be worn.

CLEAPSS® laboratory handbook – section 15.5 Plants and seeds (choosing suitable plant material, growing and cultivating plants, sources and suppliers of plants)

CLEAPSS® Hazcards 40C (Carbohydrates)



# Current Biofuels

## Method

1. The sugar beet should be cleaned and peeled.
2. The sugar beet should be sliced into strips about half a centimetre wide and weighed.
3. The strips of sugar beet should be added to a glass beaker along with enough water to cover it. Note: fill the beaker to the half way mark to avoid boiling over.
4. Set up a Bunsen burner with heatproof mat, tripod and gauze.
5. Heat the beaker of sugar beet and water with the Bunsen burner until almost boiling and simmer for 15 minutes with occasional stirring.
6. Weigh an evaporating basin on a balance that is accurate to at least two decimal points before adding any of the beet extract and make a note of the weight.
7. Carefully add enough of the liquid from the beaker to half fill the evaporating basin. Note: use heatproof gloves and take care to avoid splashes, as the liquid will be hot.
8. Heat the evaporating basin gently over the Bunsen burner to evaporate the water.
9. Leave the solution to cool then check for crystals.
10. Add more beet extract solution to the evaporating basin and repeat steps 7-9.
11. Once you have evaporated all the water from the beet extract weigh the evaporating basin containing the sugar crystals.

You can then calculate the actual yield of sugar produced using the equation below.

Yield = weight of sugar extracted/weight of sugar beet

Mass of sugar beet	g
Mass of evaporating basin	g
Mass of evaporating basin and sugar crystals	g
Actual yield of sugar	%

Make a flow chart of your extraction procedure. Use words and diagrams to show each step.

## Extension activities

Test the extract for the presence of sugars, see [activity 1F](#) Carbohydrate testing.

Calculate the relative concentrations of sucrose and glucose extracted from the sugar beet. A refractometer can be used to measure sugar content and glucose test strips or blood glucose monitors used to measure the glucose solutions.

Discuss the requirements of sugar beet plants and how farmers increase yields while coping with drought, pests, disease and climate change.

The sugar together with oil from [activity 1B](#), biodiesel from [activity 1D](#), and ethanol from [activity 1G](#) can be collected and tested for their combustion energy – see Gatsby SEP: Biofuels activity A7 'How much energy is

# Current Biofuels

released when a fuel burns?’ or ‘Energy values of food’ from Practical Chemistry [www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html](http://www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html) . These activities should be carried out in a fume cupboard.

## Suppliers

Sugar beet can be grown from seed or obtained as complete beets during the harvesting season September to Christmas (contact Ian Pettitt at Brooms Barn Research Centre [www.bertiethethebeet.co.uk](http://www.bertiethethebeet.co.uk)).

## Further reading and links

Jaggard, K. W. Qi, A. and Armstrong, M. J. 2009. A meta-analysis of sugarbeet yield responses to nitrogen fertilizer measured in England since 1980. *Journal of Agricultural Science*, **147** 287-301

Jaggard, K. W. Qi, A. and Ober, E. S. 2009. Capture and use of solar radiation, water, and nitrogen by sugar beet (*Beta vulgaris* L.). *Journal of Experimental Botany*, **60** (7) 1919-1925

[www.bertiethethebeet.co.uk/bertie-the-beet](http://www.bertiethethebeet.co.uk/bertie-the-beet) Broom’s Barn Model Farm project has been set up in a field at Broom’s Barn designed so that it is managed in exactly the same way as the rest of the farm, using commercial methods and machinery. This will ensure that the plots will be representative of commercially grown crops and the production of sugar beet from year to year can be monitored.

[www.britishsugar.co.uk/Education-Resources.aspx](http://www.britishsugar.co.uk/Education-Resources.aspx) Education resources from British Sugar covering the topics of sugar production and processing.

Norbert Rillieux and the sugar industry, Royal Society of Chemistry, Chemists in a social and historical context <http://media.rsc.org/Chemists%20in%20a%20social%20&%20historical%20context/CSHC-sugar.pdf>

Renewable Fuels Association (2010) Climate of opportunity: 2010 ethanol industry outlook, available at: [http://ethanolrfa.org/page/-/objects/pdf/outlook/RFAoutlook2010\\_fin.pdf?nocdn=1](http://ethanolrfa.org/page/-/objects/pdf/outlook/RFAoutlook2010_fin.pdf?nocdn=1) .

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

Broom’s Barn is the UK’s national centre for sugar beet research, located 20 miles east of Cambridge. Research spans a range of crops and scientific disciplines and includes liaison/extension work with growers and the industry. [www.rothamsted.ac.uk/broom/sbrindex.php](http://www.rothamsted.ac.uk/broom/sbrindex.php)

# Current Biofuels

## Activity 1F - Carbohydrate testing

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

- Use a variety of chemical tests to identify carbohydrates in plant material.
- Evaluate the merits of the sugar content of different biofuel feedstocks.
- Suggest suitable crops for bioethanol production.

## Background

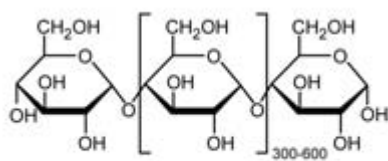
The ability of microorganisms to carry out fermentation is dependent upon the type of sugar substrate. The yeast *Saccharomyces cerevisiae* readily produces ethanol by fermentation of **sucrose** or **glucose** but is less efficient at fermenting other sugars and is unable to ferment pentose (C5) sugars. Current biofuels utilise monosaccharides in the form of sucrose from sugar beet and sugar cane or **polysaccharides** which must be broken down by hydrolytic enzymes prior to fermentation, in the form of starch from maize. Advanced biofuels utilise the polysaccharide cellulose, made up of glucose monomers, and hemicelluloses which are primarily made up of **pentose** sugars such as xylose. Both cellulose and hemicelluloses must be broken down into mono- and di-saccharide sugars before the sugar they contain is accessible for fermentation.

Testing plant material to identify the sugars and carbohydrates present is essential to determine suitable uses of a feedstock to produce biofuel and the enzymes needed to convert them to fermentable substrates.

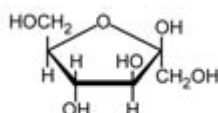
Plants produce a diverse range of sugars which provide energy stores in seeds and form the backbone of plant cell walls. Polysaccharides, such as glucomannans and glucuronoxylans, are of considerable structural complexity in ways that we are only beginning to understand. Recent efforts to unravel the biosynthetic machinery that constructs individual polysaccharides are beginning to provide fundamental insights into plant development, and could hold the key to optimising fermentation processes.

Understanding how sugars are locked into plant cell walls will enable researchers to select the right plants and the right enzymes to release the maximum amount of sugars for conversion to biofuels.

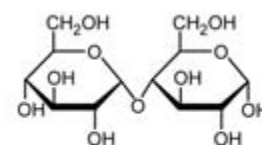
The following set of experiments enables identification of carbohydrates based on the properties of functional groups and the differences between structures.



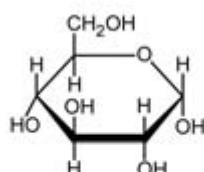
Starch  $[C_6H_{12}O_6]_n$



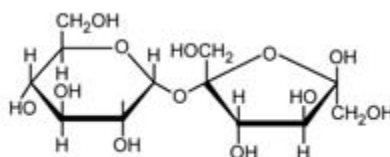
Fructose  $C_6H_{12}O_6$



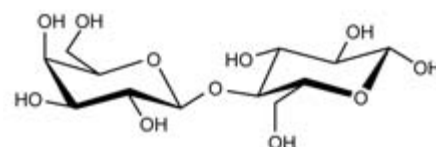
Maltose  $C_{12}H_{22}O_{11}$



Glucose  $C_6H_{12}O_6$



Sucrose  $C_{12}H_{22}O_{11}$



Lactose  $C_{12}H_{22}O_{11}$

# Current Biofuels

## Test for starch

The starch content of a variety of biofuel feedstocks can be compared. This activity could be carried out at a science fair or similar event.

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** 10-15 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

## What you will need

- Iodine solution
- Dropping pipette
- Spotting tile
- Plant material

## Health and Safety

Ensure students are not allergic to any of the plant material being tested. Wash off any iodine that comes into contact with skin immediately.

CLEAPSS® laboratory handbook – section 20.3.1 Carbohydrate tests page 2006

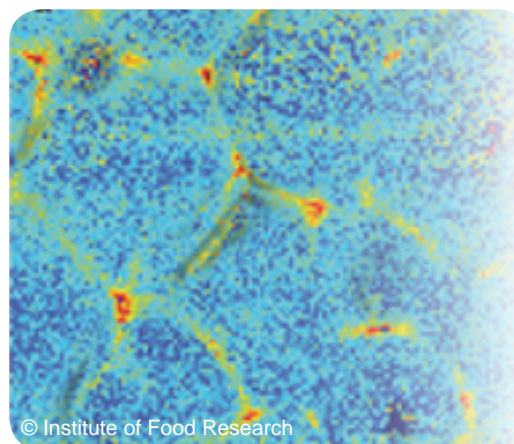
CLEAPSS® Recipe book RB50 (Iodine solution), RB93 (Stains for plant material).

CLEAPSS® Hazcards 40C (Carbohydrates), 54 (Iodine).

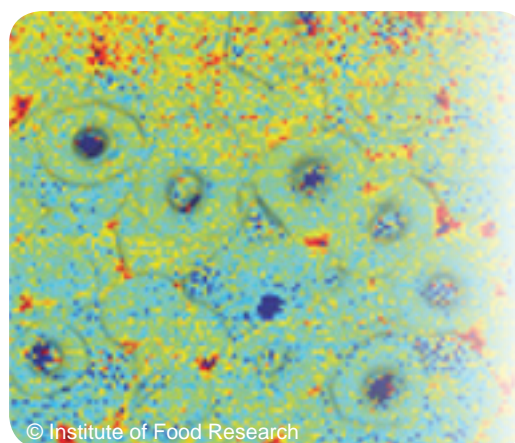
CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

## Method

1. Grind or mash some of the plant material using a pestle and mortar.
2. Add a small amount of the plant material (about 1-2 g) to the spotting tile.
3. Add a drop of iodine solution.
4. A change in colour to blue/black indicates the presence of starch.



**Starch branching in wild type and mutant maize kernels.** Blue areas depict a normal ratio of linear amylose and branched amylopectin



# Current Biofuels

## Test for reducing sugars including glucose

Reducing sugars include the monosaccharides as well as the disaccharides maltose and lactose. They are able to carry out the reduction of copper ions.

Benedict's reagent is a blue solution of copper sulphate containing copper(II) ions ( $\text{Cu}^{2+}$ ), that produces an insoluble red-brown precipitate of copper(I) oxide on reaction with reducing sugars. The copper II ions are reduced to copper I ions by the aldehyde group that is formed on isomerisation of the cyclic to linear form of the sugar.

Non-reducing disaccharides such as sucrose can be broken down to monosaccharides by heating in an acidic solution. This hydrolysis reaction breaks the bond between the two sugars. The resulting monosaccharides will then be able to reduce the blue Benedict's reagent to produce a colour change.

Sucrose is the only common example of a non-reducing sugar, and starch is a poor reducing agent with only the end of the carbohydrate chains having aldehyde groups.

This activity requires a science laboratory.

An alternative method for testing for reducing sugars can be carried out with Fehling's solution. Fehling's solution does work faster but it is more corrosive and must be stored as two separate solutions and made up fresh.

**Age Range:** This experiment is suitable for GCSE and post-16 students.

**Duration:** 50-60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

### What you will need

- Bunsen burner
- Heatproof mat
- Tripod
- Gauze
- Pestle and mortar
- Boiling tubes
- Boiling tube rack
- Glass beaker
- Graduated pipette or syringe
- Spatula
- Funnel
- Test tube holder
- Dropping pipette
- Benedict's solution (1.73 g of copper(II) sulfate pentahydrate, 10 g of anhydrous sodium carbonate and 17.3 g of sodium citrate made up to 100 ml)
- Selection of plant material or sugar beet extract from [activity 1E](#)
- Glucose solution
- Timer
- Eye protection

### Optional

- A variety of known carbohydrate solutions including starch, fructose, sucrose, maltose and lactose

# Current Biofuels

## Health and Safety

Eye protection must be worn. Ensure that a heatproof mat is used. Fill the beaker a third to half full with water to avoid boiling over and splashing hot water. Take care with hot liquids and glassware. Place the test tube in the water bath and remove it after heating using the test tube holder. Do not overheat the solution or heat the boiling tube directly with the Bunsen burner as this may lead to the ejection of hot liquids. Use Benedict's solution rather than Fehling's solution.

CLEAPSS® Laboratory handbook – section 20.3.1 Carbohydrate tests page 2006.

CLEAPSS® Recipe book, RB11 (Benedict's qualitative reagent), RB12 (Benedict's quantitative reagent).

CLEAPSS® Hazcards 27C (Copper salts), 40C (Carbohydrates), 95C (Sodium and Potassium salts).

CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

## Method

1. Grind or mash some of the plant material using a pestle and mortar.
2. Add a small volume of distilled water (approx. 5 ml) and continue to grind the plant material for another couple of minutes.
3. Transfer approximately 5 g of the extract from the plant material into a boiling tube.
4. Carry out the following steps with a positive glucose control solution in addition to the test samples.
5. Add 10 drops or 3 ml of Benedict's reagent using a pipette or syringe.
6. Place the boiling tube into a beaker of warm water.
7. Heat the beaker until the water boils and maintain the heat for 8-10 minutes.
8. Carefully remove the boiling tube from the beaker using a test tube holder and transfer to a boiling tube rack.
9. The presence of glucose is indicated by a change in colour from blue to green, yellow, orange and brick-red colours depending on the amount of reducing sugar present.

# Current Biofuels

## Extension activity: Testing glucose concentrations using Benedict's reagent

### What you will need

- Bunsen burner
- Heatproof mat
- Gauze
- Tripod
- Boiling tubes
- Boiling tube rack
- Glass beaker
- Graduated pipette or syringe
- Spatula
- Test tube holder
- Dropping pipette
- Benedict's solution (1.73 g of copper(II) sulfate pentahydrate, 10 g of anhydrous sodium carbonate and 17.3 g of sodium citrate made up to 100 ml)
- 10% glucose solution
- Selection of solutions prepared for glucose testing (ensure the same volume of distilled water is added to each sample)
- Distilled water
- Timer
- Eye protection

### Method

1. Make up a dilution series using the 10% glucose solution and the following dilutions  
1 ml of 10% glucose + 9 ml of water = 1%  
1 ml of 1% glucose + 9 ml of water = 0.1%  
1 ml of 0.1% glucose + 9 ml of water = 0.01%
2. Test each dilution by adding ten drops of Benedict's reagent to 1 ml of the glucose solution.
3. Place the boiling tube into a beaker of warm water.
4. Heat the beaker until the water boils and maintain the heat for 8-10 minutes.
5. Carefully remove the boiling tube from the beaker using a test tube holder and transfer to a boiling tube rack.
6. The colour of the solutions from blue to green, yellow, orange and brick-red colours indicates the concentration of glucose present.

Repeat steps 2-5 with the test samples and compare with the colour of the standards to determine the concentration.

# Current Biofuels

## Alternative Method: Testing glucose concentrations using potassium permanganate

This technique uses an acidified solution of potassium permanganate as the indicator. The purple pink solution of potassium permanganate ( $\text{MnO}_4^-$ ) is reduced to a colourless solution of manganese ions ( $\text{Mn}^{2+}$ ) by glucose.



The concentration of glucose can be determined by the time taken for the colour change. Using a standard solution of potassium permanganate and a set of standard glucose solutions, the rate of glucose oxidation can be calculated and compared to unknowns. The rate of reaction is directly related to the glucose concentration.

To ensure accurate results, concentrations and measurements need to be carefully made and clean glassware used.

See the Science and Plants for Schools (SAPS) Glucose concentration protocol: Estimating glucose concentration in solution. For further details: [www.saps.org.uk/secondary/teaching-resources/103-estimating-glucose-concentration-in-solution](http://www.saps.org.uk/secondary/teaching-resources/103-estimating-glucose-concentration-in-solution)

**Age Range:** This experiment is suitable for GCSE and post-16 students.

**Duration:** 50-60 minutes

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

### What you will need

- Glass beakers
- Boiling tubes
- Boiling tube rack
- Graduated pipette or syringe
- Dropping pipette
- 1M sulfuric acid
- Potassium permanganate solution (0.4 g in 1 litre)
- 12% glucose solution
- Selection of solutions prepared for glucose testing (ensure the same volume of distilled water is added to each sample)
- Distilled water
- Timer
- Glass rod
- Eye protection
- Gloves



# Current Biofuels

## Health and Safety

Eye protection must be worn and gloves are recommended. Potassium permanganate is harmful and oxidising, avoid contact with the skin. In the case of contact with skin wash off immediately. Potassium permanganate solution presents a low hazard but stains hands and clothing. Prepare the potassium permanganate and sulphuric acid solutions for the students and avoid contact of the solid with concentrated sulphuric acid.

CLEAPSS® Student Safety sheet 48, Recipe book RB73 and Hazcard 81 Potassium manganate(VII)

CLEAPSS® Hazcards 40C (Carbohydrates) and 98a (Sulfuric(VI) acid)

CLEAPSS® Laboratory handbook – section 20.3.1 Carbohydrate tests page 2006

CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

## Method

1. Label the beakers and pipettes to avoid cross contamination of potassium permanganate, sulphuric acid and glucose.
2. Make up a series of glucose solutions (2%, 4%, 6%, 8%, 10%, and make up a 12% stock solution of glucose).  
10 ml of 12% glucose + 2 ml of water = 10%  
10 ml of 12% glucose + 5 ml of water = 8%  
7.5 ml of 12% glucose + 7.5 ml of water = 6%  
5 ml of 12% glucose + 10 ml of water = 4%  
2.5 ml of 12% glucose + 12.5 ml of water = 2%
3. Make up a fresh stock indicator solution of equal volumes of sulphuric acid and potassium permanganate.
4. In the following order place 10 ml of the first glucose solution, 5 ml of sulphuric acid then 2 ml of potassium permanganate into the boiling tube.
5. Start the timer.
6. Stir with a stirring rod and stop the timer as soon as the pink colour disappears.
7. Record the time and the glucose solution used.
8. Rinse the pipette used for the glucose solution.
9. Repeat using the other glucose solutions of known concentration.
10. Plot a standard curve of glucose solution against time taken for complete colour change.
11. Repeat for a solution of unknown concentration and record the time.
12. Use the standard curve to estimate the concentration of the unknown solution.

# Current Biofuels

## Testing for non-reducing sugars including sucrose

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** 50-60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of properties of carbohydrates, chemical reactions and the carbon cycle.

### What you will need

- Bunsen burner
- Heatproof mat
- Tripod
- Gauze
- Pestle and mortar
- Boiling tube
- Boiling tube rack
- Beaker
- Spatula
- Test tube holder
- Graduated pipette or syringe
- Dilute hydrochloric acid (1 mol per dm<sup>3</sup>)
- Sodium hydrogen carbonate solution
- Benedict's solution (1.73 g of copper(II) sulfate pentahydrate, 10 g of anhydrous sodium carbonate and 17.3 g of sodium citrate made up to 100 ml)
- Selection of plant material or sugar beet extract from [activity 1E](#)
- Glucose solution
- Sucrose solution
- Eye protection

### Health and Safety

Eye protection must be worn. Ensure that a heatproof mat is used. Fill the beaker a third to half full with water to avoid boiling over and splashing hot water. Take care with hot liquids and glassware. Place the test tube in the water bath and remove it after heating using the test tube holder. Do not overheat the solution or heat the boiling tube directly with the Bunsen burner as this may lead to the ejection of hot liquids. Use Benedict's solution rather than Fehling's solution.

CLEAPSS® Student Safety sheet 48, Recipe book RB73 and Hazcard 81 Potassium manganate(VII).

CLEAPSS® Hazcards 40C (Carbohydrates) and 98a (Sulfuric(VI) acid).

CLEAPSS® Laboratory handbook – section 20.3.1 Carbohydrate tests page 2006.

CLEAPSS® Model risk assessment 3.002 (Chemical testing of food).

# Current Biofuels

## Method

1. Grind or mash some of the plant material using a pestle and mortar.
2. Add a small volume of distilled water (approx. 5 ml) and continue to grind the plant material for another couple of minutes.
3. Transfer the extract from the plant material into a boiling tube.
4. Carry out the following steps with a positive sucrose control solution and a negative glucose control solution in addition to the test samples.
5. Add about 2 ml of dilute hydrochloric acid using a pipette or syringe.
6. Place the boiling tube into a beaker of warm water.
7. Heat the beaker until the water boils and maintain the heat for 10 minutes.
8. Carefully remove the boiling tube from the beaker using a test tube holder and transfer to a boiling tube rack.
9. Allow the boiling tube to cool for 5 minutes before adding 2 ml of sodium hydrogen carbonate solution or slowly adding small amounts of solid sodium hydrogen carbonate until the fizzing stops.
10. Then follow steps 5–7 from the reducing sugar test.

## Extension activity

How could you investigate whether a solution contains both sucrose *and* glucose?

## Suppliers

Glucose, sucrose, as granulated sugar, and fructose can be obtained from most supermarkets. Maltose and lactose can be obtained from brewery suppliers.

## Further reading and links

Benedict, S. R. (1 December 1908). "[A Reagent For the Detection of Reducing Sugars](#)". *J. Biol. Chem.* **5** (6) 485–487.

Goubet F., Barton C.J., Mortimer J.C., Yu X., Zhang Z., Miles G.P., Richens J., Liepman A.H., Seffen K., Dupree P. 2009 Cell wall glucomannan in Arabidopsis is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis. *Plant J.* **60**(3) 527-38.

Mortimer J.C., Miles G.P., Brown D.M., Zhang Z., Segura M.P., Weimar T., Yu X., Seffen K.A., Stephens E., Turner S.R., Dupree P. 2010 Absence of branches from xylan in Arabidopsis gux mutants reveals potential for simplification of lignocellulosic biomass. *PNAS* **107**(40) 17409-14.

Plant sugars provide clues to sustainable bioenergy production [www.bbsrc.ac.uk/news/business-magazine/2010/winter/feature-sustainable-bioenergy-production.aspx](http://www.bbsrc.ac.uk/news/business-magazine/2010/winter/feature-sustainable-bioenergy-production.aspx)

# Current Biofuels

Detecting starch in food, Practical chemistry [www.practicalchemistry.org/experiments/detecting-starch-in-food.223.EX.html](http://www.practicalchemistry.org/experiments/detecting-starch-in-food.223.EX.html)

Science and Plants for Schools (SAPS) Estimating glucose concentration in solution [www.saps.org.uk/secondary/teaching-resources/103-estimating-glucose-concentration-in-solution](http://www.saps.org.uk/secondary/teaching-resources/103-estimating-glucose-concentration-in-solution)

Novel imaging technique looks inside starch granules [www.bbsrc.ac.uk/news/research-technologies/2011/110211-pr-novel-imaging-inside-starch-granules.aspx](http://www.bbsrc.ac.uk/news/research-technologies/2011/110211-pr-novel-imaging-inside-starch-granules.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

Prof. Paul Dupree, BSBEC Cell Wall Sugars Programme, Department of Biochemistry, University of Cambridge [www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html](http://www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html) [www.bioenergy.cam.ac.uk](http://www.bioenergy.cam.ac.uk)

# Current Biofuels

## Activity 1G - Yeast fermentation

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

- Describe the production of ethanol from renewable sources.
- Describe the process of fermentation.
- Carry out fermentation to produce ethanol.
- Analyse the rate of fermentation of different sugars.
- Evaluate the use and economic advantages of producing liquid biofuels (gasohol) from sugar.

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, bioethanol, microbes, yeast, enzyme, fermentation, varieties, sugar.

## Background

Bioethanol is produced by **fermentation** of sugars by **yeast** or *Escherichia coli*. The bacterium *Zymomonas mobilis* is a promising alternative to yeast due to its greater sugar uptake, yields and resistance to ethanol concentrations. Currently sugar beet and sugar cane are the main sources of sugar for bioethanol. Starches from maize or grain feedstocks are hydrolysed with amylase enzymes (saccharification) to produce sugar that can be fermented by yeast. Yeast have been used for centuries in brewing alcoholic drinks. The yeast *Saccharomyces cerevisiae* produces ethanol by fermentation of sucrose or glucose but, like *Zymomonas mobilis*, is unable to ferment pentose (C5) sugars. *Saccharomyces diastaticus* is able to utilise starch for fermentation. The National Collection of Yeast Cultures (NCYC) recommends certain strains for the production of bioethanol, such as *Pachysolen tannophilus*, *Candida succiphilia*, *Candida tenuis* and *Pichia stipitis*, due to their ability to degrade cellulose or ferment xylose.

In this activity students can compare the fermentation rates of yeast (*Saccharomyces cerevisiae*) under a variety of conditions. In order to calculate the rate of fermentation the amount of carbon dioxide produced can be measured over time. This can be done in a number of ways including the use of bubble counters, collection of carbon dioxide (CO<sub>2</sub>) in inverted water-filled measuring cylinders or with balloons attached to the neck of the conical flask or boiling tubes. Choose the method used according to the equipment and time available for the experiment. The volume of carbon dioxide produced can be calculated by multiplying the number of bubbles recorded by a bubble counter and the volume of one bubble. If using balloons, the volume can be measured by carefully tying off the balloon used to collect the gas produced, immersing it in a large measuring cylinder and measuring the displaced volume or by weighing the balloon, as the carbon dioxide is relatively dense. This experiment is suitable for public demonstrations and science fairs, providing appropriate risk assessment is carried out.

Students can investigate a number of variables that affect the rate of fermentation. It is suggested students are split into groups to assess the effects of different variables and report their results back to the rest of the class. The following variables can be easily compared-type of yeast e.g. fresh, dried, fast-acting, glucose concentration, temperature, pH and agitation. Research on the abilities of yeast to ferment different feedstocks and sugars is essential to the development of industrial bioethanol production. Students should investigate the ability of baker's yeast to ferment common sugars such as sucrose, glucose, fructose and maltose. The recovery and reuse of resources is important in making biofuel production economic and environmentally friendly and students could investigate the rate of fermentation with and without immobilising yeast in sodium alginate balls.

# Current Biofuels

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** Approximately two sessions of 30-50 minutes. Allow up to a week between sessions to enable sufficient fermentation for measurable levels of carbon dioxide to be produced. The experiment can be set up and run in one day for a science fair or exhibition with adjustment of the fermentation conditions.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, fermentation, alcohols, fuels and the properties of gases.

## What you will need

- Conical flask (100 ml) or boiling tubes
- Boiling tube rack
- 8% sugar solutions (Glucose, Sucrose, Fructose, Lactose, Maltose)
- 0.1M phosphate buffer pH 7
- Brewer's or baker's yeast (*Saccharomyces cerevisiae*)
- Deionised or distilled water
- Stirrers
- Balloons or bubble counters
- Measuring cylinder (50 ml)
- Thermometer
- Timers
- Beaker of disinfectant
- Eye protection

### Optional

- Water bath
- Sodium alginate
- Syringe
- 1.5% calcium chloride (CaCl) solution
- Buffer solutions at varying pH
- Strainer
- Fermentation locks
- Universal indicator solution
- Cotton wool
- Magnetic stirrer and fleas
- Alternative yeast strains

## Health and Safety

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. It is recommended that incubation is not carried out above 30°C to avoid the growth of potential human pathogens. 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".

Eye protection should be worn.

# Current Biofuels

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

CLEAPSS® Recipe book RB3 (Alginate beads), RB19 (Calcium chloride and nitrate(V) solutions), RB99 (Testing for gases).

CLEAPSS® Hazcards 19A (Calcium salts), 20 (Carbon dioxide), 40A (Ethanol), 40C (Carbohydrates).

CLEAPSS® Guidance PS 04 (COSHH: risk assessments in situations where microorganisms might be involved), PS 15 (Ventilation and levels of carbon dioxide and other gases in the laboratory & prep room), PS 89 (Measurement of anaerobic respiration in yeast).

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).

## Method

1. Prepare the fermentation stock solutions in phosphate buffer.
2. If immobilising yeast in sodium alginate, ideally the solution of sodium alginate is prepared the day before.
3. Resuspend the yeast in a small amount of distilled or deionised water in order to make a final 3% solution in the fermentation reaction. To ensure active cultures, incubate in nutrient broth for 48 hours at room temperature before inoculating. To ensure pure cultures, streak out the yeast on malt agar plates and inoculate from single colonies.
4. Label the conical flasks, add the yeast, sugar solutions and buffers.

						Control
<b>Yeast</b>	✓	✓	✓	✓	✓	✓
<b>Sugar</b>	Sucrose	Glucose	Fructose	Lactose	Maltose	✗

5. Stopper the flasks with bungs holding fermentation locks and attached bubble counters or add balloons to the neck of the flask or boiling tubes.

# Current Biofuels

## Preparing immobilised yeast

1. Prepare a 2% sodium alginate solution with warm distilled or deionised water, mix thoroughly and leave overnight in a fridge. The initial mixture can be very lumpy but will become smooth overnight.
2. Resuspend the yeast in a small amount of distilled or deionised water so that the final solution of immobilised yeast-alginate is not too runny.
3. Add the resuspended yeast solution to the sodium alginate solution and mix thoroughly.
4. Prepare a 1.5% calcium chloride solution with calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ). The calcium ions cause the sodium alginate to set and hence using distilled or deionised water for the alginate and yeast solutions is important as is avoiding contact of the syringe with the calcium chloride solution.
5. Draw the yeast-alginate solution up into a syringe.
6. Add the yeast-alginate solution into a 1.5%  $\text{CaCl}_2$  solution drop by drop. Carefully observe the shape of the drops. If the drops take on a 'comet' shaped appearance add a small amount of distilled or deionised water to the yeast-alginate solution, mix and retry.
7. Allow the yeast-alginate beads to set for at least 10 minutes.
8. Carefully strain the beads and rinse with distilled or deionised water.

## Extension activities

This activity can be carried out using immobilised yeast or different varieties of yeast.

The fermentation reaction may also be set up as a bioreactor with recording of pH and temperature with data logging software.

Students can test the gas produced during fermentation for the presence of carbon dioxide ( $\text{CO}_2$ ) using lime water. The gas can be poured into a test tube with lime water as  $\text{CO}_2$  is denser than air.

If a suitable location, time and equipment are available, the ethanol produced in the fermentation can be distilled. See the Gatsby SEP: Biofuels activity A3: Using fermentation to make ethanol.

The ethanol, oil from [activity 1B](#), biodiesel from [activity 1D](#), and sugar from [activity 1E](#) can be collected and tested for their combustion energy – see Gatsby SEP: Biofuels activity A7 'How much energy is released when a fuel burns?' or 'Energy values of food' from Practical Chemistry [www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html](http://www.practicalchemistry.org/experiments/energy-values-of-food.225.EX.html). These activities should be carried out in a fume cupboard.

## Suppliers

Bioreactors, bubble counters and enzymes 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

Brewer's or baker's yeast can be obtained from local supermarkets, brewery stores, or bakeries.

Dried yeast can also 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.

Sucrose, glucose and fructose can be obtained from local supermarkets, maltose can be obtained from brewery suppliers.

Sodium alginate and universal indicator can be obtained from [Philip Harris Education](#), Hyde Buildings, Hyde, Cheshire, SK14 4SH tel: 0845120 4520 fax: 0800 138 8881.



# Current Biofuels

## Further reading and links

James S. A., Cadet G. M., Carvajal E. J. C., Barahona P. P., Cross K., Bond C. J., Roberts I. N. 2011 *Saturnispora quitensis* sp. nov., a yeast species isolated from the Maquipucuna cloud forest reserve in Ecuador [DOI: 10.1186/1475-2875-33-3](#)

Ravella S. R., James S. A., Bond C. J., Roberts I. N., Cross K., Retter A., Hobbs P. J. 2009 *Cryptococcus shivajii* sp. nov.: a novel basidiomycetous yeast isolated from biogas reactor, [DOI: 10.1186/1475-2875-33-3](#)

Roberts, I.N. and Oliver, S 2011. The yin and yang of yeast: biodiversity research and systems biology as complementary forces driving innovation in biotechnology, [DOI: 10.1186/1475-2875-33-3](#), 477-487.

Yeast fermentation and distillation instructions from Practical Chemistry [www.practicalchemistry.org/experiments/fermentation-of-glucose-using-yeast,109,EX.html](http://www.practicalchemistry.org/experiments/fermentation-of-glucose-using-yeast,109,EX.html)

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](http://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](http://www.microbiologyonline.org.uk/teachers/resources)

Immobilised yeast- Immobilisation of yeast in calcium alginate beads, Dean Madden, 2007. [www.eurovolvox.org/Protocols/immobilisedyeast.html](http://www.eurovolvox.org/Protocols/immobilisedyeast.html)

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

National Collection of Yeast Cultures (NCYC) [www.ncyc.co.uk/](http://www.ncyc.co.uk/) The NCYC collects and preserves a wide variety of yeast cultures with applications in industry and academia; research at NCYC has shed new light on yeast evolution and genetic interrelationships and resulted in novel tools for identifying and characterising yeasts. Research associated with the Collection aims to improve techniques to identify and classify yeasts. The NCYC is involved in the following research projects:

- EEDA “Biomass to Bioalcohol Innovation in the East of England” (2009-2010).
- DEFRA-LINK “Production of BioAlcohols from Lignocellulosic waste materials in the Agri-Food chain” (2008-2012).

# Advanced Biofuels

## Lignocellulosic Bioethanol

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, bioethanol, lignocellulose, lignin, microbes, yeast, enzyme, fermentation, gribbles, varieties, pentose, hexose.

## Background

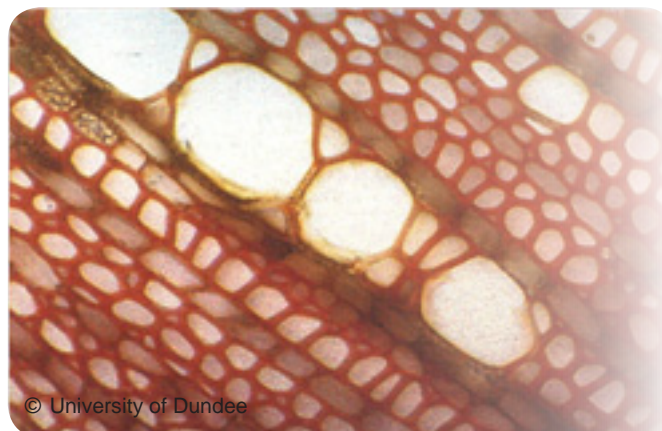
**Bioethanol** is produced by fermentation of sugars by yeast. Currently sugar beet and sugar cane are the main sources of sugar for bioethanol. In the future the biomass locked up in plant cell walls (**lignocellulose**) may be released for fermentation and production of bioethanol. This would enable bioethanol to be produced from wood, straw and waste materials.

Research to achieve this is going on in many areas including

- **Improving perennial biomass crops**
- **Manipulating lignin to optimise sugar release**
- **Improving release of sugars from plant cell walls**
- **Discovering new enzymes for sugar release**
- **Developing yeast strains to ferment sugars**
- **Bacterial fermentation of sugars to butanol**

We can use **enzymes** to break down plant biomass to release sugars for fermentation. In plants the sugars are locked into the cell walls as long chain polymers in ways we currently do not fully understand, preventing effective digestion by enzymes. If we can understand better how the plant sugars are arranged in the cell walls, we can select plants and match them with the most appropriate enzymes for more effective biofuel production.

Improving the properties of lignin in barley straw will make it easier to produce biofuel (or bioenergy) from this material without detrimental effects on the yield or quality of the crop. Lignin is a strengthening and waterproofing polymer that encrusts the sugar-based polymers in plant cell walls, making them hard to access for biofuel production. Lignin and its by-products are also toxic to microorganisms used in fermentation. Feedstocks rich in lignocellulose require treatment with acids, alkalis or steam explosion methods to hydrolyse hemicellulose and break down lignin, enabling access to the cellulose by enzymes. Steam explosion has significant potential as chemical methods have to be managed with recovery and waste water processes and can inhibit enzymes and fermentation.



© University of Dundee

Plant cell wall lignins under the light microscope



© Institute of Biological, Environmental and Rural Sciences

Willow planting measurements

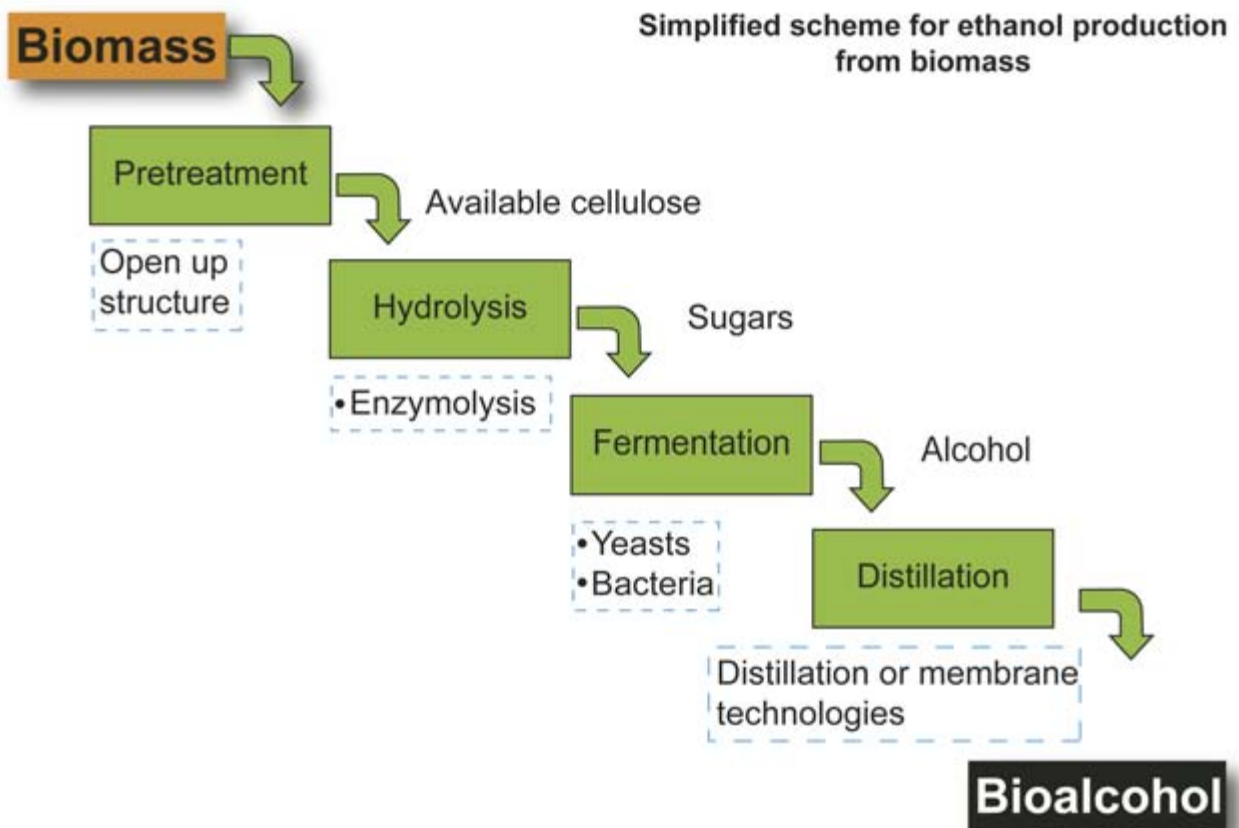
# Advanced Biofuels

Discovering new enzymes in bacteria, fungi and marine wood borers (gribbles) will enable the conversion of non-food plant biomass into biofuels. Wood and straw contain polysaccharides (polymers of sugars) that can be converted into simple sugars suitable for fermentation to produce liquid biofuels. Currently we lack effective enzymes to digest these woody materials. However, marine wood borers are voracious consumers of lignocellulose and have all the enzymes needed for digestion of wood and straw. Scientists have already sequenced the genes that are expressed in the marine wood borer gut and which encode the digestive enzymes. Scientists will study the digestive process in borers as well as a range of microorganisms and investigate the industrial applications of their enzymes for biofuel production.

To harness the potential of lignocellulosic (plant cell wall) materials for sustainable production of bioethanol, we need to optimise energy output without negative environmental, social or economic impacts. We need to optimise the release of sugars from plant cell walls in agricultural and wood-industry wastes to produce a fermentable feedstock that microorganisms can use to produce fuels. Developing robust microbial strains that can use these feedstocks will enable sustainable production of bioethanol.



Marine wood borer (gribble)



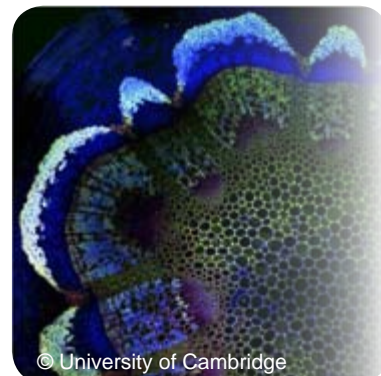
# Advanced Biofuels

## Activity 2A - Plant material testing

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

- Describe the main constituents of plant cells.
- Carry out staining for lignin and cellulose in the cell walls.
- Compare the constituents of different plant material and suggest the ideal components of biofuel crops.

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, bioethanol, starch, lignocellulose, lignin, cellulose, cell wall, miscanthus, willow, perennial.



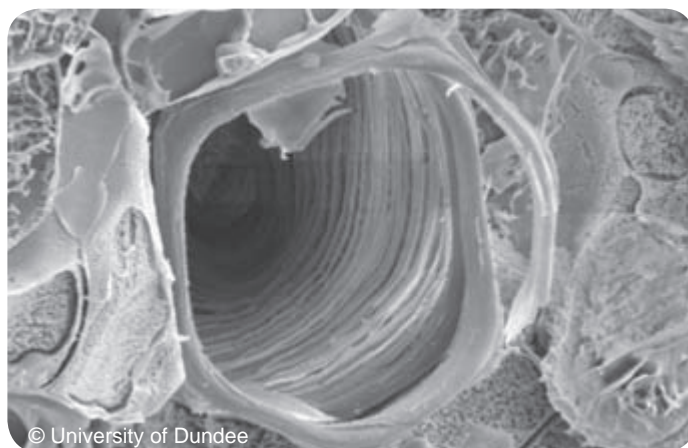
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Stained cross-section of plant stem

## Background

Perennial bioenergy crops could offer a more sustainable alternative to biofuels produced from food crops such as sugar beet, wheat or oilseed rape, which are coming under increasing criticism due to their impact on global food security. They don't require much in the way of fertilisers and can be grown on land that is unsuitable for food production. There is a drawback however, in that woody plants, such as miscanthus and willow, convert much of the carbon that they capture into lignocellulosic polymers, which are not a readily fermentable form of carbohydrate.

Lignocellulose is an important component of plants, giving them strength and rigidity. One of the main components of lignocellulose is a polymer called xylan. Xylan in wood and straw is made up of xylose sugar and represents about a third of the sugars that could potentially be used to make bioethanol, but it is locked away. Releasing the energy from lignocellulose is an important challenge to tackle as it will allow the production of fuels from plants in a sustainable way that does not affect the food chain.



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Xylem imaged with a Scanning Electron Microscope

This activity will enable students to visualise the different constituents of plant cells and assess their relative merits as fermentable biofuel feedstocks. Students prepare microscope slides of plant stems with stains that distinguish the components of plant cell structures such as lignin (aniline or phloroglucinol), starch (iodine) and cellulose (Schulze's solution).

# Advanced Biofuels

FABIL (fuchsin, aniline blue and iodine in lactophenol) is a reagent which stains and differentiates plant sections. Cytoplasm and nuclei are stained dark blue, cellulose walls a lighter blue and lignin yellow, orange, red or pink, xylem brown and starch black, depending on the nature of the plant material. A variety of alternative stains are available such as phloroglucinol which stains lignin red, Toluidine Blue O which stains lignin and tannins green to blue-green as well as pectins pink to purple, Methylene Blue which stains cellulose blue and the Safranin O-Fast Green technique that stains chromosomes, nuclei, lignin, and cell walls red while the Fast Green stains the cytoplasm and cellulosic cells green. Students should practice with iodine stains as these are less hazardous and most secondary students should be familiar with using iodine to stain for starch. For science fairs and similar events prepare slides in advance and provide microscopes or monitors connected to microscopes to enable the slides to be observed.

**Age Range:** This experiment is suitable for post-16 students. Restricted use of stains would be suitable for all secondary students.

**Duration:** 60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of cells, microscopy, plant anatomy and transport in plants including the function of xylem and phloem. An understanding of the role of photosynthesis in making the structures and substances in plants will help as will previous experience using stains to identify substances or visualise cells.

## What you will need

- Variety of plant material, preferably biofuel feedstocks such as miscanthus, willow and straw
- Variety of stains
- Microscopes
- Glass microscope slides
- Coverslips
- Tweezers
- Scalpel
- Chopping board or white tile
- Petri dish
- Dropping bottles
- Paintbrush
- Absorbent paper
- Mounted needle
- Beaker or sharpsafe
- Gloves

## Health and Safety

Students should wear gloves when using stains and take extra care with scalpels. If students have difficulty cutting sections it may be easier if the stems are held in place by inserting into a carrot, potato or polystyrene. If a microtome is available stems could be embedded in wax. Keep the stems moist to soften tissues and ensure the cells do not dry out.

CLEAPSS® laboratory handbook – section 15.5 Plants and seeds (choosing suitable plant material, growing and cultivating plants, sources and suppliers of plants).

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CLEAPSS® Recipe book RB50 (Iodine solution) and RB93 (Stains for plant material). Where possible source prepared solutions. If making up the stains take the following precautions:

Iodine is HARMFUL, see Hazcards 54a and b. Use disposable gloves and eye protection when preparing.

For aniline (phenyl-ammonium) sulfate(VI): see Hazcard 4. Wear eye protection and disposable nitrile gloves when making up the solution. Label the stain HIGHLY FLAMMABLE.

For lactophenol, see Hazcard 38C. Wear goggles and chemical-resistant gloves. Label the stain TOXIC.

Phloroglucinol is an IRRITANT. Ethanol is HIGHLY FLAMMABLE see Hazcards 12, 40 and 47. Label solution HIGHLY FLAMMABLE. Wear eye protection.

Zinc chloride is CORROSIVE see Hazcard 108. Wear eye protection, and chemical-resistant gloves, and carry out the procedure in a fume cupboard. Label the solution CORROSIVE.

The FABIL solution is TOXIC. Wear goggles and chemical-resistant gloves. Label the stain TOXIC.

Toluidine Blue O. Use disposable gloves and eye protection, use a respirator if preparing the solution.

Methylene Blue. Use disposable gloves and eye protection, use a dust mask if preparing the solution.

Safranin O. Use disposable gloves and eye protection.

Fast Green. Use disposable gloves and eye protection.

## Method

1. Prepare the stains to be used in advance (see below).
2. Carefully using a pair of tweezers and a scalpel, slice a thin transverse section off the stem of the plant. Demonstrate this step to students and emphasise the need to take care and cut sections as thinly as possible. Many sections may be too thick but with practice some sections will be thin enough to use.
3. Place the stem sections in a petri dish of water to keep moist.
4. Repeat steps 2 and 3 to produce a number of sections from each plant stem being investigated.
5. Remove stem section from the petri dish and place on a slide.
6. Remove excess water by carefully touching the edge of the section with absorbent paper.
7. Add 1 to 2 drops of stain to the section. Phloroglucinol should be left for 4 minutes before removing excess stain and adding a drop of HCl. Detailed instructions for other stains are provided below.
8. Remove excess stain with absorbent paper as before.
9. Slowly lower the coverslip onto the section using the mounted needle making sure that air bubbles are removed from the slide.
10. Label the slide and examine under the microscope.

Place any broken or used coverslips and slides in the beaker or sharpsafe.

# Advanced Biofuels

## Stain preparation and use

The following instructions for stain preparation are taken from the CLEAPSS® Recipe book. The stains should be prepared by a technician in advance of the practical and stored appropriately.

**Iodine stain for starch (also known as Lugol's solution)** Use 0.01 M iodine ( $I_2$ ) solution. 8 g of potassium iodide + 2.54 g of  $I_2$  in 100 ml of water, add the  $I_2$  to moistened KI, make up to 100 ml then dilute tenfold. See CLEAPSS® *Recipe Sheet* 50. Starch will turn blue to black.

**Aniline (phenyl-ammonium) sulfate stain for lignin** Mix 89 ml of ethanol, 10 ml of 0.05 M sulfuric acid and 1 g of phenylammonium sulfate [aniline sulfate(VI)]. Stains lignin yellow

**Phloroglucinol for pentoses and lignin** Dissolve 5 g of phloroglucinol (benzene-1,3,5-triol) in 75 ml of ethanol and 25 ml of water. Ligneous tissue should be well-flooded and staining continued for about 4 minutes after which 1 drop of concentrated hydrochloric acid should be added. Phloroglucinol stains lignin red.

**Schulze's solution (Chlor-zinc-iodide) for cellulose** Dissolve by warming 20 g of anhydrous zinc chloride in 8.5 ml of water and allow the mixture to cool. In a separate container, dissolve 1 g of potassium iodide and 0.5 g of iodine in 20 ml of water. Add this solution drop wise to the zinc chloride solution until iodine precipitate persists on agitation. Stains cellulose blue-violet, lignin yellow, cutin and suberin yellow or brown and starch blue.

**FABIL for plant tissues** Prepare 3 solutions: 0.5 % solution of aniline blue in lactophenol, 0.5% solution of basic fuchsin in lactophenol and a solution containing 0.3 g of iodine and 0.6 g of potassium iodide in 100 ml of lactophenol. When required, mix in the proportions of 4:1:5 and allow to stand overnight. Filter before use. (Cell contents stain blue, cellulose walls stain light blue and lignin stains yellow.)

**Toluidine Blue O for lignin, tannins and pectins** Use 0.05% aqueous solution. Leave the stain on for 2-4 minutes.

**Methylene Blue for cellulose.** Use 0.1% aqueous methylene blue. Leave the stain on for 15-20 minutes.

**Safranin O-Fast Green technique.** Use a 1% solution. Safranin stains chromosomes, nuclei, lignin, and cell walls red while the Fast Green stains the cytoplasm and cellulosic cells green.

## Extension activities

Recording the results of the staining procedures is an essential part of histological analysis and the method may well depend on the microscopes and facilities available. The simplest approach is to provide pencils and paper for the students to sketch what they can observe on their prepared slides. Alternatively if images can be taken with a camera or saved on to a computer connected to the microscope they can be analysed later. If there are only a few microscopes available it can be far quicker and easier to process a whole class of results by taking images of the slides for students and then allowing the students to analyse them in their own time.

Quantitative calculations can be carried out such as calculating the lignification index: see Science and Plants for Schools (SAPS) [Student Sheet 16 - What is Wood?](#) for further details.

# Advanced Biofuels

## Suppliers

Phloroglucinol, basic fuchsin, lactophenol and aniline (also known as cotton blue) solutions are available from [Sigma-Aldrich](#). Iodine solution is available from [Philip Harris Education](#), Hyde Buildings, Hyde, Cheshire, SK14 4SH, tel: 0845120 4520 fax: 0800 138 8881.

[Timstar Laboratory Suppliers Ltd](#), Timstar House, Marshfield Bank, Crewe, Cheshire, CW2 8UY tel: 01270 250459 fax:01270 250601

A general botanical staining kit insert is available from Philip Harris [www.philipharris.co.uk/secondary/biology/microbiology/botanical-staining-kit/?itemcode=B8A14126](http://www.philipharris.co.uk/secondary/biology/microbiology/botanical-staining-kit/?itemcode=B8A14126)

## Further reading and links

Prepare and examine microscopically the transverse section of a dicotyledonous stem, a prescribed biology activity from the Republic of Ireland National Council for Curriculum and Assessment Senior Cycle Leaving Certificate. [www.curriculumonline.ie/en/Post-Primary\\_Curriculum/Senior\\_Cycle\\_Curriculum/Leaving\\_Certificate\\_Established/Biology/Biology\\_Support\\_Materials/Prescribed\\_Activities/Detailed\\_Templates/Prepare\\_and\\_examine\\_microscopically\\_the\\_transverse\\_section\\_of\\_a\\_dicotyledonous\\_stem.html](http://www.curriculumonline.ie/en/Post-Primary_Curriculum/Senior_Cycle_Curriculum/Leaving_Certificate_Established/Biology/Biology_Support_Materials/Prescribed_Activities/Detailed_Templates/Prepare_and_examine_microscopically_the_transverse_section_of_a_dicotyledonous_stem.html)

Testing leaves for starch, Practical Biology. [www.practicalbiology.org/areas/introductory/energy/photosynthesis/testing-leaves-for-starch-the-technique.73.EXP.html](http://www.practicalbiology.org/areas/introductory/energy/photosynthesis/testing-leaves-for-starch-the-technique.73.EXP.html)

What sorts of carbohydrates do plants make? Science and Plants for Schools (SAPS) [Photosynthesis - A Survival Guide](http://www.saps.org.uk/secondary/teaching-resources/134-photosynthesis-a-survival-guide) <http://www.saps.org.uk/secondary/teaching-resources/134-photosynthesis-a-survival-guide>

What is wood? Science and Plants for Schools (SAPS) [Student Sheet 16 - What is Wood?](#)

Histochemical tests for fresh tissue slices, University of Illinois [http://boneslab.bio.ntnu.no/old\\_root/histochemicaltests.htm](http://boneslab.bio.ntnu.no/old_root/histochemicaltests.htm)

Photosynthesis and starch production in Pelargonium leaf discs, Science and Plants for Schools (SAPS). [www.saps.org.uk/secondary/teaching-resources/145-photosynthesis-and-starch-production-in-pelargonium-leaf-discs-](http://www.saps.org.uk/secondary/teaching-resources/145-photosynthesis-and-starch-production-in-pelargonium-leaf-discs-)

Growing the bioenergy field. BBSRC business spring, 2011 [www.bbsrc.ac.uk/news/industrial-biotechnology/2011/110314-f-growing-the-bioenergy-field.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2011/110314-f-growing-the-bioenergy-field.aspx)

Rothamsted Research Willow Power [www.rothamsted.bbsrc.ac.uk/Research/Centres/Content.php?Section=ForThePublic&Page=WillowPower](http://www.rothamsted.bbsrc.ac.uk/Research/Centres/Content.php?Section=ForThePublic&Page=WillowPower)

NNFCC Miscanthus crop fact sheet [www.nnfcc.co.uk/publications/nnfcc-crop-factsheet-miscanthus](http://www.nnfcc.co.uk/publications/nnfcc-crop-factsheet-miscanthus)

Sweet success for sustainable biofuel research [www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100125-pr-success-sustainable-biofuel-research.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100125-pr-success-sustainable-biofuel-research.aspx)

Biofuel from inedible plant material easier to produce following enzyme discovery [www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100913-pr-biofuel-from-inedible-plant-material.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100913-pr-biofuel-from-inedible-plant-material.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)



# Advanced Biofuels

## Research groups

Prof. Paul Dupree, BSBE Cell Wall Sugars Programme, Department of Biochemistry, University of Cambridge [www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html](http://www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html) [www.bioenergy.cam.ac.uk](http://www.bioenergy.cam.ac.uk)

Dr Angela Karp, BSBE Perennial Bioenergy Crops Programme, Rothamsted Research [www.bsbec.bbsrc.ac.uk/programmes/perennial-bioenergy-crops.html](http://www.bsbec.bbsrc.ac.uk/programmes/perennial-bioenergy-crops.html)

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/)

# Advanced Biofuels

## Activity 2B - Hydrolysis of biofuel feedstocks

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

- Describe the enzymatic breakdown of cellulose.
- Analyse the effectiveness of enzymatic breakdown of plant material.
- Suggest effective enzymes and conditions for the production of fermentable sugars.

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, bioethanol, sugar, lignocellulose, microbes, yeast, enzyme, fermentation, gribbles, varieties, hydrolysis.



© 2011 American Chemical Society  
The structure of the newly identified lignin-degrading enzyme from *Rhodococcus*

## Background

Sustainable liquid biofuels can be produced from lignocellulosic biomass such as wood and straw. These materials contain polysaccharides that can be converted through enzymatic hydrolysis into simple sugars which can be fermented to produce liquid biofuels.

Bioethanol produced on a large scale in Brazil and the USA is made from sugar cane or maize respectively. Sugars from sugar cane can be fermented by *Saccharomyces cerevisiae* without prior treatment as they are already disaccharides, but starch polymers from maize or wheat need conversion to di- or monosaccharide sugars, by a hydrolysis reaction known as saccharification, prior to fermentation. The enzyme mixtures used in saccharification of starch are amylases, enzymes also found in human saliva and secreted by the pancreas.

In plants the majority of sugars are locked into the cell walls in ways we do not fully understand, preventing effective digestion by enzymes. Currently we lack effective enzymes to digest these woody materials as amylases hydrolyse a different type of linkage between individual sugars to the linkages found in cell wall polysaccharides. One of the aims of current research is to discover enzymes that can release sugars from currently indigestible cell wall components. Lignocellulose and hemicelluloses are broken down by the actions of a range of enzymes including cellulases and hemicellulases.

In this activity students can compare the effectiveness of enzymes at hydrolysing a variety of feedstocks. Straw, maize and rapeseeds are recommended substrates. The popcorn mimics the process of steam explosion that can be used to open up plant cell walls to allow enzymes access to polysaccharides. In the table below the two enzymes that are compared for their ability to produce fermentable sugars from the feedstocks are cellulase and pectinase. Cellulase breaks down accessible cellulose molecules whereas pectinases break down the pectin in cell walls that holds the cellulose molecules in place. Pectin is predominantly found in non-woody parts of plants (as it is associated with the primary cell wall found around all plant cells) and holds cells together.

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**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** 50-60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of enzymes, carbohydrates and sugars.

## What you will need

- Cellulase, pectinase (Pectinex®)
- Variety of biofuel feedstocks: straw, popcorn, rapeseeds
- Boiling tubes or conical flasks
- Beaker
- Stirrers
- Water bath
- Timer
- Mortar and pestle
- Buffers across a pH range of 3-8
- Glucose test strips

### Optional

- Blood glucose monitor
- Amylase

## Health and Safety

Care should be taken with enzymes particularly due to their allergenic nature and ability to act as sensitisers. CLEAPSS® Recipe book RB37 (Enzymes), Hazcard 33 (Enzymes), RB3 (Alginate beads), RB19 (Calcium chloride and nitrate(V) solutions), Guide 3.015 (Enzymes), Laboratory handbook page 1441-1443. Solutions equal to or stronger than 1% (w/v) should be labelled as irritant - CLEAPSS® Recipe book.

## Method

1. The biofuel feedstocks should be ground down in a mortar and pestle to enable the enzymes to access the cellulose.
2. Students should weigh out 1 g of each feedstock and add it to a boiling tube.
3. Add 10 ml of buffer.
4. Add 0.5 ml of enzyme.
5. Remove a 1 ml aliquot at 5 minute intervals and test the glucose concentration using a glucose test strip, such as 'Diabur Test® 5000' (semi-quantitative) or 'Diastix' (qualitative) or blood glucose monitor.
6. Graph the results.

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							Control	Control	Control
<b>Feedstock</b>	straw	popcorn	rapeseeds	straw	popcorn	rapeseeds	straw	popcorn	rapeseeds
<b>Enzyme</b>	Cellulase	Cellulase	Cellulase	Pectinase	Pectinase	Pectinase	×	×	×
<b>pH</b>	5	5	5	5	5	5	5	5	5
<b>Temperature</b>	35°C	35°C	35°C	35°C	35°C	35°C	35°C	35°C	35°C

									Control
<b>Feedstock</b>	popcorn	popcorn	popcorn	popcorn	popcorn	popcorn	popcorn	popcorn	popcorn
<b>Enzyme</b>	Cellulase	Cellulase	Cellulase	Cellulase	Pectinase	Pectinase	Pectinase	Pectinase	×
<b>pH</b>	3	4	5	6	3	4	5	6	5
<b>Temperature</b>	35°C	35°C	35°C	35°C	35°C	35°C	35°C	35°C	35°C

## Extension activities

Repeating the experiment with amylases will allow comparison of the effectiveness of saccharification versus breakdown of the cellulose.

					Control
<b>Feedstock</b>	starch	starch	starch	starch	popcorn
<b>Enzyme</b>	Amylase	Amylase	Amylase	Amylase	×
<b>pH</b>	3	4	5	6	5
<b>Temperature</b>	35°C	35°C	35°C	35°C	35°C

Students can immobilise the enzyme in alginate beads and investigate the effect on the reaction rate. Recover the enzyme and repeat to investigate the viability of use in continuous flow processes. Placing the enzyme-alginate beads in a column or syringe will enable student to replicate a continuous flow process and test the effect of repeated passage through the column.

## Preparing immobilised enzymes

1. Prepare a 2% sodium alginate solution with warm distilled or deionised water, mix thoroughly and leave overnight in a fridge. The initial mixture can be very lumpy but will become smooth overnight.
2. Add the stock enzyme solution to the sodium alginate solution to obtain the correct final concentration desired and mix thoroughly. If required add a small amount of distilled or deionised water but take care to ensure the final solution of immobilised enzyme-alginate is not too runny.
3. Prepare a 1.5% calcium chloride solution with calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ). The calcium ions cause the sodium alginate to set and hence using distilled or deionised water for the alginate and enzyme solutions is important as is avoiding contact of the syringe with the calcium chloride solution.
4. Draw the enzyme-alginate solution up into a syringe.

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5. Add the enzyme-alginate solution into a 1.5% CaCl<sub>2</sub> solution drop by drop. Carefully observe the shape of the drops. If the drops take on a 'comet' shaped appearance add a small amount of distilled or deionised water to the enzyme-alginate solution, mix and retry.
6. Allow the enzyme-alginate beads to set for at least 10 minutes.
7. Carefully strain the beads and rinse with distilled or deionised water.

The enzyme kinetics of cellobiase can be investigated with older students. This activity would be suitable for A-level students. Bio-Rad produce a kit that enables students to investigate cellobiase rates of reaction under different conditions by observing a colour change using a spectrophotometer.

## Suppliers

A variety of enzymes including cellulase (*Celluclast*<sup>®</sup>) 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.

Diastix and sodium alginate, can be obtained from [Philip Harris Education](http://Philip Harris Education), Hyde Buildings, Hyde, Cheshire, SK14 4SH tel: 0845120 4520 fax: 0800 138 8881 and [Timstar Laboratory Suppliers Ltd](http://Timstar Laboratory Suppliers Ltd), Timstar House, Marshfield Bank, Crewe, Cheshire, CW2 8UY tel: 01270 250459 fax:01270 250601 Daibur-Test<sup>®</sup> 5000 strips as well as a wide range of blood glucose monitors and detection strips can be obtained from local chemists.

Biofuel enzyme kit for investigating the activity of cellobiase can be obtained from Bio-Rad Laboratories [www.bio-rad.com](http://www.bio-rad.com).

## Further reading and links

King, A.J. *et al*, 2010 Molecular insight into lignocellulose digestion by a marine isopod in the absence of gut microbes. *PNAS*, **107**(12), 5345-5350.

Seafarers' scourge provides hope for biofuel future [www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100308-pr-seafarers-biofuel-future.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100308-pr-seafarers-biofuel-future.aspx)

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)

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Glucose detector. National Centre for Biotechnology Education (NCBE) Practical Biotechnology. [www.ncbe.reading.ac.uk/ncbe/protocols/PRACBIOTECH/PDF/glucudet.pdf](http://www.ncbe.reading.ac.uk/ncbe/protocols/PRACBIOTECH/PDF/glucudet.pdf)

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

# Advanced Biofuels

## Research groups

Professor Simon McQueen-Mason, BSBECE Marine Wood Borer Enzyme, Discovery Programme, The University of York, Heslington, York, YO10 5DD [www.bsbec.bbsrc.ac.uk/programmes/marine-wood-borer-enzyme-discovery.html](http://www.bsbec.bbsrc.ac.uk/programmes/marine-wood-borer-enzyme-discovery.html)

Professor Paul Dupree, BSBECE Cell Wall Sugars Programme, Department of Biochemistry, University of Cambridge [www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html](http://www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html) [www.bioenergy.cam.ac.uk](http://www.bioenergy.cam.ac.uk)

Professor David Archer, BSBECE 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)

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/)

# Advanced Biofuels

## Activity 2C – Fermentation of lignocelluloses

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

- Describe the process of ethanol production from lignocelluloses.
- Carry out pretreatment and yeast fermentation of a range of substrates.
- Assess the effectiveness of pretreatment and enzymatic hydrolysis of lignocelluloses substrates.

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, residues, bioethanol, lignocellulose, microbes, yeast, enzyme, fermentation, gribbles, varieties, pentose, hexose, pretreatment.

## Background

Current bioethanol production uses food crops such as sugar cane and maize. Large amounts of sugar molecules are present in the **lignocellulose** of plant material and current research aims to 'unlock' the fermentable sugars in agricultural or forestry wastes and residues from cereal production such as straw, bran, brewers grain and wood as well as from *Brassic*as and food-chain waste.

**Fermentation** by yeast currently uses sugar beet and sugar cane as the main sources of sugar for bioethanol. Starches from maize or grain feedstocks are hydrolysed with amylase enzymes (saccharification) to produce sugar that can be fermented. Yeast have been used for centuries in brewing alcoholic drinks. The yeast *Saccharomyces cerevisiae* produces ethanol by fermentation of sucrose or glucose (hexose, C6 sugars) but is unable to ferment pentose (C5) sugars. *Saccharomyces diastaticus* is able to utilise starch for fermentation. The National Collection of Yeast Cultures recommends certain strains for the production of bioethanol, such as *Pachysolen tannophilus*, *Candida succiphilia*, *Candida tenuis* and *Pichia stipitis*, due to their ability to degrade cellulose or ferment xylose. To make use of a greater proportion of lignocelluloses it may be possible to genetically engineer yeast to ferment pentose (five carbon) and hexose (six carbon) sugars with equal efficiency. Most yeast varieties use hexose sugars as a substrate for fermentation. It may also be possible that scientists out in the field can discover new varieties of yeast that are able to preferentially ferment pentose sugars. To enable yeast to carry out fermentation the sugars trapped in plant cell wall lignocellulose, must be 'released' by **pretreatment** with steam or chemicals followed by hydrolytic breakdown of the released polysaccharides with enzyme cocktails. Currently chemical treatment involves either strong acid or mild alkali but due to the requirement for specialised equipment to carry out the procedure as well as the treatment of waste chemicals before disposal, research is focusing on steam treatments. Pretreatments change the structure of cell walls and polymers by disrupting intermolecular forces holding them together, allowing greater access by enzymes and water. Enzymatic digestion of exposed polysaccharides can produce mono-, di- and tetrasaccharides. Enzymes are expensive and need to be recovered from industrial processes. Immobilising enzymes enables easier recovery and development of more efficient continuous processing. However, it restricts the ability of the enzymes to carry out cleavage of the insoluble polysaccharides.



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Steam Explosion Unit

# Advanced Biofuels

In this activity students can compare the effectiveness of a variety of pretreatments on fermentation rates of yeast. The recovery and reuse of resources is important in making biofuel production economic and environmentally friendly and students could investigate the rate of fermentation with and without immobilising enzymes or yeast in sodium alginate balls.

1. Fermentation of polysaccharides with a range of pretreatments (steam explosion or alkali treatment).
2. Fermentation of polysaccharides with or without enzyme digestion (Popcorn, starch and oatbran, with or without amylase or cellulase).
3. Fermentation of substrates with immobilised yeast or co-immobilised yeast and cellulase.
4. Fermentation rates for monosaccharide versus polysaccharide substrates using *Saccharomyces cerevisiae*, *Saccharomyces diastaticus* or more specialised yeast strains.

It is suggested students are split into groups to assess the effects of different variables and report their results back to the rest of the class.

This activity is based on ones described in the NCBE booklet Practical Fermentation – A guide for Schools and Colleges: Yeast cells and enzyme together they can do it, bioreactor practical (replacing lactose/ lactase with cellulose/cellulase) [www.ncbe.reading.ac.uk/ncbe/protocols/pdf/fermsg.pdf](http://www.ncbe.reading.ac.uk/ncbe/protocols/pdf/fermsg.pdf), a resource developed by Dr Jen Bromley in conjunction with Science and Plants for Schools (SAPS) [www.sapa.org.uk](http://www.sapa.org.uk) and the work of the Society of General Microbiology [www.microbiologyonline.org.uk](http://www.microbiologyonline.org.uk).

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** Set up 60 minutes, analysis of results and reporting back 60 minutes. Allow up to a week between sessions to enable sufficient fermentation for measurable levels of carbon dioxide to be produced. The experiment can be set up and run in one day for a science fair or exhibition with adjustment of the fermentation conditions.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, fermentation, enzymes, alcohols, fuels and the properties of gases.

## What you will need

- Conical flask (100 ml) or boiling tubes
  - 8% glucose solution
  - 0.1M phosphate buffer pH 7
  - Brewer's or baker's yeast (*Saccharomyces cerevisiae*)
  - Deionised or distilled water
  - Stirrers
  - Balloons or bubble counters
  - Measuring cylinder (50 ml)
  - Pressure cooker or autoclave
  - Popcorn maker
  - Alkali (1M NaOH)
  - Cellulase (*Celluclast*)
  - Amylase
  - Oatbran or popcorn
  - Cornflour or potato starch
  - Thermometer
  - Timers
  - Beaker of disinfectant
  - Eye protection
- Optional*
- Water bath
  - Sodium alginate
  - Syringe
  - 1.5% Calcium chloride (CaCl) solution
  - Buffer solutions at varying pH
  - Strainer
  - Fermentation locks
  - Universal indicator solution
  - Cotton wool
  - Magnetic stirrer and fleas
  - Alternative yeast strains



# Advanced Biofuels

## Health and Safety

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 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

CLEAPSS® Recipe book RB3 (Alginate beads), RB19 (Calcium chloride and nitrate(V) solutions), RB99 (Testing for gases).

CLEAPSS® Hazcards 19A (Calcium salts), 20 (Carbon dioxide), 40A (Ethanol), 40C (Carbohydrates).

CLEAPSS® Guidance PS 04 (COSHH: risk assessments in situations where microorganisms might be involved), PS 15 (Ventilation and levels of Carbon dioxide and other gases in the laboratory & prep room), PS 89 (Measurement of anaerobic respiration in yeast)

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).

Care should be taken with enzymes particularly due to their allergenic nature and ability to act as sensitisers. CLEAPSS® Recipe book RB37 (Enzymes), Hazcard 33 (Enzymes), Guide 3.015 (Enzymes), Laboratory handbook page 1441Q-1443. Solutions equal to or stronger than 1% (w/v) should be labelled as irritant.

# Advanced Biofuels

## Method

You may want to set up one simple experiment to show the rate of fermentation with and without pretreatment, alternatively you may want to divide a class up into groups to investigate different variables and report back after they have conducted their experiments. The tables below show a number of possible experiments that can be conducted. *Celluclast* catalyses the breakdown of the glucose polymers that comprise cellulose to glucose, cellobiose (i.e. pairs of glucose units) and longer chains of glucose units. The optimum conditions for activity of this enzyme preparation are in the range pH 4.5-6.0, and about 50-60 °C.

1. The biofuel feedstocks should be pretreated with either steam (using a pressure cooker, autoclave or popcorn maker) or alkali (1M NaOH for 1 hour at room temperature) prior to the activity. Ensure the alkali-treated solution is neutralised before fermentation with acetic acid. This may require repeated additions of acetic acid with agitation and time for the feedstock to equilibrate.
2. Prepare the fermentation stock solutions in phosphate buffer.
3. Students should label the conical flasks then add the yeast, feedstocks and buffers.
4. If immobilising yeast in sodium alginate ideally the solution of yeast and sodium alginate is prepared the day before.
5. Resuspend the yeast in a small amount of distilled or deionised water in order to make a final 3% solution in the fermentation reaction. To ensure active cultures incubate in nutrient broth for 48 hours at room temperature before inoculating. To ensure pure cultures streak out the yeast on malt agar plates and inoculate from single colonies.
6. Add the following to the conical flasks or bioreactor
  - 100 ml of phosphate buffer
  - Feedstock equivalent to 10 g of original dry weight
  - 5 ml of enzyme.
7. Inoculate with yeast
8. Stopper the flasks with bungs holding fermentation locks and attached bubble counters or add the balloons.

<b>Yeast</b>	✓	✓	✓	✓	✓	✓
<b>Feedstock</b>	Oatbran	Oatbran	Oatbran	Popcorn	Popcorn	Popcorn
<b>Pretreatment</b>	✗	Alkali	Steam	✗	Alkali	Steam
<b>Enzyme</b>	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase

<b>Yeast</b>	✓	✓	✓	✓	✓	✓	✓	✓	✓
<b>Feedstock</b>	Oatbran	Oatbran	Oatbran	Popcorn	Popcorn	Popcorn	Starch	Starch	Glucose
<b>Pretreatment</b>	✗	Alkali	Alkali	✗	Steam	Steam	✗	✗	Alkali
<b>Enzyme</b>	Cellulase	Cellulase	✗	Cellulase	Cellulase	✗	Amylase	✗	Cellulase

# Advanced Biofuels

In order to calculate the rate of fermentation the amount of carbon dioxide produced can be measured over time. This can be done in a number of ways including the use of bubble counters available from National Centre for Biotechnology Education (NCBE), collection of carbon dioxide (CO<sub>2</sub>) in inverted water-filled measuring cylinders, with balloons attached to the neck of the conical flask or boiling tubes or with gas syringes. Choose the method used according to the equipment and time available for the experiment. The volume of carbon dioxide produced can be calculated by multiplying the number of bubbles recorded by a bubble counter and the volume of one bubble. If using balloons the volume can be measured by carefully tying off the balloon used to collect the gas produced, immersing it in a large measuring cylinder and measuring the displaced volume. Note: the gas in the balloon is not likely to be predominantly carbon dioxide and is instead displaced air, with the denser carbon dioxide produced remaining at the bottom of the flask. This experiment is suitable for public demonstrations and science fairs providing appropriate risk assessment is carried out.

## Extension activities

Enzymes and microbes can prove to be expensive components in an industrial bioreactor operation. Being able to recover and reuse them is enhanced if they are immobilised. Students can immobilise yeast and enzymes in sodium alginate and assess the impact on the ability of the reactions to carry out fermentation.

<b>Yeast</b>	✓	✓	✓	✓	✓	✓	✓	✓	✓
<b>Feedstock</b>	Oatbran	Oatbran	Oatbran	Oatbran	Popcorn	Popcorn	Popcorn	Popcorn	Glucose
<b>Pretreatment</b>	Alkali	Alkali	Alkali	Alkali	Steam	Steam	Steam	Steam	Alkali
<b>Enzyme</b>	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase	Cellulase
<b>Immobilisation</b>	Yeast	Yeast and enzyme	Enzyme	✗	Yeast	Yeast and enzyme	Enzyme	✗	✗

## Preparing immobilised yeast

1. Prepare a 2% sodium alginate solution with warm distilled or deionised water, mix thoroughly and leave overnight in a fridge. The initial mixture can be very lumpy but will become smooth overnight.
2. Resuspend the yeast in a small amount of distilled or deionised water so that the final solution of immobilised yeast-alginate is not too runny.
3. Add the resuspended yeast solution to the sodium alginate solution and mix thoroughly.
4. Prepare a 1.5% calcium chloride solution with calcium chloride dihydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O). The calcium ions cause the sodium alginate to set and hence using distilled or deionised water for the alginate and yeast solutions is important as is avoiding contact of the syringe with the calcium chloride solution.
5. Draw the yeast-alginate solution up into a syringe.
6. Add the yeast-alginate solution into a 1.5% CaCl<sub>2</sub> solution drop by drop. Carefully observe the shape of the drops. If the drops take on a 'comet' shaped appearance add a small amount of distilled or deionised water to the yeast-alginate solution, mix and retry.
7. Allow the yeast-alginate beads to set for at least 10 minutes.
8. Carefully strain the beads and rinse with distilled or deionised water.

Students could investigate the ability of yeast to ferment different sugar substrates, see [activity 1G - Yeast fermentation](#).

The fermentation reaction may also be set up as a bioreactor with recording of pH and temperature with data logging software.

# Advanced Biofuels

## Suppliers

Bioreactors, bubble counters and a variety of enzymes including cellulose (*Celluclast*<sup>®</sup>) 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

Popcorn and oatbran can be obtained from local supermarkets.

Brewer's or baker's yeast can be obtained from local supermarkets, brewery stores, or bakeries.

Dried yeast can also 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.

Sodium alginate and universal indicator can be obtained from [Philip Harris Education](http://Philip Harris Education), Hyde Buildings, Hyde, Cheshire, SK14 4SH, tel: 0845120 4520 fax: 0800 138 8881.

## Further reading and links

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Yeast fermentation and distillation instructions from Practical Chemistry [www.practicalchemistry.org/experiments/fermentation-of-glucose-using-yeast,109.EX.html](http://www.practicalchemistry.org/experiments/fermentation-of-glucose-using-yeast,109.EX.html)

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](http://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 [www.microbiologyonline.org.uk/teachers/resources](http://www.microbiologyonline.org.uk/teachers/resources)

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)

# Advanced Biofuels

## Research groups

Professor Katherine Smart, BSBEC 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/)

Professor Simon McQueen-Mason, BSBEC Marine Wood Borer Enzyme, Discovery Programme, The University of York, Heslington, York, YO10 5DD [www.bsbec.bbsrc.ac.uk/programmes/marine-wood-borer-enzyme-discovery.html](http://www.bsbec.bbsrc.ac.uk/programmes/marine-wood-borer-enzyme-discovery.html)

Prof. Paul Dupree, BSBEC Cell Wall Sugars Programme, Department of Biochemistry, University of Cambridge [www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html](http://www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html) [www.bioenergy.cam.ac.uk](http://www.bioenergy.cam.ac.uk)

National Collection of Yeast Cultures, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA [www.ncyc.co.uk/](http://www.ncyc.co.uk/)

Professor Keith Waldron, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA [www.hooch.org.uk](http://www.hooch.org.uk)

# Advanced Biofuels

## Bacterial Biofuels

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, bioethanol, biobutanol, lignocellulose, microbes, bacteria, enzyme, fermentation, varieties, soil, synthetic biology, nutrient broth.

## Background

Genetically modified strains of *Escherichia coli* are able to break down cellulose and produce bioethanol, and some strains of bacteria, such as *Clostridium acetobutylicum*, are able to produce biobutanol. Many naturally occurring strains of bacteria and fungi, such as those found in soil, produce cellulases that can convert lignocellulose to fermentable sugars. Research to identify cellulases in bacteria and fungi as well as optimising the lignocellulosic conversion process will hopefully lead to more efficient production of biofuels.

Biobutanol is widely recognised as a superior biofuel to ethanol, in terms of energy content, ease of distribution, versatility and applications. However, the strains of bacteria currently used to produce biobutanol generate unwanted by-products and are inefficient. *Clostridium acetobutylicum* is able to convert starch to butanol but yields are three times less efficient than for ethanol production. Currently Clostridia form spores when the butanol levels get too high and fermentations need to be carried out in the absence of oxygen, making the development of an easy to manage industrial process harder.

Bioethanol is produced by fermentation of sugars by yeast or *Escherichia coli*. The bacterium *Zymomonas mobilis* is a promising alternative to yeast due to its greater sugar uptake, yields and resistance to ethanol concentrations. Neither yeast nor bacteria are capable of efficiently fermenting pentose sugars and research to modify their metabolism to provide this ability may enable the fermentation of lignocellulosic derived sugars in the future.

Moreover, they are unable to utilise lignocellulose directly as a feedstock. Research aims to generate bacterial strains, using synthetic biology, which can efficiently utilise lignocellulose to produce butanol and ethanol. Once developed, these strains must be tested for their ability to work on an industrial scale in an environmentally friendly and sustainable process using non-food biomass.

Researchers have identified the gene for breaking down lignin in a soil-living bacterium called *Rhodococcus jostii*. Although such enzymes have been found in fungi, bacteria can be modified more easily to produce large amounts of the required enzyme. In addition, bacteria are quick and easy to grow, making it easier to produce enzymes which can break down lignin on an industrial scale. There is hope that similar enzymes can be found in bacteria which live in very hot environments such as near volcanic vents. Enzymes in these bacteria have evolved to work best at high temperatures meaning they are ideally suited to be used in industrial processes.



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Bacterial cultures examined in a containment cabinet



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Experimental industrial scale bioreactors

# 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

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# 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/)

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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/buggroup/research/](http://www2.warwick.ac.uk/fac/sci/chemistry/research/bugg/buggroup/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)

# Advanced Biofuels

## Activity 2E - Cellulase enzymes

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

- Describe the breakdown of cellulose by cellulases and cellulase producing microbes.
- Carry out quantitative assays of enzyme activity.
- Assess the relative merits of immobilised cellulases and microbe produced cellulases.

**Keywords** Bioenergy, biofuel, sustainable, renewable, biomass, yield, waste, bioethanol, lignocellulose, cellulase, microbes, yeast, bacteria, gribbles, enzyme, varieties.

### Background

In this activity two methods can be used to assess the activity of cellulase enzyme, gel diffusion and viscosity reduction. It is recommended that four sources of cellulase are compared: fruit extracts, commercially sourced cellulase, yeast and bacteria (immobilised in sodium alginate or on sterile paper discs). If yeast and bacteria are to be tested it is essential that strains producing cellulase are used. If enzymes and microbes cannot be obtained the experiment can easily be carried out comparing fruit sources of cellulase. During the ripening of some fruits cellulases are produced that break down the cellulose in cell walls causing softening.

This activity is based on the Science and Plants for Schools activity - [Cellulase assays](#). A similar activity investigating starch and bacteria rather than cellulose called [Breakdown of starch by microbes](#) published in *Practical Microbiology for Secondary Schools*, Society for General Microbiology, may also be carried out.

### Gel diffusion method

The activity of cellulase enzyme can be determined by the degree of breakdown of carboxymethylcellulose (CMC). A zone of cellulose breakdown can be observed and measured when cellulase, cellulase-producing microorganisms or cellulase-containing material is added to a well cut in agar containing CMC. The more active or concentrated the enzyme the larger the zone of cellulose destruction.

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** 50 minutes to prepare samples and assays, 24 hour incubation and 20 minutes to observe and record results.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, enzymes, carbohydrates and the properties of solids and liquids.

### What you will need

- Petri dishes
- Agar
- Carboxymethylcellulose (CMC)
- Cork borer
- Congo red solution
- Sodium chloride solution
- Fruit extracts
- Mortar and pestle

### Optional

- Cellulase
- Bacteria expressing cellulase
- Yeast expressing cellulase
- Sodium alginate
- Paper discs

# Advanced Biofuels

## Health and Safety

Care should be taken with enzymes particularly due to their allergenic nature and ability to act as sensitisers. CLEAPSS® Recipe book RB37 (Enzymes), Hazcard 33 (Enzymes), Guide 3.015 (Enzymes), Laboratory handbook page 1441-1443. Solutions equal to or stronger than 1% (w/v) should be labelled as irritant.

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

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).

CLEAPSS® Hazcard 32 (Dyes, indicators and stains). Congo red solutions above 0.1% are TOXIC and require special risk assessments.

## Method

1. Prepare an agar gel containing 1.7% agar and 0.5% CMC (carboxymethylcellulose). Pour this gel into petri dishes and allow it to set.
2. Prepare the fruit extracts by mashing a variety of ripe fruits in a mortar and pestle.
3. If testing enzymes or microbes using paper discs, prepare the paper discs by cutting them out of filter paper using a narrow cork borer or using a hole punch. Sterilise the discs by autoclaving wrapped in foil.
4. If testing enzymes or microbes immobilised in sodium alginate, make up the alginate the night before to allow it to fully dissolve.
5. After the agar gel has set, use a narrow cork borer to punch small cylinders in the gel. Then, using a mounted needle, remove each of these cylinders to create a series of similar sized wells in the agar. Four or more wells can be put in a single dish, provided they are spaced apart.
6. Place similar volumes of extracts of fruits in each of the wells. In one well, place some distilled water, as a control.
7. Mix the enzyme or microbes with the alginate shortly before adding it to the wells in the agar using a syringe.
8. Incubate the dishes for at least 24 hours at 30 °C.
9. After the incubation period is finished, use tap water to rinse out the contents of the wells, and then flood the dishes with Congo red solution for 15 minutes. Then rinse the dishes with 1 M sodium chloride solution for at least 10-15 minutes.

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Wells containing cellulase should have a clear zone around them, and the diameter of the zone gives a measure of the cellulase activity in that well. If investigating cellulase production by microbes you may need to add nutrients to the sodium alginate mix and carry out the Congo red staining on behalf of the students, ensuring the plates are sealed with tape before they are examined. As a comparison the production of an extracellular enzyme, glucoamylase, can be investigated by using starch agar gels and *Saccharomyces diastaticus*. Glucoamylase is produced by *S. diastaticus* and carries out cleavage of single glucose molecules by breaking  $\alpha$ -1,4 glycosidic bonds in starch.

## Viscosity reduction method

Cellulase enzymes degrade cellulose fibres by cleaving glucose molecules predominantly from the ends of the polysaccharide chains. Wallpaper paste provides a source of cellulose fibres that can be degraded by cellulases resulting in a change in viscosity. This property can be exploited to set up a quantitative assay of cellulase activity. By loading samples of wallpaper paste mixed with a source of cellulase into a syringe and measuring the time taken for it to run out of the syringe a quantitative measure of cellulase activity can be made.

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** 60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, enzymes, carbohydrates and the properties of solids and liquids.

## What you will need

- Boiling tubes
- Wallpaper paste (without fungicide)
- Syringe
- Retort stand
- Beaker
- Stirrers
- Water bath
- Timer
- Fruit extract

## Method

1. Make up a 2% (w/v) wallpaper paste solution, sufficient to provide 25 ml for each sample to be tested.
2. Place 25 ml of the paste in a boiling tube and add 2 to 5 ml of fruit extract. Mix thoroughly.
3. Then pour the mixture into the barrel of a syringe, held in a retort stand, pointing downwards into a small beaker. Note the time taken for all the mixture to drain through the syringe nozzle into the beaker.
4. Incubate the fruit or enzyme-wallpaper paste mixture at different temperatures, such as in a water-bath at 30°C, allow to return to room temperature and repeat the investigation, checking the change in viscosity.
5. To speed up the investigation for students, provide samples that have been incubated prior to the lesson or assign students to groups to investigate different variables.

The more active the enzyme the greater the reduction in viscosity, and so the shorter the drainage times. If investigating cellulase production by microbes you may need to provide nutrients and carry out an overnight incubation in the wallpaper paste prior to assessing the viscosity.

# Advanced Biofuels

## Extension activities

Effects on the zone of destruction can be tested under different conditions such as temperature and pH. Natural sources of enzyme have some advantages and if using commercial sources to investigate the effects of pH or temperature, check that they are not heat stable or have unusual pH profiles.

Samples can be taken from the wallpaper paste prior to and following degradation to quantify the levels of sugar produced and evaluate the efficiency of the enzymes for producing fermentable sugars.

## 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

Whatman paper discs can be obtained from Sigma-Aldrich.

## Further reading and links

King, A.J. *et al*, 2010 Molecular insight into lignocellulose digestion by a marine isopod in the absence of gut microbes. *PNAS*, **107**(12), 5345-5350.

Cellulase assays, Science and Plants for Schools (SAPS) [Cellulase assays. www.saps.org.uk/students/projects/179-student-project-enzymes-and-their-activity-in-fruits-and-vegetables](http://www.saps.org.uk/students/projects/179-student-project-enzymes-and-their-activity-in-fruits-and-vegetables)

Breakdown of Starch by microbes, *Practical Microbiology for Secondary Schools*, Society of General Microbiology [Breakdown of starch by microbes](http://www.practicalbiology.org/areas/intermediate/bio-molecules/bacterial-enzymes/digestion-of-starch-microbes.104.EXP.html)

Seafarers' scourge provides hope for biofuel future [www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100308-pr-seafarers-biofuel-future.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2010/100308-pr-seafarers-biofuel-future.aspx)

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/)

*Breakdown of starch by microbes* published in *Practical Microbiology for Secondary Schools*, Society for General Microbiology [www.practicalbiology.org/areas/intermediate/bio-molecules/bacterial-enzymes/digestion-of-starch-microbes.104.EXP.html](http://www.practicalbiology.org/areas/intermediate/bio-molecules/bacterial-enzymes/digestion-of-starch-microbes.104.EXP.html)

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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)

# Advanced Biofuels

## Research groups

Professor Simon McQueen-Mason, BSBECC Marine Wood Borer Enzyme, Discovery Programme, The University of York, Heslington, York YO10 5DD [www.bsbec.bbsrc.ac.uk/programmes/marine-wood-borer-enzyme-discovery.html](http://www.bsbec.bbsrc.ac.uk/programmes/marine-wood-borer-enzyme-discovery.html)

Professor Paul Dupree, BSBECC Cell Wall Sugars Programme, Department of Biochemistry, University of Cambridge [www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html](http://www.bsbec.bbsrc.ac.uk/programmes/cell-wall-sugars.html) [www.bioenergy.cam.ac.uk](http://www.bioenergy.cam.ac.uk)

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/)

# Advanced Biofuels

## Algal Biofuels

**Keywords** Bioenergy, biofuel, biodiesel, biogas, sustainable, renewable, biomass, yield, waste, microbes, enzyme, photosynthesis, algae, varieties, unicellular, multicellular, eukaryotic, oil, carbon dioxide, biodiesel, hydrocarbons, carbohydrates, carbon partitioning, bioprospecting, somatic fusion, hybrid, heterokaryon, directed evolution, synthetic biology, genetic modification.

## Background

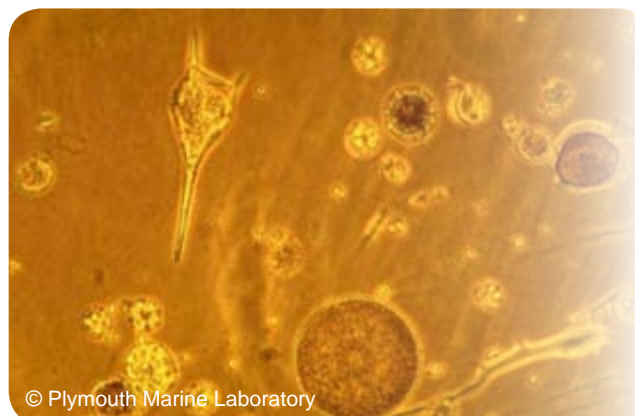
**Algae** are a diverse group of eukaryotic photosynthetic organisms that constitute over 40,000 species. They can be single-celled (unicellular) or multicellular such as seaweed. **Microalgae** have been described as nature's very own power cells and could provide alternatives to petroleum-based fuels without competing with crops.

Algae can harvest the power of the sun through **photosynthesis** and convert this into biomass including oil. Many species are fast growing and efficient at absorbing **carbon dioxide** (CO<sub>2</sub>) being more productive than land plants per unit area. This makes them an important part of the carbon cycle and they are able to produce complex molecules, such as hydrocarbons and carbohydrates, including cellulose, proteins, fats and oils, from the carbon dioxide they absorb. How algae and plants convert carbon dioxide into different molecules, known as carbon partitioning, is of great interest to researchers who aim to develop algae that can produce the substances we require. Research is being undertaken to uncover novel microalgal compounds that could provide alternatives to those from petrochemical sources. Algae produce more oils when they are starved of nitrogen but we don't yet understand why this should be. Uncovering the metabolic mechanisms behind such behaviour will be important if we want to harness the full potential of algae.

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.

As well as producing hydrocarbons that can be converted into fuels or plastics some microalgae have unique abilities such as being able to produce hydrogen gas which can be used in fuel cells to produce electricity. While still others, such as cyanobacteria, might one day be used in solar panels to generate electricity directly. 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.

Unlike land plants microalgae produce only one cell type and don't divert resources into multicellular structures such as flowers, roots or vasculature, and so they grow much more quickly than land plants. Microalgae can be grown in large bioreactors and continually harvested unlike crops or macroalgae. They could be grown using the waste carbon dioxide from industrial processes, power stations or waste treatment plants. The oil they produce can then be converted into liquid fuel such as biodiesel. The ability of microalgae to grow using waste CO<sub>2</sub> emissions and sunlight, and not compete for land with crops, makes them an attractive proposition both economically and sustainably. Unfortunately, the culture of algae on a large commercial scale (mainly for biomass for aquaculture or specialised products such as natural food colourants, omega-3 oils and antioxidants) has so far been restricted to sunny climates, and mainly to those species that are tolerant to extreme environments such as high light or saline conditions.



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Algae



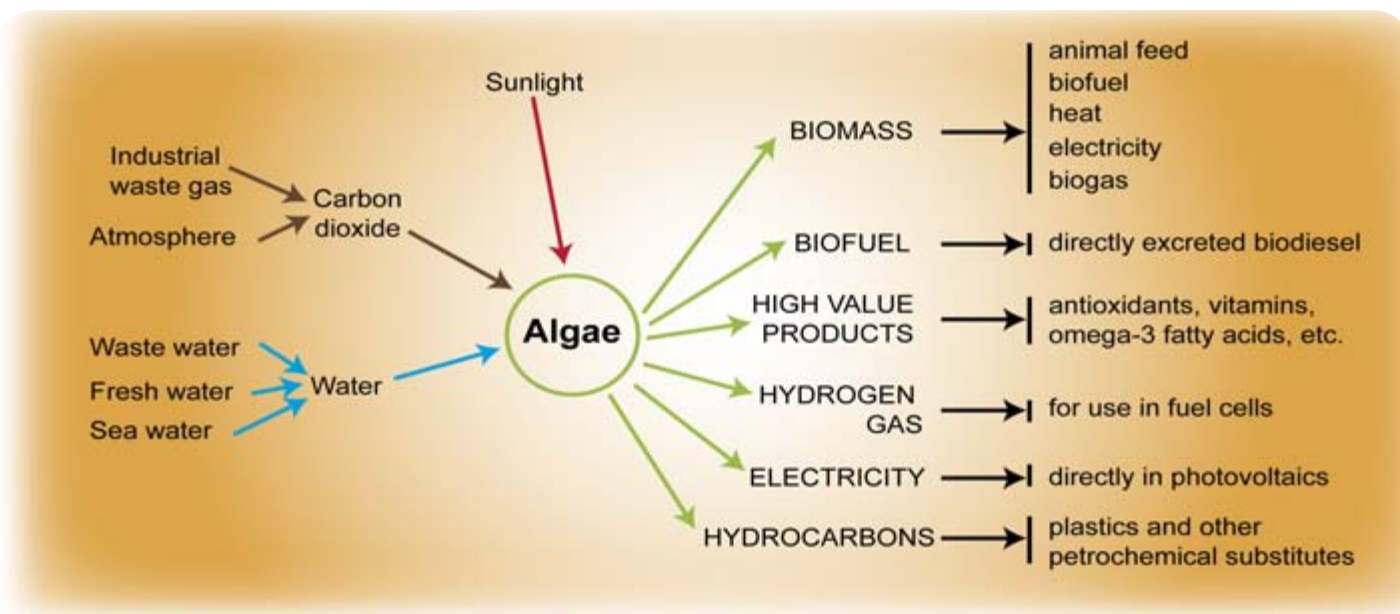
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SEM image of algae



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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<sub>2</sub> from flue gas emissions.



The strains of algae eventually used for processes outside of the research lab will depend on many factors: economics, engineering practicalities, overcoming scientific barriers, adoption of industrial standards, local planning applications, government incentives, social acceptance and more. Depending on the outcome of research and experimental pilot plants it is feasible that many of the individual algal applications can be combined in one facility such as waste water treatment, energy generation, animal feed production and the removal of carbon dioxide. The algae may be cultured in self-contained bioreactors, in open-air ponds or harvested from the environment. The process of harvesting algae is currently a significant challenge to obtaining economical yields. The problems associated with culturing and harvesting differ in bioreactors, marine environments or large ponds that could cover hectares of otherwise unproductive land. 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.

There may well be naturally occurring algae that can perform many of the tasks that we might want and researchers carrying out bioprospecting hope to identify suitable strains by selective screening. When growing algae in open systems it is inevitable that the ponds will get contaminated with algae from the environment, so many developers of algal technologies are hoping to harness these environmental algae as the main source of the algal biomass in their ponds. Strain selection will be key to successfully developing algal derived biofuels.

There are a number of methods for developing suitable strains of algae, including breeding, somatic fusion, genetic modification, synthetic biology and directed evolution. Breeding algae is theoretically possible but currently faces significant scientific challenges, such as identifying why algal species possess the genes for sexual reproduction but are only observed to reproduce asexually in the lab. Therefore, alternative techniques such as somatic fusion and synthetic biology are being investigated. Somatic or protoplast fusion involves combining the cells of different strains of algae. The technique has been used on plants and yeast and researchers are now investigating if stable algal hybrids can be created using this technique. This

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somatic breeding approach is particularly attractive since it allows the creation of novel strains by crossing species boundaries and exploiting the diversity found amongst the microalgae, but without using GM technology, which is currently rather limited for algae. Nevertheless, research is being conducted to develop methods for genetic modification to introduce desirable traits into algae, and synthetic biology approaches to re-engineer algal cells. One further option would be to use a technique called directed evolution. Here many algae are subjected to conditions that cause their DNA to change very slightly, the change in each individual alga's DNA will be subtly different from the changes in any other alga's DNA. The algae are then selected based on some condition, perhaps how much oil they produce, and these selected 'best' algae then go through the process again. So in a gradual stepwise fashion algae that are 'better' at producing oils are selected – in a process analogous to evolution.

Another key issue faced in the development of algal biofuels is harvesting. The oil can be collected from algae in a variety of ways but may involve growing algae in batches rather than continuously. Separating the algae from culture, concentrating the algae, drying algae, extracting oils mechanically or chemically and recycling the nutrients and water to reduce waste, all present difficulties and potential energy costs. Some strains of algae, such as *Scenedesmus*, form thick sediments whereas others are extremely small or motile.

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## 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<sub>2</sub>) 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<sub>2</sub> emissions and sunlight, and not compete for land with crops, makes them an attractive proposition both economically and sustainably.

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<sub>2</sub> 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



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Photobioreactor

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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  $\mu\text{m}$  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  $\mu\text{m}$  in length and often found in ponds or farmyard puddles.

**Pinnularia nobilis.** Very small single celled alga about 3  $\mu\text{m}$  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

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## Health and Safety

Take care with powdered nutrient algae medium (HARMFUL, IRRITATING, 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](http://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](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. 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.

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**For liquid cultures just omit the agar.**

## BG11 – 2x base:

NaNO <sub>3</sub>	1.5 g
K <sub>2</sub> HPO <sub>4</sub>	0.04 g
MgSO <sub>4</sub> •7H <sub>2</sub> O	0.075 g
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
NaCO <sub>3</sub>	0.02 g

## Trace metal A5\_1000x mix:

H <sub>3</sub> BO <sub>3</sub>	2.86 g
MnCl <sub>2</sub> •4H <sub>2</sub> O	1.81 g
ZnSO <sub>4</sub> •7H <sub>2</sub> O	0.222 g
NaMoO <sub>4</sub> •2H <sub>2</sub> O	0.39 g
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.079 g
Co(NO <sub>3</sub> ) <sub>2</sub> •6H <sub>2</sub> O	49.4 mg
Distilled water	1.0 L

Autoclave the final solution.

Make up to 500 ml with distilled H<sub>2</sub>O and autoclave.  
The pH should be 7.1 after sterilisation.

## 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
- Cafetière
- Coffee filter and filter papers

# Advanced Biofuels

## 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<sub>2</sub> 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. Data 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. Cafetières 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.

# Advanced Biofuels

## Suppliers

Algae and culture media 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

Algae can also 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.

Bioreactors 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 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/](http://www.saps.org.uk/) Cambridge University Botanic Garden 1 Brookside, Cambridge CB2 1JE tel: 01223 748455, [saps@hermes.cam.ac.uk](mailto: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](http://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](http://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](http://www.bbsrc.ac.uk/news/food-security/2011/110328-pr-photosynthesis-for-food-fuel-production.aspx)

[www.bioenergywiki.net/Algae\\_for\\_bioenergy](http://www.bioenergywiki.net/Algae_for_bioenergy)

[www.bioenergy.cam.ac.uk/abc.html](http://www.bioenergy.cam.ac.uk/abc.html)

[www.biofuelstp.eu/algae.html](http://www.biofuelstp.eu/algae.html)

[www.biomara.org/](http://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/](http://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/](http://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](http://www.nuffieldbioethics.org/biofuels-0)



# Advanced Biofuels

## 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)

The Algal Bioenergy Consortium [www.bioenergy.cam.ac.uk/abc.html](http://www.bioenergy.cam.ac.uk/abc.html)

Professor Johnathan Napier, Rothamsted Research [www.rothamsted.ac.uk](http://www.rothamsted.ac.uk)

Dr Saul Purton, University College London [www.ucl.ac.uk/biology/academic-staff/purton/purton.htm](http://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](http://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](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](http://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](http://www.sheffield.ac.uk/mbb/staff/gilmour)

BioMara project [www.biomara.com](http://www.biomara.com) at The Scottish Association for Marine Science, Dunstaffnage marine laboratory, Oban [www.sams.ac.uk](http://www.sams.ac.uk)

Culture Collection of Algae and Protozoa (CCAP) national algae collection [www.ccap.ac.uk](http://www.ccap.ac.uk)

# Advanced Biofuels

## Activity 3B - Algal photosynthesis

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

- Describe the requirements of photosynthesis.
- Take measurements to assess the rate of photosynthesis under varying conditions.
- Evaluate the benefits of producing biofuels from algae and the conditions required.

**Keywords** Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, yield, waste, photosynthesis, carbon dioxide, algae, varieties, photobioreactor.

### Background

**Algae** are a diverse group of eukaryotic photosynthetic organisms that constitute over 40,000 species. They can be single-celled (unicellular) or multicellular such as seaweed. Microalgae have been described as nature's very own power cells and could provide alternatives to petroleum-based fuels without competing with crops.

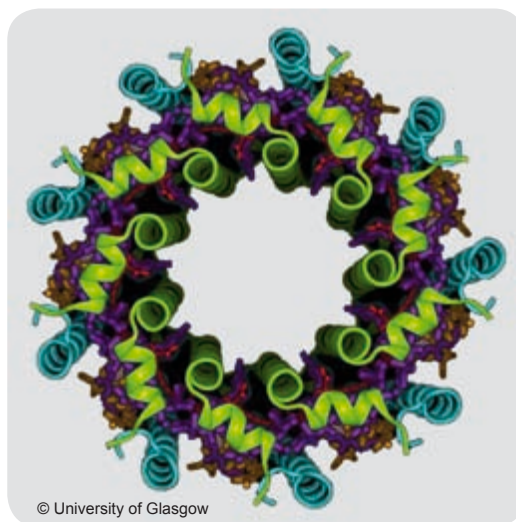
Algae can harvest the power of the sun through **photosynthesis** and convert this into biomass including oil. Many species are fast growing and more productive than land plants. This makes them an important part of the carbon cycle and they are able to produce complex molecules, such as hydrocarbons and carbohydrates, including cellulose, proteins, fats and oils, from the carbon dioxide they absorb. How algae and plants convert carbon dioxide into different molecules, known as carbon partitioning, is of great interest to researchers who aim to develop algae that can produce the substances we require. Photosynthesis is only 6% efficient and it may be possible to improve this to produce faster growing algae, higher yielding plants or develop novel ways of capturing solar energy.

For efficient and economic production of algal biofuels a number of conditions are required and suitable algae varieties need to be identified and developed. The UK has a relatively cold climate that is not particularly sunny, slowing growth of algae and reducing productivity. The possible solutions to this are culturing algae in self-contained photobioreactors, rather than open air ponds, or using surplus heat from industrial activity to warm ponds and thereby increase growth rates. In this activity students can investigate the most suitable conditions for algal photosynthesis and compare the photosynthetic ability of varieties of algae.

This activity will enable students to observe the photosynthesis reaction in algae using an indicator method for carbon dioxide uptake.

A range of photosynthesis experiments have been developed by Science and Plants for Schools (SAPS) including photosynthesis using algae wrapped in jelly balls:

[www.saps.org.uk/secondary/teaching-resources/235-student-sheet-23-photosynthesis-using-algae-wrapped-in-jelly-balls](http://www.saps.org.uk/secondary/teaching-resources/235-student-sheet-23-photosynthesis-using-algae-wrapped-in-jelly-balls)



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Light Harvesting complex 2

# Advanced Biofuels

**Age Range:** This experiment is suitable for secondary and post-16 students.

**Duration:** 60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of microbes, photosynthesis, plants, the properties of gases, indicators and chemical reactions. Knowledge of photosynthesis and the properties of light will help students interpret the results of their investigations.

## What you will need

- Varieties of unicellular algae
- Hydrogen carbonate indicator
- Universals
- Light source
- Pipettes

### Optional

- Sodium alginate
- Syringe
- Beaker
- Strainer
- CaCl<sub>2</sub> solution
- Colorimeter

## Health and Safety

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 RB3 (Alginate beads), RB48 (Indicators-carbon dioxide)

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

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.

# Advanced Biofuels

## Method

1. Different species of algae should be cultured prior to the activity (you may want to carry out **activity 3A** - culturing algae first).
2. Prepare solutions of equal algal density, a dark green coloured solution will provide sufficient algae to enable measurement of photosynthesis over a short period. Some cultures will readily settle if placed in the dark overnight, other cultures will require filtering or centrifugation. The concentration of algae in culture can be assessed by cell counts, relative colour or using a densitometer. Larger concentrated vials of *Scenedesmus* algae sufficient to produce a class set of algal balls are available from Sciento if there is insufficient time to culture algae.
3. Add 2 ml of algal solution to the universal and 2 ml of hydrogen carbonate indicator.
4. Alternatively the algae can be immobilised in sodium alginate balls prior to adding to the indicator.
5. The algae are exposed to equivalent levels of light using a light box.
6. Photosynthesis can be measured as the indicator turns from yellow to purple as carbon dioxide is removed from the solution by the algae. Hydrogen carbonate indicator is very sensitive to the levels of carbon dioxide (CO<sub>2</sub>) present.
7. The relative levels of dissolved carbon dioxide can be compared to a set of prepared standard solutions *or* if the algae have been immobilised in alginate balls a colourimeter can be used.
8. The concentration of carbon dioxide in the indicator can be measured using a colourimeter to test the absorbance of light at 550nm.

The amount of light that will pass through the indicator decreases as it turns purple and the % of carbon dioxide is calculated by the colourimeter.



## Extension activities

If the time and sufficient equipment is available students can immobilise the algae in the sodium alginate prior to the activity. Prepared algal balls will keep for some time in distilled water in a fridge. Allow them time to warm up and acclimatise to the light before checking that they are still active.

# Advanced Biofuels

## Preparing immobilised algae

1. Prepare a 2-3% sodium alginate solution with warm distilled or deionised water, mix thoroughly and leave overnight in a fridge. The initial mixture can be very lumpy but will become smooth overnight. Sodium alginate from different suppliers can vary in viscosity. A magnetic stirrer can be used to stir the mixture overnight but do not use a heated stirrer as this will reduce the efficacy of the gel matrix.
2. Concentrate the algae suspension by gentle centrifugation or allowing to settle overnight in a dark room. Resuspend in a small amount of distilled or deionised water so that the final solution of immobilised algae-alginate is not too runny.
3. Add the resuspended algae to the sodium alginate solution and mix thoroughly. Ideally a dark green solution will be produced in order to have enough algae to be able to carry out a sufficient rate of photosynthesis to be easily measured in relatively short timescales.
4. Prepare a 1.5% calcium chloride solution with  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ . The calcium ions cause the sodium alginate to set and hence using distilled or deionised water for the alginate solution and resuspending the algae is important as is avoiding contact of the syringe with the calcium chloride solution.
5. Draw the algae-alginate solution up into a syringe.
6. Add the algae-alginate solution into a 1.5%  $\text{CaCl}_2$  solution drop by drop. Carefully observe the shape of the drops. If the drops take on a 'comet' shaped appearance add a small amount of distilled or de-ionised water to the algae-alginate solution, mix and retry.
7. Allow the algae-alginate beads to set for at least 10 minutes.
8. Carefully strain the beads and rinse with distilled or deionised water.

A number of variables can be investigated and the results graphed by students. The ability of algae to carry out photosynthesis with different types of lights could be assessed. Filters can be used to block specific wavelengths of light to investigate the chromophores present in the algae.

## Suppliers

Algae and culture media 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

Photosynthesis kits and hydrogen carbonate indicator 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

Colorimeters can be obtained from Philip Harris Education, Hyde Buildings, Hyde, Cheshire SK14 4SH, tel: 0845120 4520 fax: 0800 138 8881

## Further reading and links

Eldridge, D. (2004) A novel approach to photosynthesis practicals. *School Science Review*, **85** (312), 37-45.

Photosynthesis using algae wrapped in jelly balls, SAPS [www.saps.org.uk/secondary/teaching-resources/235](http://www.saps.org.uk/secondary/teaching-resources/235)

Investigating photosynthesis using immobilised algae, Practical Biology [www.practicalbiology.org/areas/advanced/energy/photosynthesis/investigating-photosynthesis-using-immobilised-algae.54.EXP.html](http://www.practicalbiology.org/areas/advanced/energy/photosynthesis/investigating-photosynthesis-using-immobilised-algae.54.EXP.html)

# Advanced Biofuels

Immobilised algae - Immobilised algae for studying photosynthesis, Debbie Eldridge [www.eurovolvox.org/Protocols/PDFs/ImmobilisedAlgae1.0\\_UK\\_eng.pdf](http://www.eurovolvox.org/Protocols/PDFs/ImmobilisedAlgae1.0_UK_eng.pdf)

Investigating Photosynthesis – students guide, National Centre for Biotechnology Education (NCBE), [www.ncbe.reading.ac.uk/ncbe/materials/METABOLISM/PDF/PhotosynthSG.pdf](http://www.ncbe.reading.ac.uk/ncbe/materials/METABOLISM/PDF/PhotosynthSG.pdf)

Investigating factors affecting the rate of synthesis, Practical Biology [www.practicalbiology.org/areas/intermediate/energy/photosynthesis/investigating-factors-affecting-the-rate-of-photosynthesis.45.EXP.html](http://www.practicalbiology.org/areas/intermediate/energy/photosynthesis/investigating-factors-affecting-the-rate-of-photosynthesis.45.EXP.html)

Investigating the light dependent reaction in photosynthesis [www.practicalbiology.org/areas/advanced/energy/photosynthesis/investigating-the-light-dependent-reaction-in-photosynthesis.60.EXP.html](http://www.practicalbiology.org/areas/advanced/energy/photosynthesis/investigating-the-light-dependent-reaction-in-photosynthesis.60.EXP.html)

Identifying the conditions needed in photosynthesis [www.practicalbiology.org/areas/introductory/energy/photosynthesis/identifying-the-conditions-needed-for-photosynthesis.74.EXP.html](http://www.practicalbiology.org/areas/introductory/energy/photosynthesis/identifying-the-conditions-needed-for-photosynthesis.74.EXP.html)

Photosynthesis and starch production in Pelargonium leaf discs, Science and Plants for Schools (SAPS). [www.saps.org.uk/secondary/teaching-resources/145-photosynthesis-and-starch-production-in-pelargonium-leaf-discs-](http://www.saps.org.uk/secondary/teaching-resources/145-photosynthesis-and-starch-production-in-pelargonium-leaf-discs-)

CLEAPSS® Recipe book Hydrogen carbonate indicator, page 48.

CLEAPSS® Guide R57 (2000) Colorimeters

Colorimetry and Higher Still, SSERC 1999, Biology Notes, Bulletin 197, 16.

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)

The Algal Bioenergy Consortium [www.bioenergy.cam.ac.uk/abc.html](http://www.bioenergy.cam.ac.uk/abc.html)

Professor Johnathan Napier, Rothamsted Research [www.rothamsted.ac.uk](http://www.rothamsted.ac.uk)

Dr Saul Purton, University College London [www.ucl.ac.uk/biology/academic-staff/purton/purton.htm](http://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](http://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](http://marine-vectors.eu/about_us/the_pml_team/staff_directory/carole_llewellyn.aspx)

BioMara project [www.biomara.com](http://www.biomara.com) at The Scottish Association for Marine Science, Dunstaffnage marine laboratory, Oban [www.sams.ac.uk](http://www.sams.ac.uk)

Culture Collection of Algae and Protozoa (CCAP) national algae collection [www.ccap.ac.uk](http://www.ccap.ac.uk)

# 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 photosynthesise. 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

# Advanced Biofuels

## 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.

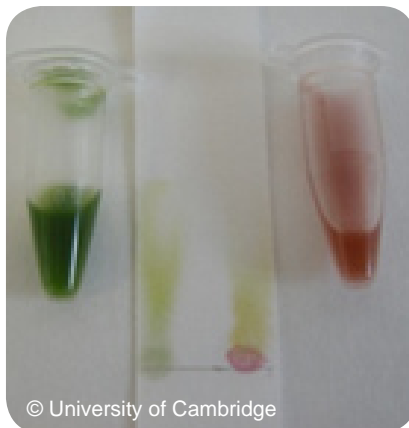
## Method

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?**



# Advanced Biofuels

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)

# Dry Activities

## Activity 4A - Making biofuel molecules

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

- Construct some simple molecules.
- Describe the molecules involved in photosynthesis and used as biofuels.
- Suggest the properties of the substances that the models represent.

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

### What you will need

Molymod<sup>®</sup> molecular model set for **basic organic chemistry**.

One class set will provide enough atoms and bonds for six groups to form carbon dioxide, water, methane, bioethanol and biobutanol molecules (but not all at the same time). The complete set will enable groups to combine their molecules and atoms to make the larger molecular structures including glucose, cellulose and biodiesel.

**Age Range:** This activity is suitable for GCSE or post-16 students.

**Duration:** 30-60 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of atoms, compounds and molecules. An understanding of natural substances and metabolism will help students contextualize the use of models to represent fuels and natural substances in the exercise.

### Instructions

Provide each group of students with a pack of **basic organic chemistry** Molymod<sup>®</sup> molecular models.

#### Each pack contains:

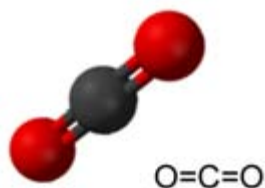
- Ten hydrogens (white)
- Four carbons (black)
- Two oxygens (red)
- Two chlorines (green)
- One nitrogen (blue)
- Twenty single bonds
- Four multiple (flexible) bonds

Start off by explaining that you will be constructing the molecules that are important for plants to grow and make biomass then you will be moving on to the more complicated molecules that are formed from plants and used as biofuels. Provide each group or pair of students with one set of molecules and bonds and the diagrams below. The diagrams can be displayed on a powerpoint or provided on the activity sheet. Once the students have made each molecule and it has been checked they will need to take it apart in order to construct the next one. Once the group has made butanol they will need to join another group to be able to make the larger molecules.

# Dry Activities

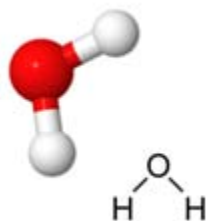
## Carbon dioxide

First of all introduce carbon dioxide and explain its role in photosynthesis. You may also want to explain the greenhouse gas properties of carbon dioxide and its production from combustion of fossil fuels. Instruct the students to make a model of carbon dioxide from two oxygen atoms (red), one carbon atom (black) and four multiple (flexible) bonds.



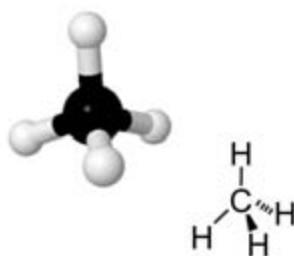
## Water

Explain the importance of water to plants and its role in photosynthesis. Instruct the students to make a model of water from two hydrogen atoms (white), one oxygen atom (red) and two single bonds.



## Methane

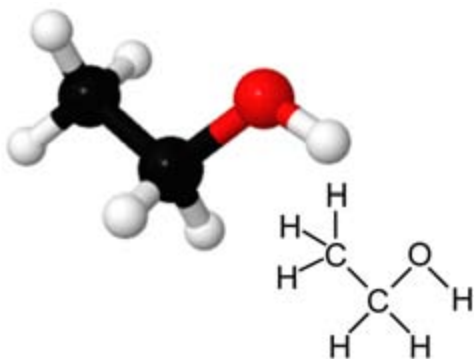
Introduce biogas and explain that the main constituent is methane. You may want to describe the production of biogas through anaerobic digestion as well as the environmental benefits and ease of production in developing countries. Instruct the students to make a model of methane from four hydrogen atoms (white), one carbon atom (black) and four single bonds.



# Dry Activities

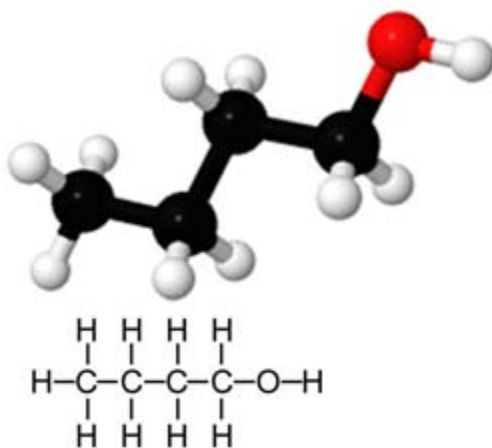
## Ethanol

Introduce bioethanol and explain that the final product is just the same as the ethanol found in alcohol. You may want to describe the production of bioethanol from fermentation of carbohydrate feedstocks. Instruct the students to make a model of ethanol from six hydrogen atoms (white), two carbon atoms (black), one oxygen atom (red) and eight single bonds.



## Butanol

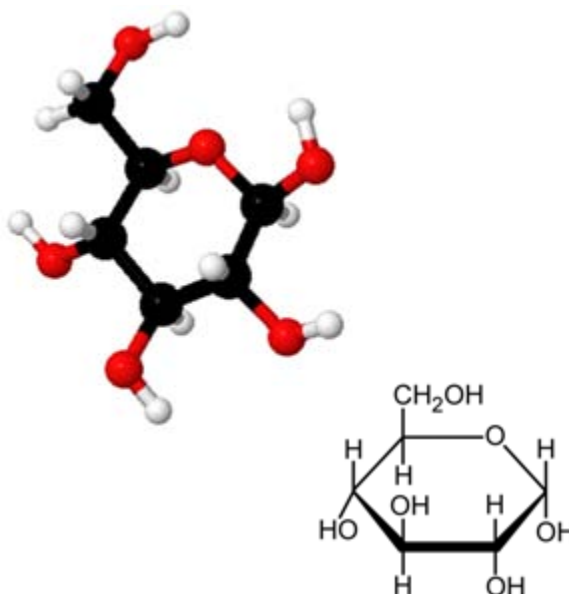
Introduce butanol and explain that it is a superior transport fuel to ethanol due to its higher energy content and the ability to use it in existing pipelines, infrastructure and engines without it needing to be blended with fossil fuels. You may also want to explain the research required to increase the range of feedstocks that can be used, the yield and the tolerance of the microorganisms to biobutanol for optimum production. Instruct the students to make a model of butanol from ten hydrogen atoms (white), four carbon atoms (black), one oxygen atom (red) and fourteen single bonds.



# Dry Activities

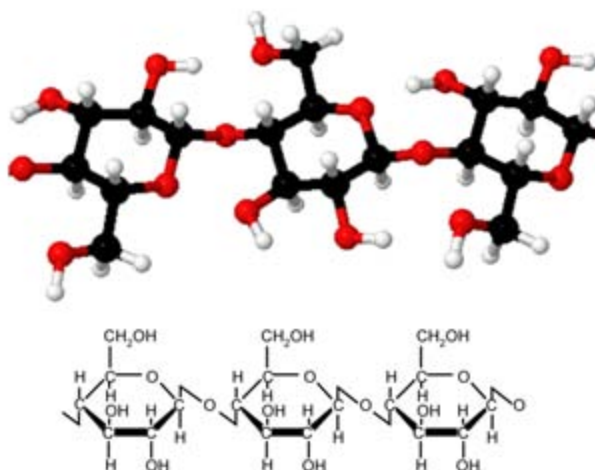
## Glucose

Introduce glucose and explain its production by plants through photosynthesis. You may also want to explain the use of glucose by yeast in fermentation. The students will now need to join together to make the glucose molecule. Three packs are required to provide enough oxygen atoms to make a molecule of glucose. Instruct the students to make a model of glucose from twelve hydrogen atoms (white), six carbon atoms (black), six oxygen atoms (red) and twenty single bonds. Important: Once completed and checked, instruct the students not to take the model apart.



## Cellulose

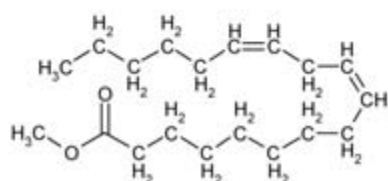
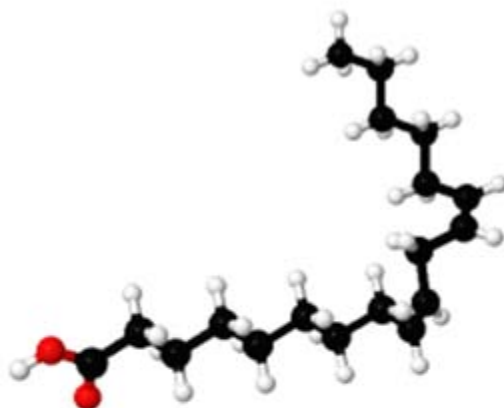
Introduce cellulose and explain its production by plants. You may also want to explain the use of cellulose in plant tissues and the research being undertaken to enable fermentation of lignocellulosic feedstocks. All the students will now need to join together to make a portion of a cellulose molecule featuring three repeating glucose subunits. Instruct the students to make the model of cellulose by joining together the glucose molecules already made. There are sufficient oxygen atoms (fourteen) to form the two connections between the glucose molecules but not the oxygens on the ends of the chain. Students will now need to take the model apart to be able to construct the model of biodiesel.



# Dry Activities

## Biodiesel (Methyl Linoleate)

Introduce biodiesel and explain its production from oils and fats. All the students will need to join together to make a biodiesel (methyl linoleate) molecule. Instruct the students to make the model from thirty four hydrogen atoms (white), nineteen carbon atoms (black), two oxygen atoms (red), six multiple (flexible) bonds and fifty single bonds.



**IMPORTANT:** Ensure that all the atoms and bonds are counted up and returned at the end of the session so that lost parts can be identified and replaced before repeating the activity.

### Suppliers

Molymod® molecular models are available from [www.molymod.com/](http://www.molymod.com/) Spiring Enterprises Limited  
Gillmans Industrial Estate, Natts Lane Billingshurst, West Sussex RH14 9EZ, UK +44 (0) 1403 782 387

### Further reading

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)

# Student activity sheet

## Making biofuel molecules




This activity will guide you through constructing models of the molecules that are important for plants to grow and make biomass as well as more complicated molecules that are formed from plants and used as biofuels.

You will need to work in small groups to begin with making the smaller molecules and then work in larger groups to make the larger molecules.

### What you will need

A pack of **basic organic chemistry** Molymod® molecular models.

Each pack contains:

- Ten hydrogens (white)
- Four carbons (black)
- Two oxygens (red)
- Two chlorines (green)
- One nitrogen (blue)
- Twenty single bonds
- Four multiple (flexible) bonds
- Carbon atoms are **Black** 
- Oxygen atoms are **Red** 
- Hydrogen atoms are **White** 

Diagrams are provided to help you construct your molecules.

Once you have made each molecule and it has been checked you will need to take it apart in order to construct the next one.

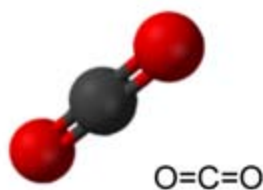
Once your group has made the butanol molecule you will need to join with another group to be able to make the larger molecules.

### Instructions

#### Carbon dioxide

Carbon dioxide is essential to photosynthesis. Photosynthesis converts carbon dioxide and water into organic compounds including sugars using the energy from sunlight. Combustion of fuels produces carbon dioxide and it is one of the greenhouse gases contributing to climate change.

To make a model of carbon dioxide you will need two oxygen atoms (red), one carbon atom (black) and four multiple (flexible) bonds. Once you have made your molecule and had it checked, take it apart.

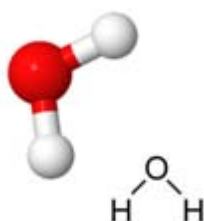


# Student activity sheet

## Water

Water is essential to photosynthesis. Water covers approximately 70% of the world's surface but only 2.5% is fresh water and most of that is frozen or found underground.

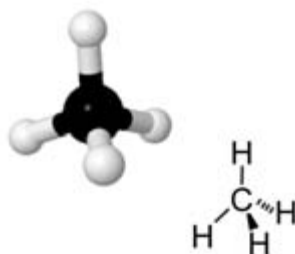
To make a model of water you will need two hydrogen atoms (white), one oxygen atom (red) and two single bonds. Once you have made your molecule and had it checked, take it apart.



## Methane

Biogas is produced by anaerobic digestion and the main constituent is methane. Biogas can be burnt to produce heat for cooking, warming homes and producing electricity. It can also be compressed and used as a transport fuel in specially converted vehicle engines.

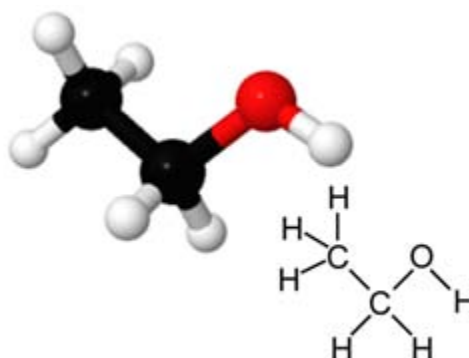
To make a model of methane you will need four hydrogen atoms (white), one carbon atom (black) and four single bonds. Once you have made your molecule and had it checked, take it apart.



## Ethanol

Bioethanol is produced from fermentation of carbohydrate feedstocks and the final product is just the same as the ethanol found in alcohol. Bioethanol is compatible with existing vehicle engines and can be mixed with fossil fuels. Up to 10% blends with petrol can be used without modifying vehicle engines.

To make a model of ethanol you will need six hydrogen atoms (white), two carbon atoms (black), one oxygen atom (red) and eight single bonds. Once you have made your molecule and had it checked, take it apart.



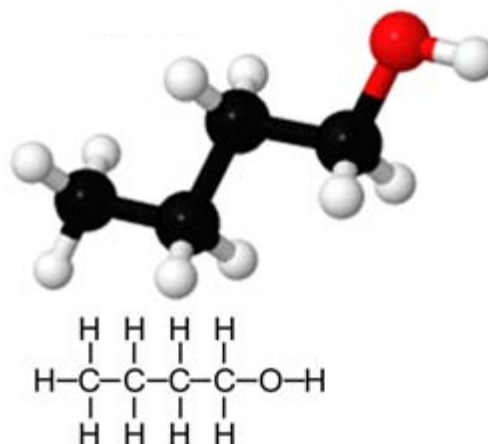


# Student activity sheet

## Butanol

Butanol is a superior transport fuel to ethanol due to its higher energy content and the ability to use it in existing pipelines, infrastructure and engines without it needing to be blended with fossil fuels. However, further research is required to increase the range of feedstocks that can be used as well as the yield and the tolerance of the microorganisms to biobutanol before it can be produced on an industrial scale.

To make a model of butanol you will need ten hydrogen atoms (white), four carbon atoms (black), one oxygen atom (red) and fourteen single bonds. Once you have made your molecule and had it checked, take it apart.

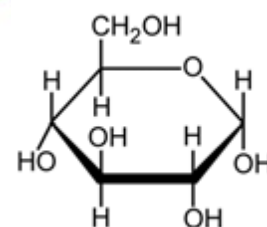
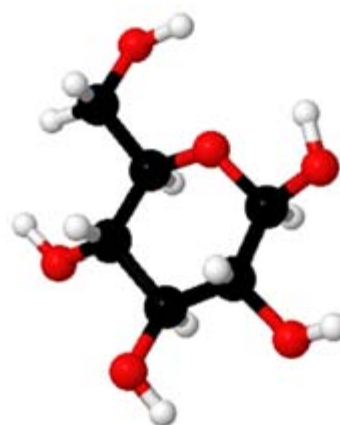


## Glucose

Glucose is a monosaccharide sugar produced by plants through photosynthesis. Glucose is the main source of energy for cells and is one of the sugars used by yeast in fermentation to produce ethanol.

You will now need to work with another two groups to make a glucose molecule. Three packs are required to provide enough oxygen atoms to make a molecule of glucose.

To make a model of glucose you will need twelve hydrogen atoms (white), six carbon atoms (black), six oxygen atoms (red) and twenty single bonds. Important: Once completed and checked, do not take the model apart.



## Cellulose

Cellulose is a polysaccharide formed by plants from glucose molecules. It is the main structural component of cell walls in the form of lignocelluloses and research is being undertaken to enable it to be fermented to produce biofuels.

To be able to make a portion of a cellulose molecule you will now need to work together with three other groups to make a cellulose molecule featuring three repeating glucose subunits.

To make the model of cellulose join together the glucose molecules already made. There are sufficient oxygen atoms (fourteen) to form the two connections between the glucose molecules but not the oxygens on the ends of the chain. You will need to take the model apart to be able to construct the next model – biodiesel!

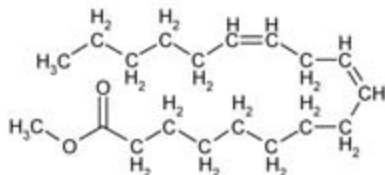
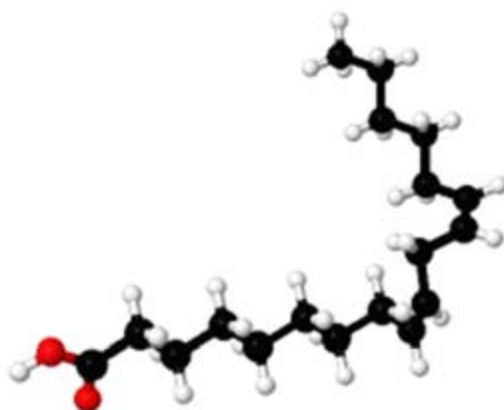


# Student activity sheet

## Biodiesel (Methyl Linoleate)

Biodiesel is produced from oils and fats mixed with methanol and a catalyst. Biodiesel can replace diesel or be further refined to produce synthetic kerosene suitable for use in aviation fuel.

You will need to work together as one large group to make a biodiesel (methyl linoleate) molecule. To make the model of biodiesel you will need thirty four hydrogen atoms (white), nineteen carbon atoms (black), two oxygen atoms (red), six multiple (flexible) bonds and fifty single bonds.



**IMPORTANT:** Ensure that all the atoms and bonds are counted up and returned at the end of the session so that lost parts can be identified and replaced before repeating the activity.

# Dry Activities

## Activity 4B - Biofuel feedstocks

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

- Classify biofuel feedstocks according to the fuels they produce, their relation to food crops or using their own classification scheme
- Compare biofuel feedstocks
- Suggest the pros and cons of the biofuel feedstocks according to their properties

**Keywords** Bioenergy, biofuel, biodiesel, sustainable, renewable, biomass, feedstock, yield, waste, varieties,

### What you will need

One set of Biofuel feedstock resin samples and a set of feedstock cards. A limited number of these feedstock packs are available from BBSRC by contacting Tristan Bunn [Tristan.Bunn@bbsrc.ac.uk](mailto:Tristan.Bunn@bbsrc.ac.uk)

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

**Duration:** 10-30 minutes.

**Suggested prior knowledge:** It is recommended that you elicit the existing student knowledge of plants, food and non-food crops, properties of materials and classification. An understanding of processing of natural substances in agriculture as well as the energy and food industries will help students imagine the way the feedstocks can be used to produce biofuels.

## Instructions

Pass the feedstock resin samples around the class to enable students to take a close look at them. Start off by explaining that biofuel feedstocks can be categorized in a variety of ways. Provide students with the images of the feedstocks or if there are a small number of students the feedstock themselves. Allow students to discuss how they would categorize the feedstocks and come up with their own classification system. Ask students to group the feedstocks according to the following levels of development or final products;

Current biofuel feedstocks are **Rapeseed, Soya Beans, Maize, Castor Beans, Sweet Sorghum, Sugar beet, Sugar cane**

Advanced biofuel feedstocks are **Barley stems, Miscanthus, Willow**

Biodiesel feedstocks are **Rapeseed, Soya Beans, Maize, Castor Beans**

Bioethanol feedstocks are **Maize, Sweet Sorghum, Sugar beet, Sugar cane**

Lignocellulosic bioethanol feedstocks are **Barley stems, Miscanthus, Willow**

# Dry Activities

**Ranking the feedstocks.** Ask students to rank the feedstocks in order of lowest to highest according to measurements such as amount used for fuels, the amount of land that would be required to grow them in the UK to replace fossil fuels, the amounts imported into the UK or the biofuel yields from different crops. The following figures may be helpful.

## Feedstocks used for fuel in the UK 2009-2010

- 28% soy
- 18% sugar cane
- 13% oilseed rape
- 11% tallow
- 6% palm oil

## UK arable land needed to produce 5% of the UK transport fuels

- 12% needed for sugar beet
- 25% needed for cereals
- 40% needed for oilseed rape derived biodiesel
- 45% needed for wheat straw lignocellulosic bioethanol

## Biofuel feedstock imports 2009-2010

- 20% Argentinean soy
- 20% Brazilian sugar cane
- 7% American soy
- 7% German oilseed rape
- 4% Malaysian palm oil

## Biofuel yields

- 6000l/ha bioethanol from sugarcane
- 5000l/ha bioethanol from sugar beet
- 3000l/ha bioethanol from maize
- 1000l/ha bioethanol from barley
- 5000l/ha biodiesel from oil palm
- 1400l/ha biodiesel from oilseed rape
- 1000l/ha biodiesel from castor beans
- 800l/ha biodiesel from soybean

# Dry Activities

Rapeseed



Maize



Soya Beans



# Dry Activities

Castor Beans



Sweet Sorghum



# Dry Activities

Barley stems



Sugar beet

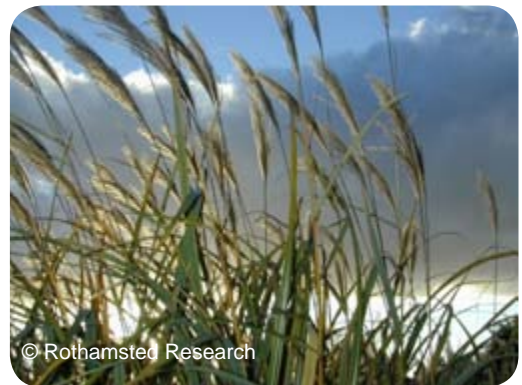


# Dry Activities

Sugar cane



Miscanthus



Willow





# Dry Activities

## Suppliers

Biofuel feedstock resin samples can be obtained from the BBSRC Inspiring Young Scientists coordinator Tristan Bunn [Tristan.Bunn@bbsrc.ac.uk](mailto:Tristan.Bunn@bbsrc.ac.uk).

## Further reading

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)

# Dry Activities

## Activity 4C – Bioenergy crosswords

Crosswords are provided for use at a range of educational levels Primary, Key Stage 3, Key Stage 4 and Post-16. The crosswords cover vocabulary and concepts associated with bioenergy research that students are expected to be familiar with at the educational level specified. There will also be a few words that are included that may be specific to bioenergy research and not covered in the curriculum, designed to stretch students and enrich their understanding of the topic. It would be advisable to introduce students to the words and their meanings in the course of the learning session.

Crosswords support the literacy component of science and will help with spelling, reading, writing, scientific communication, understanding, revision of the terminology and definitions.

### The crosswords can be used in many ways:

- introduction to the topic to elicit prior knowledge
- recapping a previous session
- to build up word lists for wall display
- a fun end of session exercise
- as a takeaway activity

Crosswords and wordsearches provide many levels of differentiation. They can be completed individually or as a group, with or without support sheets listing the words.

They can be used to differentiate by task allowing the student to select a crossword or wordsearch. If the wordsearch is completed with ease then the student can be encouraged to attempt the crossword. Students will have recently found the same words in the wordsearch and so feel more confident in attempting the crossword.

A solution for each crossword and wordsearch is provided which allows students to check their own work and thereby allow you to spend more time engaging with other students.

Word exercises such as sentence loops allow students to expand their vocabulary specific to the subject, and when completed within a group the activities promote discussion and therefore promote their oracy skills.

# Introductory Crossword Clues

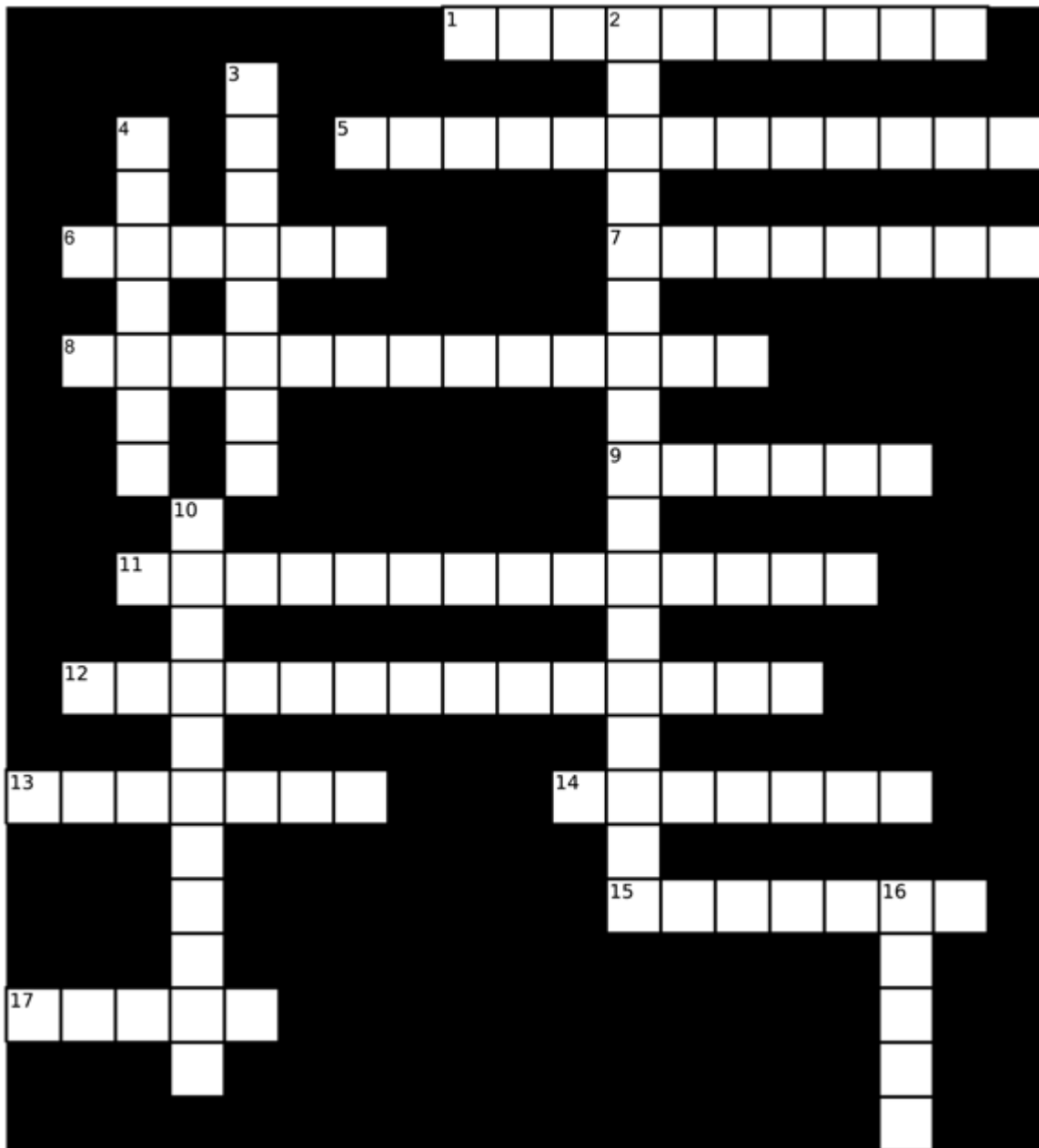
## Across

1. Non-renewable fuels, such as coal, oil and gas (2 words)
5. The steady increase in the temperature of the Earth's atmosphere (2 words)
6. An insoluble carbohydrate found in plants and plant products
7. A substance that increases the rate of a reaction
8. A gas produced by the burning of fuels and used in photosynthesis (2 words)
9. A group of proteins that speed up reactions in living things
11. A method of separating substances where they move at different speeds
12. A process carried out in green plants that uses light
13. With oxygen
14. A renewable fuel produced from biological material
15. The specific sugar made by photosynthesis
17. A measure of the amount of crop produced

## Down

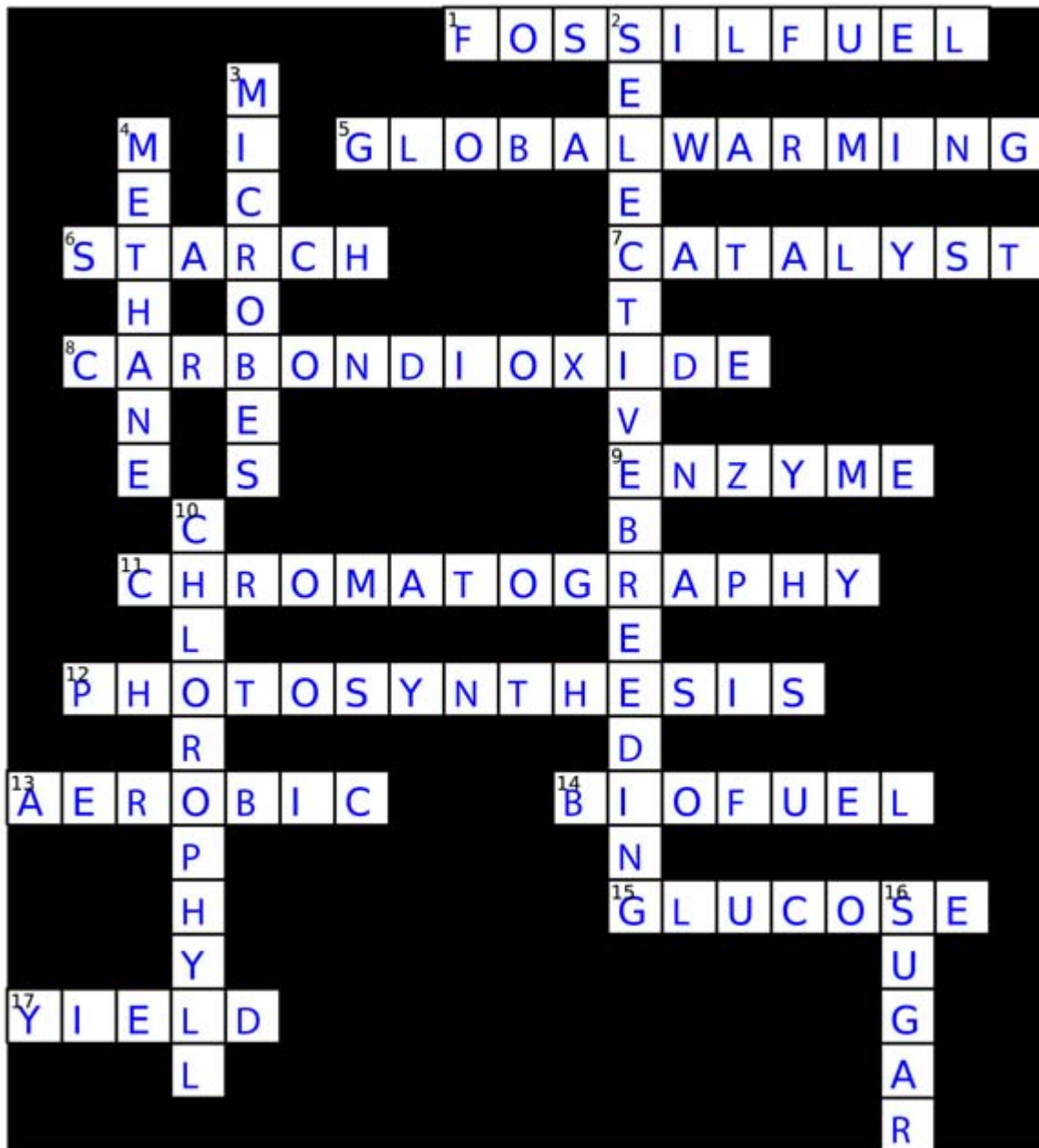
2. The process of producing desirable characteristics in the next generation (2 words)
3. Very small living things that can only be seen with a microscope
4. A gas that is found with crude oil and produced in decomposition
10. The green chemical in plants that absorbs light energy
16. A carbohydrate that is a source of energy in respiring cells

# Introductory Crossword



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# Introductory Crossword Answers



# Intermediate Crossword Clues

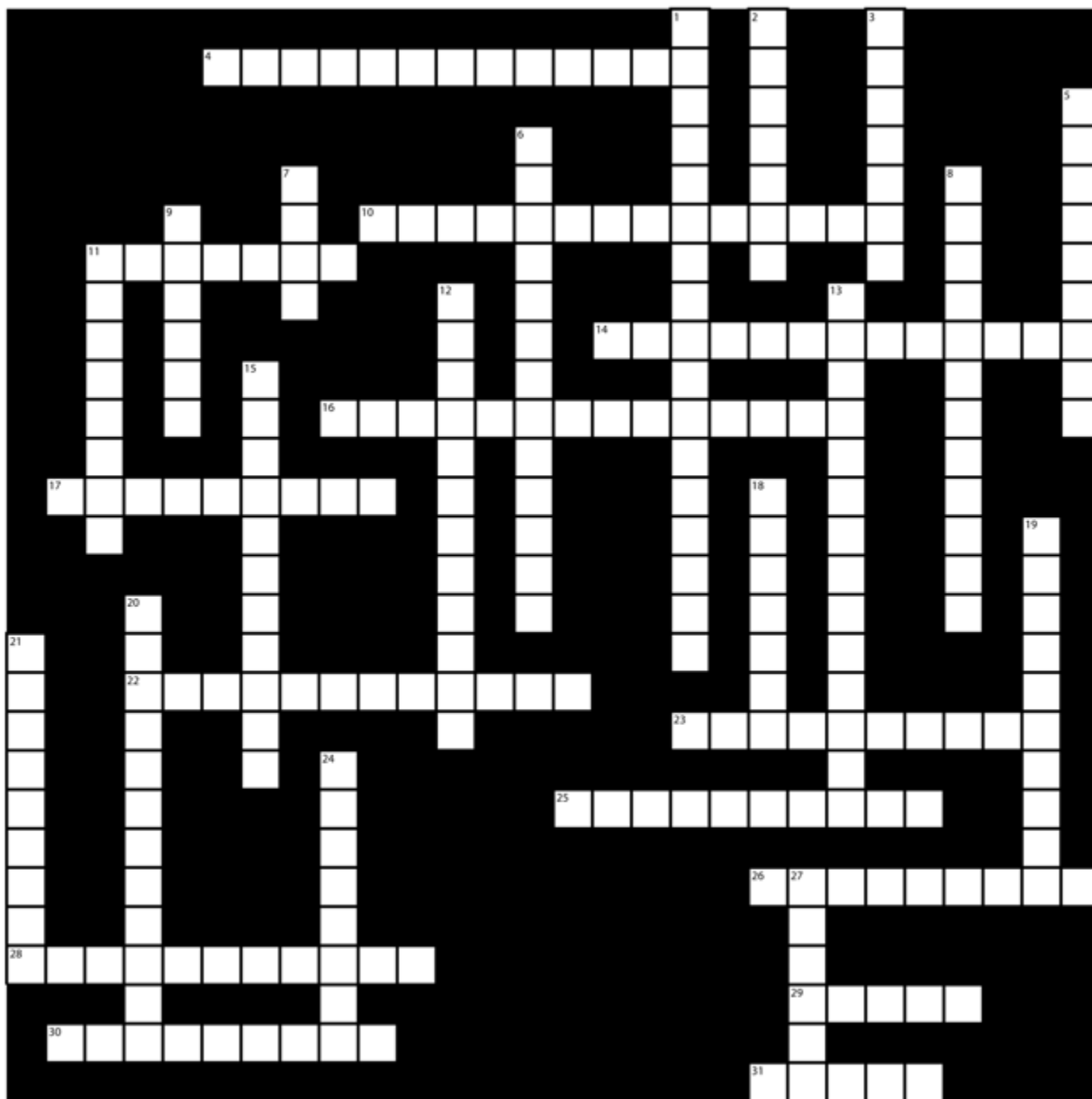
## Across

4. A gas produced by the burning of fuels and used in photosynthesis. (2 words)
10. A process carried out in green plants that uses light
11. A gas that is found with crude oil and produced in decomposition
14. Gas such as carbon dioxide that traps heat in the atmosphere (2 words)
16. A method of separating substances where they move at different speeds
17. A resource that can be replaced more quickly or at the same rate as it is being used
22. A process in which liquids are purified or separated by heating to form a gas and then condensed back into a liquid
23. Non-renewable fuels, such as coal, oil and gas (2 words)
25. A layer of gases that surrounds the Earth
26. The material from which the plant cell walls are made
28. The green chemical in plants that absorbs light energy
29. A measure of the amount of crop produced
30. A measurement of the 'thickness' of a fluid
31. Name for the fungus that is single-celled most of its life, used in the production of alcohol and capable of fermenting carbohydrates

## Down

1. The process of producing desirable characteristics in the next generation (2 words)
2. A renewable fuel produced from biological material
3. The specific sugar made by photosynthesis
5. An organic compound derived from oil or fat that can be used as a transport fuel
6. The steady increase in the temperature of the Earth's atmosphere (2 words)
7. Part of a chromosome that contains the 'instructions' for a particular characteristic such as leaf shape
8. An anaerobic cellular process in which organic foods are converted into simpler compounds such as alcohol
9. An insoluble carbohydrate found in plants and plant products
11. Very small living things that can only be seen with a microscope
12. The tiny structures inside plant cells where photosynthesis occurs
13. A complex carbohydrate composed of a chain of monosaccharides joined together by glycosidic bonds
15. A compound that contains double or triple bonds
18. The term for the dry weight of a living thing that can be used as a fuel
19. A chemical reaction where a compound, such as starch or cellulose, is broken down by reaction with water into smaller components.
20. Chemicals that are made only from hydrogen and carbon
21. Without oxygen
24. A substance that increases the rate of a reaction
27. A group of proteins that speed up reactions in living things

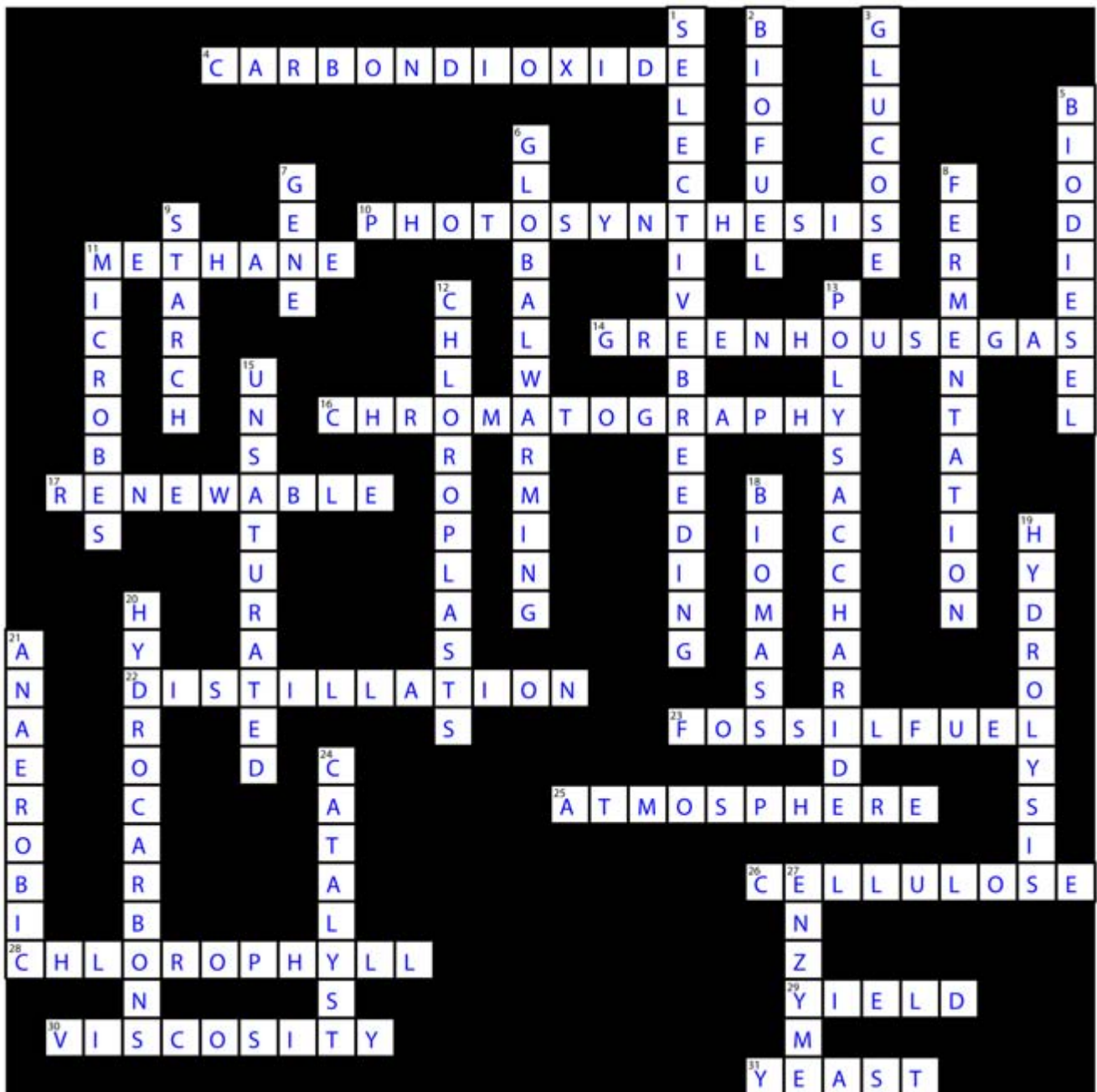
# Intermediate Crossword





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# Intermediate Crossword Answers



# Advanced Crossword Clues

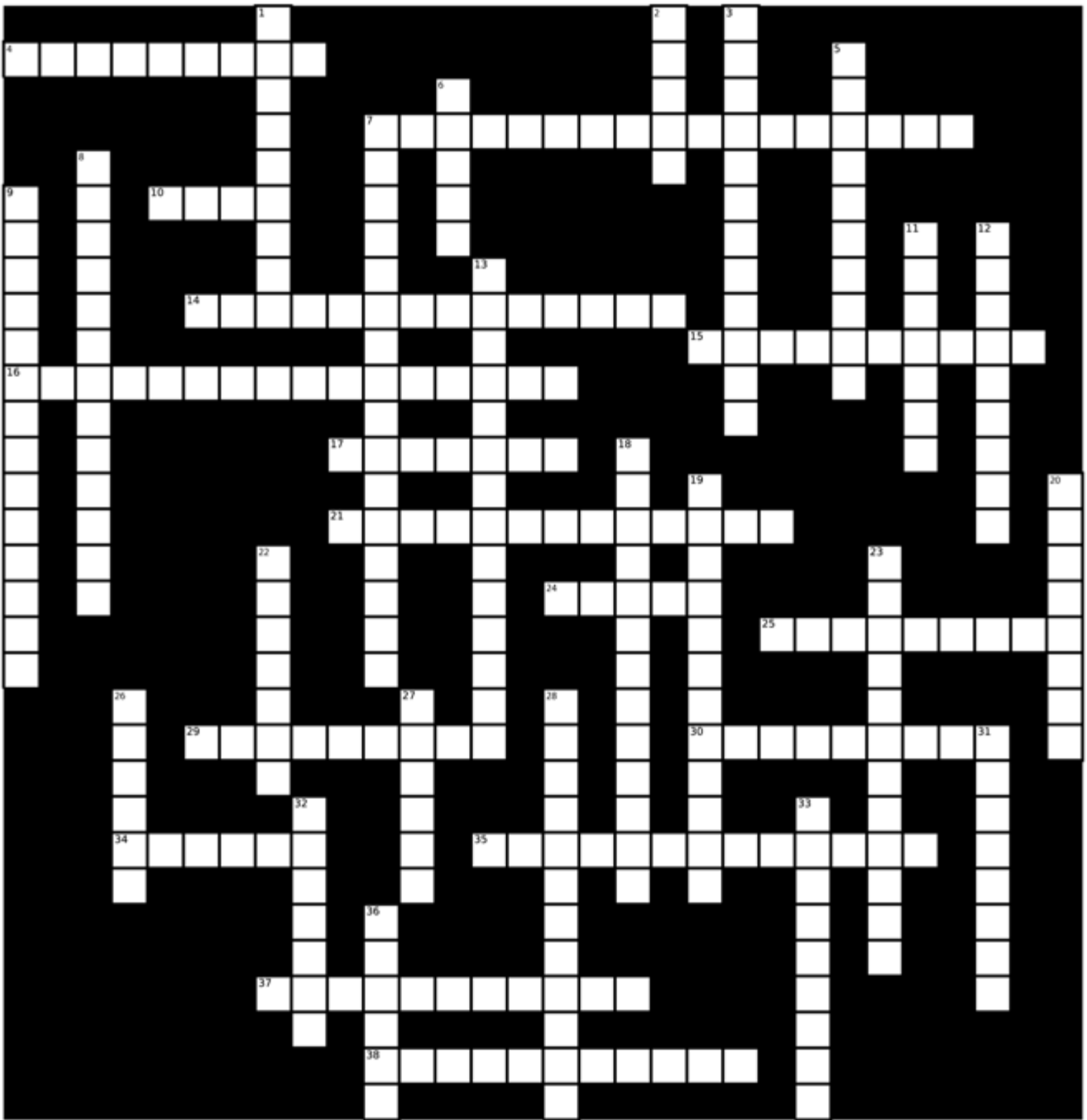
## Across

- A group of long chain hydrocarbons derived from the breakdown of fats with a single carboxylic group and aliphatic tail (2 words)
- The process of producing desirable characteristics in the next generation (2 words)
- The fundamental, physical, and functional unit of heredity
- A complex carbohydrate composed of a chain of monosaccharides joined together by glycosidic bonds
- Non-renewable fuels, such as coal, oil and gas (2 words)
- A new and growing science that focuses on re-designing and re-building natural biological systems synthetically from the ground up (2 words)
- The term for the dry weight of a living thing that can be used as a fuel
- A gas produced by the burning of fuels and used in photosynthesis (2 words)
- Name for the fungus that is single-celled most of its life, used in the production of alcohol and capable of fermenting carbohydrates
- The material from which plant cell walls are made
- A measurement of the 'thickness' of a fluid
- Without oxygen
- A group of proteins that speed up reactions in living things
- Traps heat in the atmosphere (2 words)
- A hydrocarbon that contains double or triple bonds
- The green chemical in plants that absorbs light energy

## Down

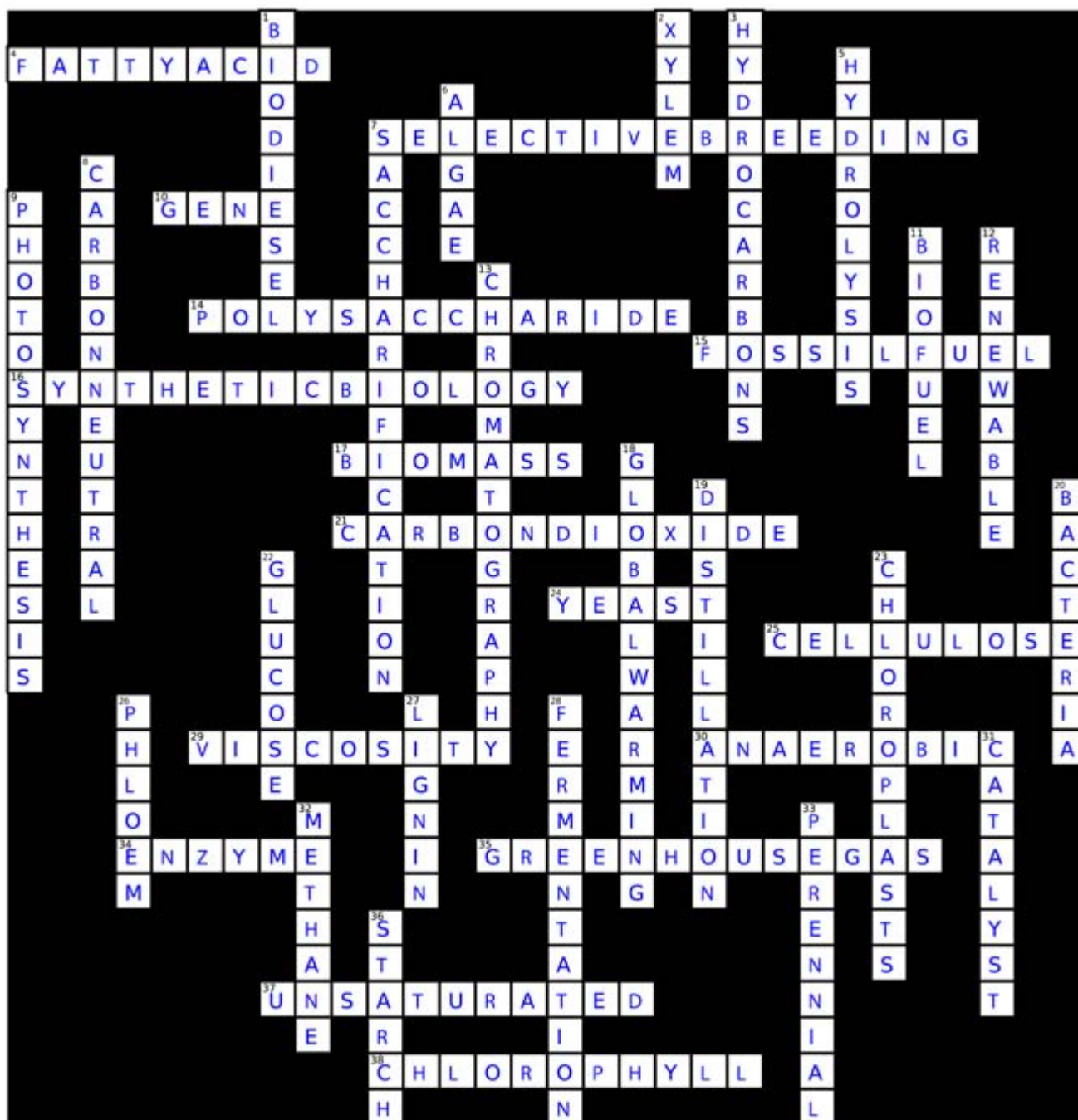
- A transport fuel derived from oil or fat
- A type of vascular tissue in terrestrial plants primarily involved transporting water and nutrient and providing structural support
- Chemicals that are made only from hydrogen and carbon
- A chemical reaction where a compound, such as starch or cellulose, is broken down by reaction with water into smaller components
- Phototrophic eukaryotic microorganisms
- The process of converting complex carbohydrate into simple monosaccharide components through hydrolysis
- Applies to a process which occurs without any change in the total amount of carbon dioxide present in the atmosphere (2 words)
- A process carried out in green plants that uses light
- A renewable fuel produced from biological material
- A resource that can be replaced more quickly or at the same rate as it is being used
- A method of separating substances where they move at different speeds
- The steady increase in the temperature of the Earth's atmosphere (2 words)
- A process in which liquids are purified or separated by heating to form a gas and then condensed back into a liquid
- Microscopic, single-celled organisms that possess a prokaryotic type of cell structure
- The specific sugar made by photosynthesis
- The tiny structures inside plant cells where photosynthesis occurs
- A tissue in a vascular plant that functions primarily in transporting organic food materials from the photosynthetic organ to all the parts of the plant
- Organic substance which act as a binder for the cellulose fibres in wood and certain plants and adds strength and stiffness to the cell walls
- An anaerobic cellular process in which organic foods are converted into simpler compounds such as alcohol
- A substance that increases the rate of a reaction
- A gas that is found with crude oil and produced in decomposition
- Lasting through the year or for several years
- An insoluble carbohydrate found in plants and plant products

# Advanced Crossword



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# Advanced Crossword Answers



# Dry Activities

## Activity 4D – Bioenergy wordsearches

Wordsearches are provided for use at a range of educational levels Primary, Key Stage 3, Key Stage 4 and Post-16. The wordsearches cover vocabulary and concepts associated with bioenergy research that students are expected to be familiar with at the educational level specified. There will also be a few words that are included that may be specific to bioenergy research and not covered in the curriculum designed to stretch students and enrich their understanding of the topic. It would be advisable to introduce students to the words and their meanings in the course of the learning session.

Wordsearches support the literacy component of science and will help with spelling, reading, writing, scientific communication, understanding, revision of the terminology and definitions.

### The wordsearches can be used in many ways:

- introduction to the topic to elicit prior knowledge
- recapping a previous session
- to build up word lists for wall display
- a fun end of session exercise
- as a takeaway activity

Crosswords and wordsearches provide many levels of differentiation. They can be completed individually or as a group, with or without support sheets listing the words.

They can be used to differentiate by task allowing the student to select a crossword or wordsearch. If the wordsearch is completed with ease then the student can be encouraged to attempt the crossword. Students will have recently found the same words in the wordsearch and so feel more confident in attempting the crossword.

A solution for each crossword and wordsearch is provided which allows students to check their own work and thereby allow you to spend more time engaging with other students.

Word exercises such as sentence loops allow students to expand their vocabulary specific to the subject, and when completed within a group the activities promote discussion and therefore promote their oracy skills.

## Introductory Wordsearch



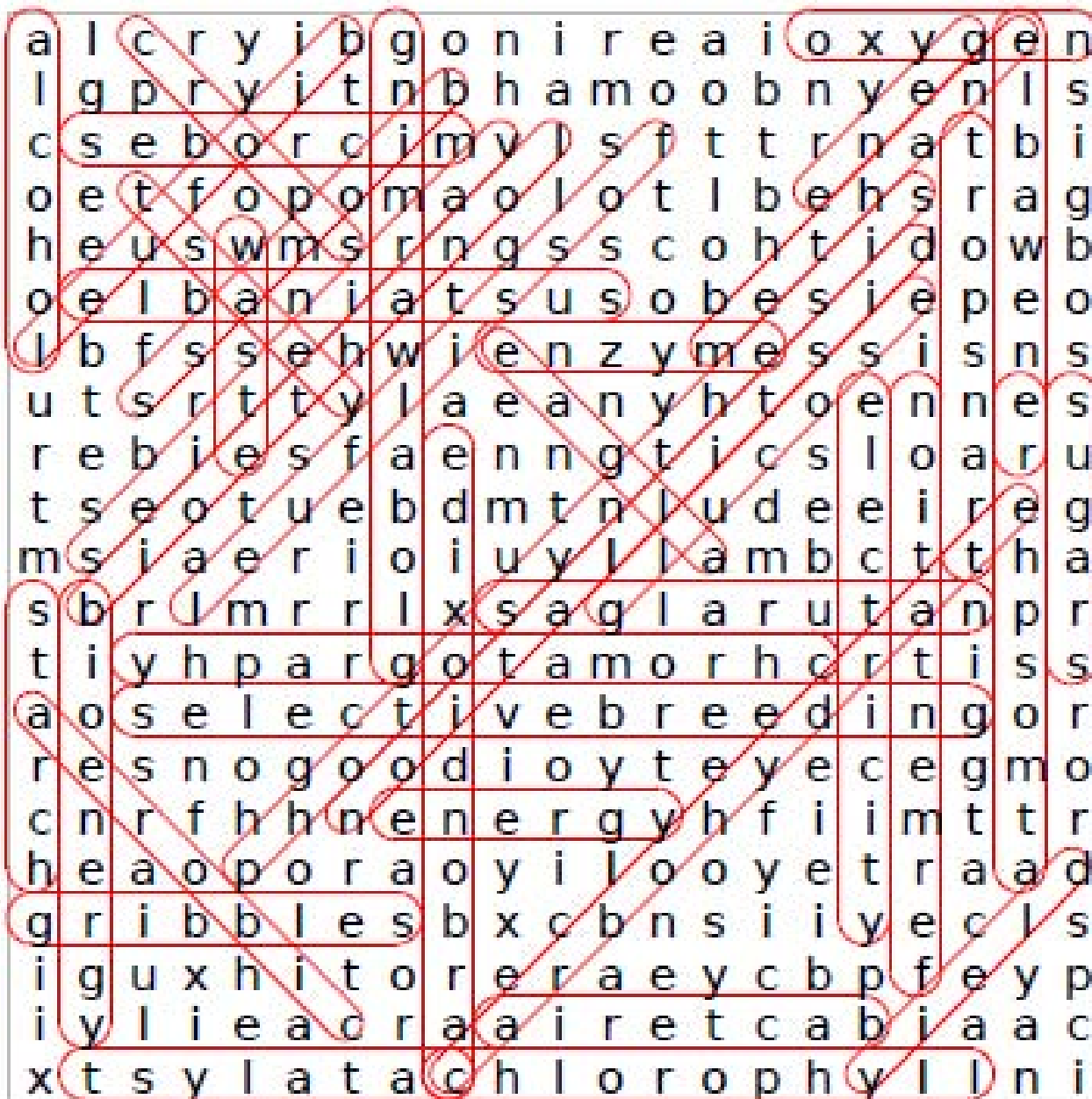
bioenergy  
 biofuel  
 fossil fuel  
 bioethanol  
 alcohol  
 carbohydrate  
 energy  
 carbon dioxide  
 oxygen  
 natural gas  
 methane  
 sustainable  
 renewable  
 transport

biomass  
 yield  
 waste  
 microbes  
 yeast  
 fermentation  
 catalyst  
 enzyme  
 gribbles  
 photosynthesis  
 algae  
 varieties  
 electricity

crops  
 starch  
 sugars  
 chromatography  
 distillation  
 atmosphere  
 aerobic  
 glucose  
 bacteria  
 gene  
 chlorophyll  
 selective breeding  
 global warming



## Introductory Wordsearch Answers



# Intermediate Wordsearch



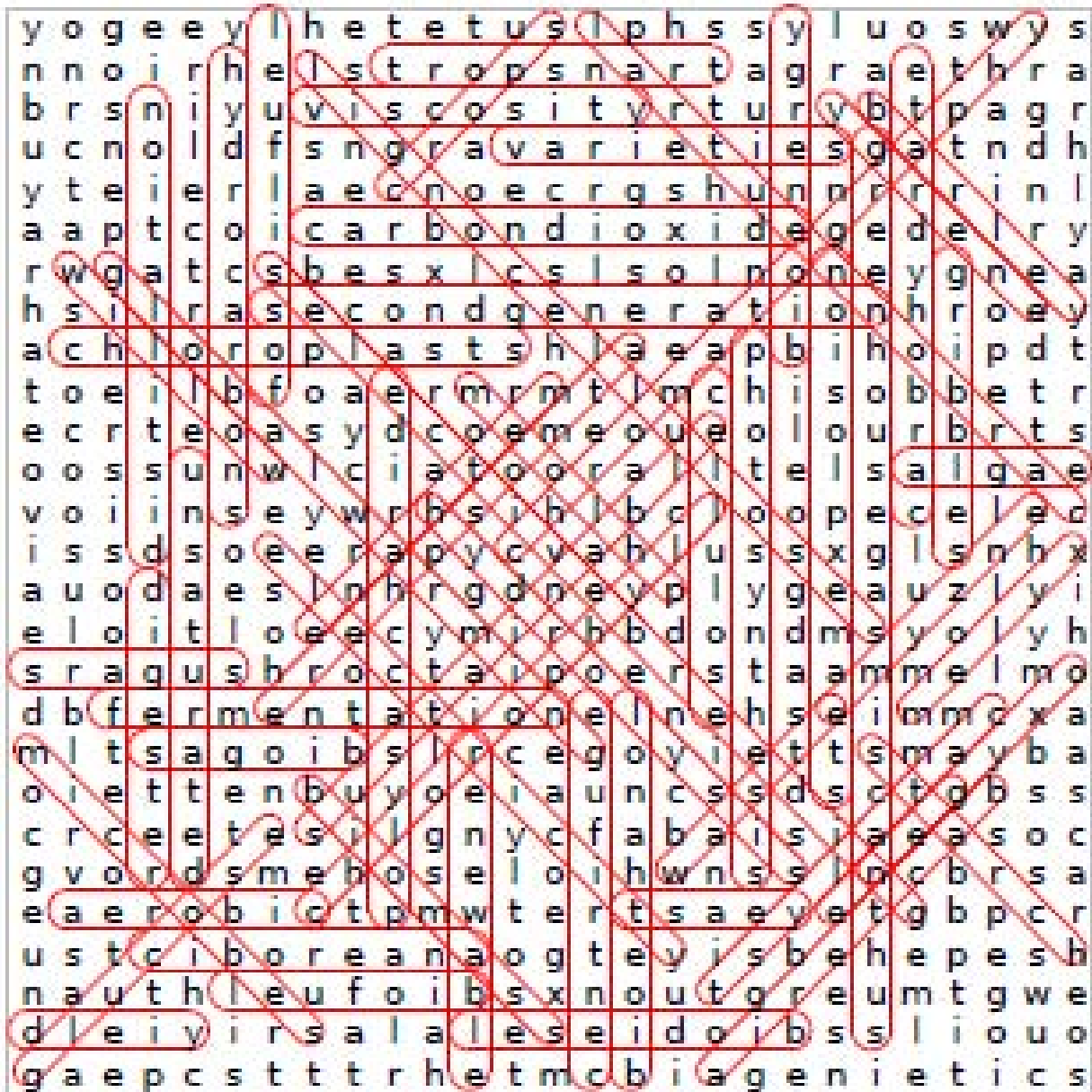
bioenergy  
biodiesel  
oxygen  
sustainable  
waste  
anaerobic  
second generation  
chloroplasts  
transport  
hydrolysis  
viscosity  
atmosphere  
gene  
phloem  
greenhouse gas

biofuel  
carbohydrate  
biogas  
renewable  
microbes  
catalyst  
lignocelluloses  
algae  
crops  
polysaccharide  
chromatography  
aerobic  
chlorophyll  
selective breeding  
miscanthus

fossil fuel  
energy  
methane  
biomass  
yeast  
enzyme  
steam explosion  
varieties  
starch  
hydrocarbons  
distillation  
glucose  
cellulose  
carbon neutral  
willow

bioethanol  
carbon dioxide  
digester  
yield  
fermentation  
gribbles  
photosynthesis  
electricity  
sugars  
unsaturated  
centrifuge  
bacteria  
xylem  
global warming  
barley

# Intermediate Wordsearch Answers



# Advanced Wordsearch



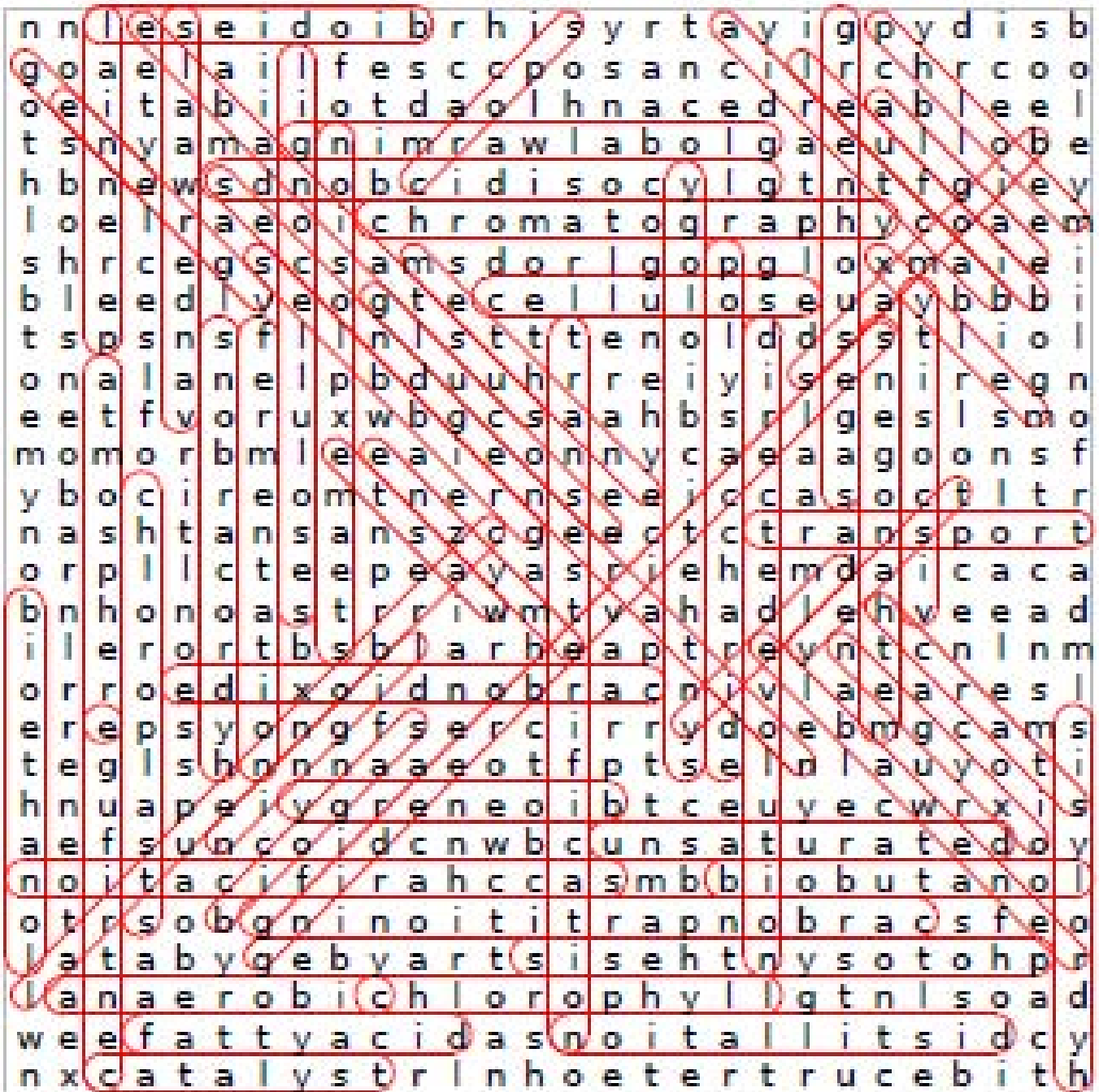
bioenergy  
 biobutanol  
 biogas  
 biomass  
 fermentation  
 glycosidic bonds  
 lignin  
 chloroplasts  
 perennial  
 hydrocarbons  
 chromatography  
 transesterification  
 methanogens  
 xylem  
 global warming  
 synthetic biology

biofuel  
 energy  
 methane  
 yield  
 anaerobic  
 enzyme  
 lignocelluloses  
 algae  
 starch  
 unsaturated  
 distillation  
 atmosphere  
 gene  
 phloem  
 greenhouse gas

bioethanol  
 carbon dioxide  
 sustainable  
 waste  
 catalyst  
 gribbles  
 steam explosion  
 transport  
 hydrolysis  
 viscosity  
 centrifuge  
 glucose  
 chlorophyll  
 selective breeding  
 carbon partitioning

biodiesel  
 oxygen  
 renewable  
 yeast  
 saccharification  
 second generation  
 photosynthesis  
 crops  
 polysaccharide  
 Van der waals  
 fatty acid  
 bacteria  
 cellulose  
 carbon neutral  
 directed evolution

# Advanced Wordsearch Answers



# Dry Activities

## Activity 4E - Bioenergy sentence loops

Sentence loops should be printed out, cut into separate strips and laminated. The words and definitions are on separate strips and students match up the correct definition and words arranging them in a loop in order to complete the exercise correctly.

# Bioenergy sentence loops

## Biodiesel

A green house gas that is used by plants in photosynthesis to create biomass.



## Carbon dioxide

A resource that can be renewed more quickly or at the same rate as it is being used or is unlikely to run out due to inexhaustible supplies.



## Renewable resource

A process carried out in green plants that uses light energy captured by chlorophyll to convert carbon dioxide and water to carbohydrates and oxygen.



## Photosynthesis

Biofuel consisting of ethanol produced by the fermentation of plant material rich in sugar or lignocellulose.



## Bioethanol

The use of resources to meet the need of present generations without compromising the need of future generations by balancing environmental, social and economic factors.



## Sustainability

A cellular process occurring without oxygen, in which organic foods such as sugars are converted into simpler compounds such as bioethanol, and chemical energy (ATP) is produced.



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# Bioenergy sentence loops

**Fermentation**

Without oxygen



**Anaerobic**

A group of proteins that speed up reactions in living things.



**Enzyme**

The green chemical in plants that absorbs light energy for photosynthesis.



**Chlorophyll**

Non-renewable fuels, such as coal, oil and gas, formed over millions of years from the decomposition, in anaerobic conditions, of plant and animal remains.



**Fossil Fuels**

Renewable fuel comprised of approximately 60% methane, produced by anaerobic digestion of organic material by microorganisms. Can be used as a transport fuel or for generating heat and electricity.



**Biogas**

An organic compound derived by processing and transesterification of plant oil or animal fats that can be used as a transport fuel in replacement of diesel derived from fossil fuel.



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# Glossary

**Adapted from Royal Society *Sustainable biofuels* publication, Nuffield bioethics publication and using [www.biology-online.org](http://www.biology-online.org) dictionary**

This glossary is designed to provide some simple definitions that will enable students to understand and explain the terminology used by BBSRC to explain bioenergy research. Some terms have been simplified and others will be too advanced for younger students. A student word list for Key Stage 2 and 3 students is also provided.

**Advanced plant breeding strategy** - a type of plant breeding strategy in which the genetic basis of a trait is screened for in the progeny of a cross using a lab-based test. This saves time and labour compared with conventional plant breeding. There are two types of advanced plant breeding strategy: *marker-assisted breeding* and *genomics-assisted breeding*.

**Aerobic** - With oxygen.

**Alcohol** - An organic chemical containing one or more hydroxyl groups.

**Algae** - Phototrophic eukaryotic microorganisms.

**Anaerobic** - Without oxygen.

**Arable land** - land that is suitable for crop production.

**Asexual reproduction** - Reproduction involving only one parent, producing offspring that are genetically identical to each other and to the parent.

**Atmosphere** - A layer of gases that surrounds the Earth.

**Bacteria** - Microscopic, single-celled organisms belonging to Kingdom Monera that possess a prokaryotic type of cell structure.

**Barley** - A valuable grain, of the family of grasses, genus *Hordeum*, used for food, and for making malt, from which are prepared beer, ale, and whisky.

**BBSRC** - Biotechnology and Biological Sciences Research Council.

**Biobutanol** - Butanol produced by some strains of bacteria, such as *Clostridium acetobutylicum*.

**Bioenergy** - Energy including, heat, electricity and liquid fuels, derived from non-food feedstocks or from inedible elements and waste from food crops.

**Biodiesel** - An organic compound derived by processing and transesterification of plant oil or animal fats that can be used as a transport fuel in replacement of diesel derived from fossil fuel.

**Biodiversity** - Shorthand for biological diversity. This is the variability among living organisms from all ecosystems and the ecological complexes of which they are part. It includes diversity within species, between species and of ecosystems.

**Bioethanol** - Biofuel consisting of ethanol produced by the fermentation of plant material rich in sugar or lignocellulose.

**Biofuel** - A renewable fuel produced from biological material such as recently dead plants, animals or their waste.

**Biogas** - Renewable gaseous fuel comprised of methane (approximately 60%) and carbon dioxide, produced by anaerobic digestion of organic material by microorganisms. Can be used as a transport fuel or, as a replacement for natural gas.

**Biomass** - Any biological material that can be used either directly as a fuel, converted to a fuel or used in industrial or fibre production.

**Biomass** - The term for the dry weight of a living thing.

**Bio-oil** - A carbon-rich liquid produced by pyrolysis of plant material, which can be used to produce chemicals and fuels.

# Glossary

**Bioprospecting** - The search for useful organic compounds or organisms in the environment.

**Carbohydrate** - An essential food group found in our diet (includes sugar, starch and fibre).

**Carbon cycle** - How carbon is cycled between living organisms and the air.

**Carbon dioxide** (CO<sub>2</sub>) - A gas produced by cell respiration and the burning of fuels. Used by plants for photosynthesis.

**Carbon neutral** - applies to a process which occurs without any change in the total amount of carbon dioxide present in the atmosphere.

**Catalyst** - A substance, including enzymes, that increases the rate of a chemical reaction but is not consumed during the process.

**Cell** - The basic unit that living things are made of.

**Cellulose** - Major material from which the plant cell walls are made.

**Centrifuge** - A piece of equipment used to separate substances according to their density by rotation.

**Chemical energy** - Energy that is stored in chemical form, such as in coal, oil or food.

**Chemical reaction** - A chemical change in which new substances are formed but there is no change in the number of atoms of each element.

**Chlorophyll** - The green chemical in plants that absorbs light energy and converts it into chemical energy through photosynthesis.

**Chloroplasts** - The compartments inside plant cells that contain chlorophyll, where photosynthesis occurs.

**Chromatography** - A method of separating substances. The substances are separated as they move, in a solvent, through a material, e.g. paper. The substances often move at different speeds.

**Combustion** - An oxidation reaction in which energy is released.

**Complete combustion** - An oxidation reaction that takes place when oxygen gas is in excess.

**Compound** - A substance containing two or more elements chemically joined together.

**Crops** - A plant grown to be harvested for agricultural use.

**Crystalline** - A material that has some regular arrangement of particles.

**Decomposers** - Organisms that break down dead organisms (e.g. bacteria and fungi).

**Digester** - A large vessel used to carry out biological decomposition.

**Directed evolution** - A method used to alter the proteins or RNA produced by organisms through mutation and selection or screening of variants with desirable properties.

**Distillation** - A process in which a liquid is converted into vapour by heating and then condensed back into a liquid. It is used to purify and to separate a liquid mixture.

**Element** - A substance that cannot be broken down into anything simpler by chemical reactions. An element consists of one type of atom.

**Energy** - The ability to do work or produce change.

**Enzyme** - A protein that speeds up reactions in living things.

# Glossary

**Extremophile** - Microorganisms that live optimally at relatively extreme conditions e.g. of acidity, salinity, temperature or pressures. Enzymes isolated from these organisms are used in some industrial manufacturing processes.

**Fatty acid** - A group of long chain hydrocarbons derived from the breakdown of fats with a single carboxylic group and aliphatic tail.

**Fermentation** - An anaerobic (without oxygen) cellular process in which organic foods are converted into simpler compounds such as alcohol, and chemical energy (ATP) is produced.

**Fertilisers** - Substances added to soil to replace lost nutrients and help plant growth

**First generation biofuels** - refers commonly to biofuels that are made from the food parts of food crops, such as sugar cane and oil palm, including bioethanol fermented from sugars and broken-down starch, and biodiesel derived from plant oils. Biogas is also known as a first generation biofuel.

**Fossil fuels** - Non-renewable fuels, such as coal, oil and gas, formed over millions of years from the decomposition, in anaerobic conditions, of plant and animal remains.

**Fuel** - A substance that can undergo a chemical change to release energy, usually as heat, in a controlled way.

**Gasification** - A process that converts materials, such as coal, petroleum or biomass, into synthesis gas (or 'syngas'), which comprises mainly carbon monoxide and hydrogen.

**Genetic modification (GM)** - The technology entailing all processes of altering the genetic material of a cell to make it capable of performing the desired functions, such as producing novel substances

**Gene** - Part of a chromosome. One gene contains the 'instructions' for a particular characteristic such as flower colour. The fundamental, physical, and functional unit of heredity

**Global warming** - The steady increase in the temperature of the Earth's atmosphere.

**Glucose** - The specific sugar made by photosynthesis.

**Glycerol** - A compound with the molecular formula  $C_3H_5(OH)_3$  which is a by-product of the production of biodiesel via transesterification. Can be used in other industries, e.g. pharmaceuticals, cosmetics etc

**Glycosidic bonds** - A type of covalent bond that joins carbohydrate (sugar) molecules together in di- or polysaccharides

**Greenhouse gas** - Gas such as carbon dioxide that traps heat in the atmosphere

**Gribbles** - Marine wood borers

**Hazard** - A property of something that could cause harm to health or the environment.

**Hexose** - Monosaccharide containing six carbon atoms

**Hydrocarbon** - Chemicals that are made only from hydrogen and carbon. Fuels contain large amounts of this chemical group.

**Hydrolysis** - A chemical reaction where a compound, such as starch or cellulose, is broken down by reaction with water into smaller components. In the case of biofuels, this can use enzymes or acid

**Incomplete combustion** - An oxidation reaction that takes place when oxygen gas is in a limited supply.

**Iodine solution** - This solution is used to indicate the presence of starch in a leaf - it turns blue-black in contact with starch.

# Glossary

**Life Cycle** - The sequence of events that happen to a material from obtaining the raw materials for its manufacture to its disposal as waste

**Life Cycle Assessment** - An examination of every stage in the manufacture and use of a material for a particular purpose, comparing its economic and environmental costs with other potential materials.

**Lignin** - Organic substance which act as a binder for the cellulose fibres in wood and certain plants and adds strength and stiffness to the cell walls.

**Lignocellulose** - Plant cell walls are composed of lignin and cellulose, which provide mechanical strength. Can be broken down to lignin and cellulose or used directly as a feedstock.

**Maize** - A cereal crop commonly known as corn that is grown predominantly in the USA, Canada and Australia.

**Methane** - A gas that is found with crude oil and produced in decomposition. At home we use cookers and boilers to react it with oxygen to provide heat.

**Methanogens** - Methane producing microorganisms.

**Microbe** - A very small living thing that can only be seen with a microscope. Some are harmful and some are useful.

**Miscanthus** - A fast-growing tall grass species that is grown as an energy crop.

**Molecule** - A particle made up of two or more atoms joined together.

**Natural Gas** - Found in association with hydrocarbon fuels, primarily coal, and consisting mainly of methane.

**Non-renewable resource** - A resource that cannot be renewed at the same rate as it is being used and will eventually run out.

**Organic compound** - A compound that contains carbon-carbon bonds.

**Pascal** - The unit for measuring pressure. It equals one Newton per m<sup>2</sup>.

**Pentose** - Any monosaccharide sugar containing five atoms of carbon per molecule.

**Perennial** - Lasting through the year or for several years.

**Phloem** - A tissue in a vascular plant that functions primarily in transporting organic food materials (e.g. sucrose) from the photosynthetic organ (leaf) to all the parts of the plant.

**pH** - A measure of the acidity of a solution; the lower the pH number the stronger the acid.

**Photosynthesis** - A process carried out in green plants that uses light energy captured by chlorophyll to convert carbon dioxide and water to carbohydrates and oxygen.

**Pollutant** - A substance present in the environment as a result of human activity that can harm the environment or health.

**Polysaccharide** - A complex carbohydrate composed of a chain of monosaccharides joined together by glycosidic bonds.

**Product** - The substances formed during a chemical reaction.

**Reactant** - The substance present at the start of a reaction A chemical that undergoes a chemical change in a chemical reaction.

**Renewable resource** - A resource that can be renewed more quickly or at the same rate as it is being used or is unlikely to run out due to inexhaustible supplies.

**Risk** - An estimate of how dangerous a hazard is in a particular situation.

# Glossary

**Saccharification** - The process of converting complex carbohydrate (e.g. starch) into simple monosaccharide components (e.g. glucose) through hydrolysis.

**Saturated compound** - A compound with only single bonds between its atoms.

**Second generation** – Bioenergy solutions that either make use of waste or rely on non-food crops that can be grown on marginal land.

**Selective breeding** - The process of allowing certain animals or plants to breed so as desirable characteristics are found in the next generation.

**Species** - (In evolution) a group of organisms with the same characteristics, (living) a group of organisms with the same characteristics that can breed with each other.

**Starch** - An insoluble carbohydrate found in plants and plant products. The storage molecule for the surplus glucose made by photosynthesis.

**Straw** - The stalks of harvested cereal crops such as wheat and barley.

**Substrate** - The substance acted upon by an enzyme.

**Sugar** - A carbohydrate that is a source of energy in respiring cells. Glucose belongs to this food group.

**Sustainable development** – A programme of developing new energy technology that does not harm the environment or use up non-renewable resources.

**Sustainability** - The use of resources to meet the need of present generations without compromising the need of future generations by balancing environmental, social and economic factors.

**Synthetic biofuels** - Fuels produced via thermochemical conversion of biological material, such as biodiesel, which have exactly the same properties as fuels derived from fossil fuels. These are defined differently to synthetic fuels, because synthetic fuels can also be made from coal, gas and oil.

**Synthetic biology** - A new and growing science that focuses on re-designing and re-building natural biological systems synthetically from the ground up.

**Transesterification** - A reaction that is catalysed by an acid or a base, where the alkoxy group of an ester compound is replaced by another alcohol. This process can be used to produce biodiesel.

**Unsaturated compound** - A hydrocarbon that contains double or triple bonds.

**van der Waals** - Electrodynamic forces arising between atoms or molecules.

**Variety** - A subgroup of a species which has a slightly different set of characteristics.

**Viscosity** - A measurement of the 'thickness' of a fluid.

**Water** - Combined with CO<sub>2</sub> in photosynthesis to produce glucose.

**Willow** - Any tree or shrub of the genus *Salix*, including many species.

**Xylem** - A type of vascular tissue in terrestrial plants primarily involved in transporting water and nutrient (from the roots to the shoot and leaves) and providing structural support.

**Yeast** - Colloquial name for the fungus that is characteristically single-celled most of its life, eukaryotic, reproduce asexually by budding or binary fission, capable of fermenting carbohydrates. used in the production of ethanol.

**Yield** - A measure of the amount of crop produced.

# Keywords in school science

To help you pitch the language used to communicate your research to students a list of keywords encountered in Key Stage 2 and 3 Science that may relate to bioenergy topics are provided below. Keywords are words that help students to communicate ideas in science clearly and with understanding. The key words in the following list are from the *Framework for teaching science: Years 7, 8 and 9 (2002, crown copyright)* and originally appeared in the 'Language for learning' sections of units in the Science Key Stage 3 schemes of work developed by the Qualification and Curriculum Authority.

## Year 6

### Sc1 Scientific enquiry

accurate, average, bar line graph, bar chart, collect, compare, conclusion, data, graph, explain, evaluate, evidence, fair test, idea, identify, interpret, limitation, line graph, observation, measurement, pattern, predict, present, record, repeat measurements, repeat observations, results, secondary data, test

### Sc2 Life processes and living things

alcohol, carbohydrate, dissolve, energy, fat, fertiliser, fibre, food chain, germ, germination/germinate, growth, health, life cycle, petal, plant food, microbe, nutrients, ovary, oxygen, pollen, pollination/pollinate, producer, reproduction/reproduce, starch, sepal, stamen, stigma, style,

### Sc3 Materials and their properties

air, ash, baking powder, bath salts, bicarbonate of soda, boiling temperature, bubbles, carbon dioxide, change, change of state, charcoal, condense, conditions, dissolve, evaporation/evaporate, filter, freeze, gas, hazard, heat, insoluble, irreversible, liquid, melt, mixture, natural gas, oxygen, reversible, solid/solidify, soluble, solution, state, steam, water cycle

### Sc4 Physical processes

air, gravity, insulator, light, light beam, newton, opaque, reflection/reflect, revolve, rotation/rotate, sound, sphere/spherical, spin, stationary, tension, water, weight

## Year 7

### Sc1 Scientific enquiry

correlation, data logger, generalisation, line of best fit, prediction, reliability, repeat reading, sample size, strength of evidence, theory

### Sc2 Life processes and living things

cell, hereditary, inherited, nucleus, tissues, consumer, dormant, food web, habitat, insulation, interdependence, light intensity, organisms, producer, association, characteristics, classify, feature, multi-cellular, species, taxonomic group, variation

### Sc3 Materials and their properties

acid, alkali, colour change, corrosive, equation, hazard, hydrochloric acid, hydroxides, indicator, litmus, neutral, pH range, reaction, risk, carbon, carbonates, combustion reactions, element, hydrogen, line graph, methane, oxide, oxygen, product, reactant, word equation, zinc

compressible, diffusion, expansion, gas pressure, particle, particle theory, proximity, attracted, chromatography, chromatogram, compound, distillation, filtration, insoluble, saturated solution, separate, solute, solution, solvent, suspension, trace



# Keywords in school science

## Sc4 Physical processes

conservation, density, energy transfer, friction, fuel, lubricants, magnitude, mass, atmosphere,

## Year 8

### Sc1 Scientific enquiry

anomalous results, data search, environmental conditions, evaluate, hypothesis, opinion, population size, precision, quadrat sampling, qualitative, quantitative, range, reliable data, repeats, sample size, sequence of events, sufficient data, transect, trial, measurements, using secondary sources, variable

### Sc2 Life processes and living things

absorption, digestion, enzyme, minerals, molecules, starch, sugars, aerobic, glucose, bacteria, fungi, microorganisms, sterilising, community, distribution, ecosystem, habitat, humidity, population sizes, pyramid of numbers, transect

### Sc3 Materials and their properties

atom, chlorides, chlorine, compound, element, equation, formula, molecule, reactants, sodium, symbol, composition, oxides, cooling rates, crystals, deposit, mineral, precipitation,

### Sc4 Physical processes

conduction, convection, insulator, joule, radiation, radiation, spectrum, translucent, transmission, transparent, frequency, wave

## Year 9

### Sc1 Scientific enquiry

carrying out a survey, control accuracy, controlling variables, dependent variable, developing a technique, independent variable, most appropriate equipment, precision, proportional, quantitative data, reliability/trustworthiness of data, sampling, scientific method, trial run, validity of conclusions

### Sc2 Life processes and living things

asexual, breed, classification, clone, characteristics, gamete, gene, genetically modified, grafting, selective breeding, species, variety, biomass, chlorophyll, Elodea, etiolation, palisade cell, photosynthesis, xylem, balance, compete, competition, deficiency, fungicide, insecticide, nitrates, nutrient, pesticide, sustainable development, toxin, weedkiller, yield

### Sc3 Materials and their properties

carbonates, product, salt, sulfates/sulphates, displacement, order of reactivity, acid rain, catalyst, global warming, neutralisation, vegetation cover, prefixes: di-, mono-, poly-; suffixes: -ate, -ide, -ite

### Sc4 Physical processes

dissipation, electric generator, kinetic energy, potential energy,

## Further Reading

The Royal Society, January 2008. *Sustainable biofuels: prospects and challenges*, Policy document 01/08, ISBN 978 0 85403 662 2. <http://royalsociety.org/Sustainable-biofuels-prospects-and-challenges/>

Nuffield Council on Bioethics, April 2011, *Biofuels: ethical issues* ISBN: 978-1-904384-22-9 [www.nuffieldbioethics.org/biofuels-0](http://www.nuffieldbioethics.org/biofuels-0)

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>

Society for General Microbiology (SGM), 2010, *Microbiology – A resource for Key Stage 5* ISBN: 0 95368 384 2 [www.microbiologyonline.org.uk/teachers/resources](http://www.microbiologyonline.org.uk/teachers/resources)

King, T. And Reiss, M. with Roberts, M., 2001, *Practical Advanced Biology*. Cheltenham, Nelson Thornes Ltd

The Association for Science Education and University of Cambridge International Examinations, 2006. *International Practical Science Guide: advice and activities for primary and secondary science in the classroom* ISBN: 0 86357 410 6 (ISBN: 978 0 86357 411 5) [www.cie.org.uk/docs/profiles/teachers/International%20Practical%20Science%20Guide%20final.pdf](http://www.cie.org.uk/docs/profiles/teachers/International%20Practical%20Science%20Guide%20final.pdf)

Renewable Fuels Agency, 2008, *The Gallagher Review of the indirect effects of biofuels production*, [www.renewablefuelsagency.gov.uk/sites/renewablefuelsagency.gov.uk/files/\\_documents/Report\\_of\\_the\\_Gallagher\\_review.pdf](http://www.renewablefuelsagency.gov.uk/sites/renewablefuelsagency.gov.uk/files/_documents/Report_of_the_Gallagher_review.pdf).

Growing the bioenergy field. BBSRC business spring 2011 [www.bbsrc.ac.uk/news/industrial-biotechnology/2011/110314-f-growing-the-bioenergy-field.aspx](http://www.bbsrc.ac.uk/news/industrial-biotechnology/2011/110314-f-growing-the-bioenergy-field.aspx)–

## Weblinks

[www.bbsrc.ac.uk/](http://www.bbsrc.ac.uk/) Biotechnology and Biological Sciences Research Council

[www.bsbec.bbsrc.ac.uk/](http://www.bsbec.bbsrc.ac.uk/) BBSRC Sustainable Bioenergy Centre

[www.cleapss.org.uk/](http://www.cleapss.org.uk/) Provides a health and safety school advisory service and teaching resources.

[www.saps.org.uk/](http://www.saps.org.uk/) Science and Plants for Schools (SAPS) - Teaching resources for photosynthesis and biofuels

[www.ncbe.reading.ac.uk/menu.html](http://www.ncbe.reading.ac.uk/menu.html) National Centre for Biotechnology Education (NCBE) – Teaching materials and resources for microbiology, photosynthesis and biofuels.

[www.practicalbiology.org/](http://www.practicalbiology.org/) Practical activities and teaching resources from the Society of Biology, Nuffield Foundation and CLEAPSS.

[www.practicalbiology.org/](http://www.practicalbiology.org/) Practical activities and teaching resources from the Society of Biology, Nuffield Foundation and CLEAPSS.

[www.microbiologyonline.org.uk/home](http://www.microbiologyonline.org.uk/home) Microbiology teaching resources and student activities from the Society for General Microbiology (SGM)

[www.bio-rad.com](http://www.bio-rad.com) Bio-Rad Laboratories – Biofuel enzyme kit for investigating the activity of cellobiase.

[www.plants4products.org.uk](http://www.plants4products.org.uk) Renewables don't run out teaching resource for 9-12 year olds from Chemical Industry Education Centre at the University of York.

[www.hgca.com/content/template/9/0/Education/Education/Education.msp](http://www.hgca.com/content/template/9/0/Education/Education/Education.msp) Posters and industrial uses for crops teaching resource for Key Stage 2 from the Home-Grown Cereals Authority.

[www.teachrenewables.co.uk/](http://www.teachrenewables.co.uk/) Teaching resources and links covering biorenewable fuels, plants and technologies from the National Non-Food Crops Centre (NNFCC)

<http://practicalaction.org/renewable-energy-5> Renewable energy teaching resources for Key Stages 2-4.

[www.nef.org.uk/greenschool/index.htm](http://www.nef.org.uk/greenschool/index.htm) National Energy Foundation

[www.decc.gov.uk/en/content/cms/meeting\\_energy/bio\\_energy/bio\\_energy.aspx](http://www.decc.gov.uk/en/content/cms/meeting_energy/bio_energy/bio_energy.aspx) Department of Energy and Climate Change

[www.defra.gov.uk/](http://www.defra.gov.uk/) The Department for Environment, Food and Rural Affairs (Defra)

[www.bis.gov.uk/](http://www.bis.gov.uk/) The Department for Business, Innovation and Skills (BIS)

[www.nnfcc.co.uk/](http://www.nnfcc.co.uk/) National Non-Food Crops centre. The UK's National Centre for Biorenewable Energy, Fuels and Materials

[www.biomassenergycentre.org.uk/](http://www.biomassenergycentre.org.uk/) The BIOMASS Energy Centre aims to be a one stop shop able to provide information, advice and guidance to UK individuals and organizations - signposting to other specialised sources of advice as necessary - on a wide range of biomass fuels and conversion technologies. The BIOMASS Energy Centre (BEC) is owned and managed by the UK Forestry Commission, via Forest Research, its research agency.

[www.dft.gov.uk/topics/sustainable/biofuels/](http://www.dft.gov.uk/topics/sustainable/biofuels/) Department for Transport. Provides links to statistics and legislation relating to biofuels as well as the Renewable Transport Fuels Obligation (RTFO).

[www.ukerc.ac.uk/](http://www.ukerc.ac.uk/) UK Energy Research Centre. The UKERC carries out research into sustainable future energy systems.

[www.environment-agency.gov.uk/](http://www.environment-agency.gov.uk/) Environment Agency. The Environment Agency is an Executive non-departmental public body (NDPB) that reports to the Secretary of State for Environment, Food and Rural Affairs. The main focus of their interest in Bioenergy is around the use of biofuels. Bioenergy is one of their designated business areas and it is split into four subsections; biogas, biodiesel, bioethanol and biomass. The Environment Agency has a position statement in relation to biofuels for transport.

[www.carbontrust.co.uk/](http://www.carbontrust.co.uk/) The Carbon Trust provide specialist support to business and the public sector to help cut carbon emissions, save energy and commercialise low carbon technologies.

# Curriculum Links

The Department for Education is conducting a review of the primary and secondary National Curriculum. The links in this document relate to the statutory programmes of study for science in the National Curriculum 2007 and specific sections of common science qualifications offered in UK schools. These qualifications include the GCSEs and iGCSE Certificates for teaching 2011, existing SQA Standard and Higher grades and revised A-levels in science subjects. Relevant links to broader topics such as global warming, fossil fuels and the carbon cycle are not routinely specified.

## Key Stage 1

### Sc1 Scientific Enquiry

#### Ideas and evidence in science

1 That it is important to collect evidence by making observations and measurements when trying to answer a question.

#### Investigative skills

Planning

b use first-hand experience and simple information sources to answer questions

Obtaining and presenting evidence

e follow simple instructions to control the risks to themselves and to others

f explore, using the senses of sight, hearing, smell, touch and taste as appropriate, and make and record observations and measurements

Considering evidence and evaluating

i compare what happened with what they expected would happen, and try to explain it, drawing on their knowledge and understanding

### Sc3 Materials and their properties

#### Grouping materials

##### Breadth of study

Health and safety

b recognise that there are hazards in living things, materials and physical processes, and assess risks and take action to reduce risks to themselves and others.

## Key Stage 2

### Sc1 Scientific Enquiry

#### Ideas and evidence in science

1 That it is important to test ideas using evidence from observation and measurement.

Investigative skills

Planning

b consider what sources of information, including first-hand experience and a range of other sources, they will use to answer questions

Obtaining and presenting evidence

e use simple equipment and materials appropriately and take action to control risks

##### Breadth of study

Health and safety

b recognise that there are hazards in living things, materials and physical processes, and assess risks and take action to reduce risks to themselves and others.

## Key Stage 3

### 1.1 Scientific thinking

### 1.2 Applications and implications of science

### 1.4 Collaboration

### 2.1 Practical and enquiry skills

### 3.1 Energy, electricity and forces

### 3.2 Chemical and material behaviour

### 3.3 Organisms, behaviour and health

### 3.4 The environment, Earth and universe

## Key Stage 4

### 1 How science works

#### 1.1 Data, evidence, theories and explanations

#### 1.2 Practical and enquiry skills

#### 1.3 Communication skills

#### 1.4 Applications and implications of science

#### 2.1 Organisms and health

#### 2.2 Chemical and material behaviour

#### 2.3 Energy, electricity and radiations

#### 2.4 Environment, Earth and universe

# Curriculum Links

## Summary of links to Key stage 4 and 5 qualifications

### Biogas generator

OCR Additional Science A and Biology A GCSE

Module B4: The processes of life B4.3 How do living organisms obtain energy?

AQA Biology GCSE

B3.4 Humans and their environment B3.4.3 Biofuels

OCR Gateway Science GCSE Biology B

Module B6: Beyond The Microscope Item B6d: Biofuels

### Oil extraction

AQA Chemistry GCSE and Science GCSE

Unit C1.4 Crude oil and Fuels C1.4.3 Hydrocarbon fuels

C1.6 Plant oils and their uses C1.6.1 Vegetable oils

OCR Chemistry and Additional Science B Gateway GCSE

Module C3: Chemical Economics Item C3g

SQA Higher Chemistry

SQA Intermediate Chemistry

AQA Biology A-level

Unit 1 BIOL1 Biology and disease 3.1.3

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health Module 1 Biological Molecules

### Oil viscosity

AQA Science B GCSE

SQA Intermediate Chemistry

OCR Chemistry A A-level

2.1.2 Alkanes Hydrocarbons from crude oil Hydrocarbons as fuels

How Science Works 6a, 7b:

4.1.3 Carboxylic Acids and Esters. Esters, triglycerides, unsaturated and saturated fats

How Science Works 7c:

OCR Chemistry B Salters A-level

Unit F331: Chemistry for Life Developing Fuels. Organic functional groups

AQA Biology B A-level

Unit 1 BIOL1 Biology and disease 3.1.3

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health Module 1 Biological Molecules

SQA Biology: Advanced Higher Course

Unit: Cell and Molecular Biology. Structure and function of cell components

SQA Chemistry Higher

Unit 2: The World of Carbon c) Reactions of carbon compounds

### Biodiesel production

AQA Chemistry GCSE and Science GCSE

Unit C1.4 Crude oil and Fuels C1.4.3 Hydrocarbon fuels

C1.6 Plant oils and their uses C1.6.1 Vegetable oils

OCR Physics and Science A 21st Century GCSE

Module P3: Sustainable energy, P3.1 How much energy do we use?

# Curriculum Links

Edexcel Chemistry A-level

Unit 4 General Principles of Chemistry I – Rates, Equilibria and Further 4.8 Further Organic Chemistry 4  
Carboxylic acid derivatives

AQA Biology A-level

Unit 1 BIOL1 Biology and disease 3.1.3

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health Module 1 Biological Molecules

## Extracting sugar from sugar beet

Edexcel Chemistry GCSE

Unit C3: Chemistry in Action Topic 5 Organic chemistry

AQA Chemistry GCSE and Science GCSE

Unit C1.4 Crude oil and Fuels C1.4.3 Hydrocarbon fuels

## Carbohydrate testing

Edexcel Chemistry GCSE

Unit C3: Chemistry in Action Topic 5 Organic chemistry

AQA Biology A-level

Unit 1 BIOL1 Biology and disease 3.1.3

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health Module 1 Biological Molecules

SQA Standard Grade Biology

SQA Access Chemistry

SQA Intermediate Chemistry

## Yeast fermentation

AQA Chemistry and Science A GCSE

C1.5 Other useful substances from crude oil

C1.5.3 Ethanol

OCR Biology Gateway GCSE

Item B6d: Biofuels

OCR Biology A 21st Century GCSE

Module B7: Further Biology

B7.5 New technologies

Edexcel Chemistry GCSE

Unit C3: Chemistry in Action Topic 5 Organic chemistry

Edexcel Biology GCSE

Unit B3: Using Biology Topic 3 Biotechnology

SQA Access and Intermediate Biology

Unit D024 09 Biotechnological Industries

SQA Access and Intermediate Chemistry

SQA Higher Biotechnology

Unit: D042 12 Microbiological Techniques

SQA Higher Chemistry

# Curriculum Links

## Plant Material Testing

SQA Standard Grade Biology

OCR Biology A-level

3.1 AS Unit F211: Cells, Exchange and Transport Module 1: Cells

WJEC Biology A-level

BY2 : Biodiversity and physiology of Body Systems. Adaptations for Transport

## Hydrolysis of biofuel feedstocks

OCR Biology A 21st Century GCSE

Module B7: Further Biology B7.5 New technologies

SQA Intermediate Chemistry

AQA Biology A-level

Unit 1 BIOL1 Biology and disease 3.1.3

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health Module 1 Biological Molecules

SQA Higher Chemistry

## Fermentation of lignocelluloses

OCR Biology A 21st Century GCSE

Module B7: Further Biology, B7.5 New technologies

AQA Biology GCSE

B3.4.3 Biofuels

OCR Biology and Additional Science B Gateway GCSE

Module B4: It's A Green World, Item B4b: Photosynthesis

SQA Access and Intermediate Biology

Unit D024 09 Biotechnological Industries

SQA Higher Biotechnology

Unit: D042 12 Microbiological Techniques

SQA Higher Chemistry

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health

## Bacterial cellulase

Gateway science suite GCSE Biology B

Module B6: Beyond The Microscope Item B6e: Life in soil

OCR Biology A-level

3.2 AS Unit F212: Molecules, Biodiversity, Food and Health

WJEC Biology and Human Biology A-level

Unit BY4: Metabolism, Microbiology and Homeostasis. 4.4 Microbiology

SQA Biotechnology Higher

Unit 1 Microbiology

Unit 2 Microbiological Techniques

Unit 3 Biotechnology

# Curriculum Links

## Cellulase enzyme activity

AQA Biology and Additional Science GCSE  
B2.3 Photosynthesis

AQA Biology A-level  
Unit 1 BIOL1 Biology and disease 3.1.3

OCR Biology A-level  
3.2 AS Unit F212: Molecules, Biodiversity, Food and Health

SQA Higher Chemistry

## Culturing Algae

AQA Biology and Science GCSE  
B1.5.1 Energy in biomass  
B1.6.2 The carbon cycle

AQA Biology, Science and Additional Science GCSE  
B2.3.1 Photosynthesis

AQA Science B  
3.3.2.3 The importance of carbon

OCR Biology A-level  
Module 3: Photosynthesis

## Algal photosynthesis

AQA Biology and Science GCSE  
B1.5.1 Energy in biomass  
B1.6.2 The carbon cycle

AQA Biology, Science and Additional Science GCSE  
B2.3.1 Photosynthesis

AQA Science B  
3.3.2.3 The importance of carbon

OCR Biology A-level  
Module 3: Photosynthesis

## Algae chromatography

OCR Additional Science B Gateway GCSE  
Module C3: Chemical Economics Item C3g: Batch or continuous?

AQA Biology GCSE  
B2.3 Photosynthesis

AQA Biology and Science GCSE  
B1.5.1 Energy in biomass  
B1.6.2 The carbon cycle

AQA Biology, Science and Additional Science GCSE  
B2.3.1 Photosynthesis

AQA Science B GCSE  
3.3.2.3 The importance of carbon



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