

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 (CO₂) 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.

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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.

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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 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
Yeast	✓	✓	✓	✓	✓	✓
Sugar	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.

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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% 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 (CO_2) using lime water. The gas can be poured into a test tube with lime water as 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. 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 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 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.

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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 *International Journal of Systematic and Evolutionary Microbiology*, **61**(12), 3072-3076.

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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, *Biotechnology Letters*, **33** (3), 477-487.

Yeast fermentation and distillation instructions from Practical Chemistry 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

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

Immobilised yeast- Immobilisation of yeast in calcium alginate beads, Dean Madden, 2007. 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

Research groups

National Collection of Yeast Cultures (NCYC) 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).