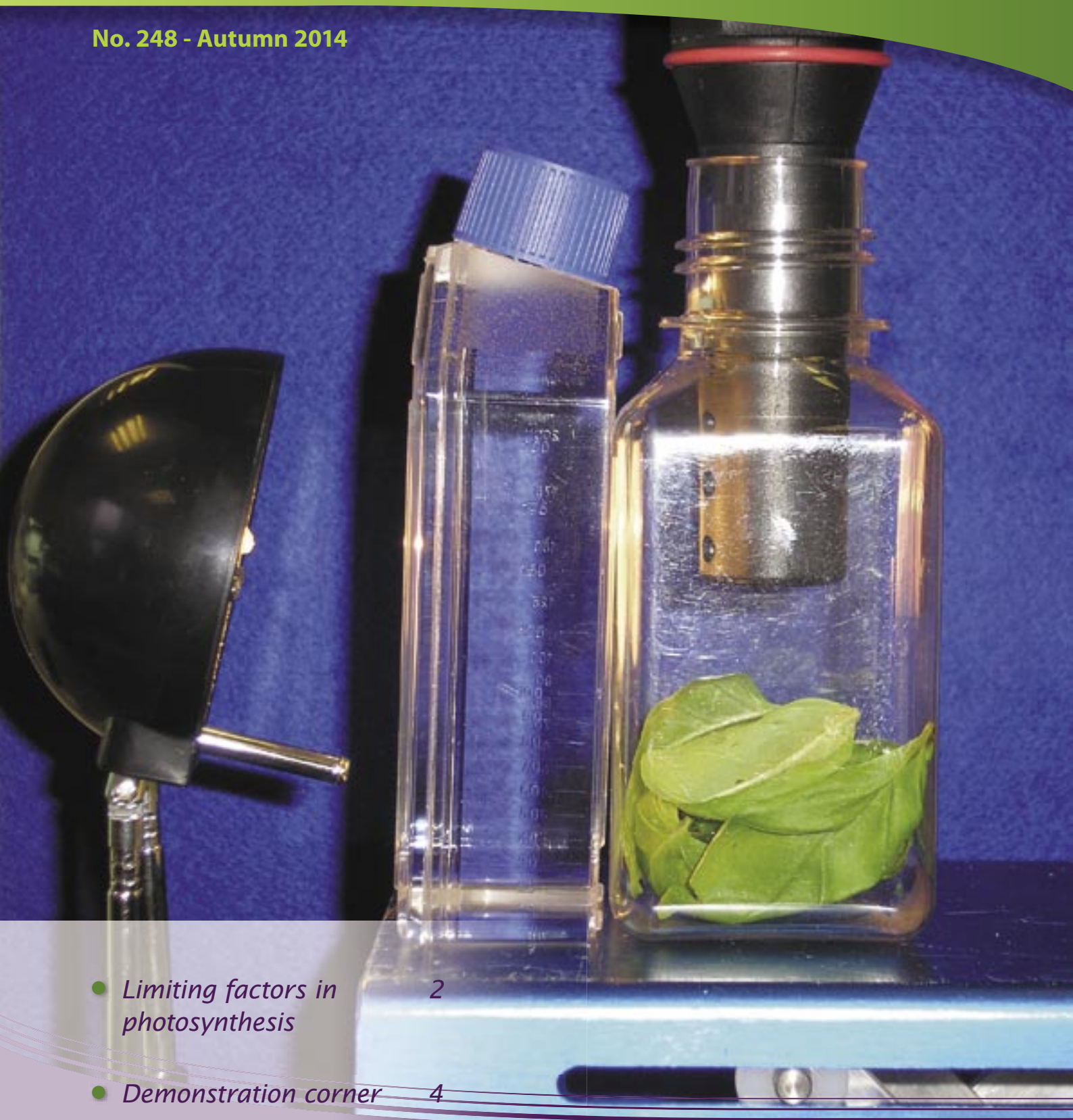
SSERC *Bulletin*

Ideas and inspiration supporting science and technology for all Local Authorities

No. 248 - Autumn 2014



 - ISSN 1759-1082

Limiting factors in photosynthesis – carbon dioxide

In a previous issue of this Bulletin [1] we described how the effect of light intensity on the rate of photosynthesis could be measured using a variety of different methods. We finished that previous article by suggesting that the use of carbon dioxide probes would, in principle, allow the measurement of the rate of photosynthesis with varying carbon dioxide concentrations present. In this article we present results from such experiments.

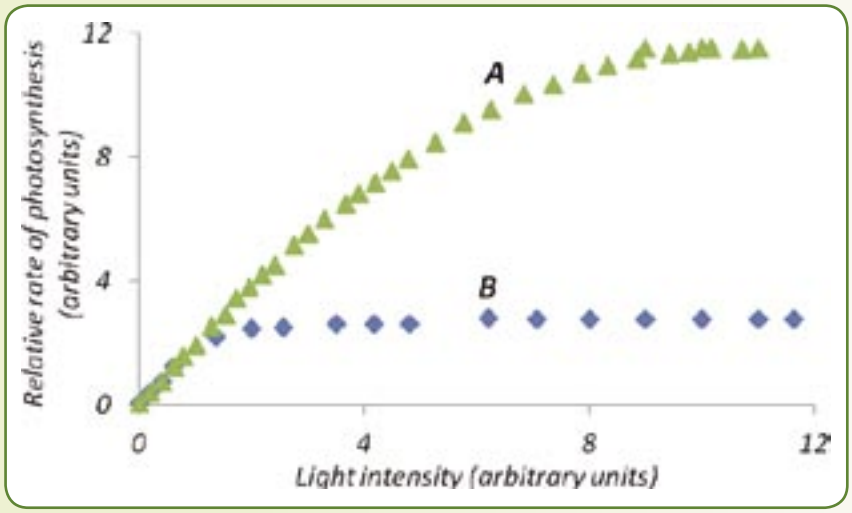


Figure 1 *- Effect of external factors on the rate of photosynthesis in Chlorella (plot adapted from [2]).*
A) Effect of light intensity at 25°C, 0.04% CO_2.
B) Effect of light intensity at 25°C, 0.01% CO_2.

A number of factors are known to affect photosynthetic rates in plants. Classically the variation of factors is as shown in Figure 1 [2].

The interpretation [2] of the plots in Figure 1 can be summarised as:

- At low light intensities the rate of photosynthesis increases linearly as a function of light intensity.
- At higher light intensities the rate of photosynthesis is limited by the available CO_2 concentration (Curve B).

The implication is that if we remove the effect of all other variables (temperature, chlorophyll concentration and light intensity) then we should be able to show experimentally that the rate of photosynthesis will be increased if we increase carbon dioxide concentration (assuming the light intensity is sufficiently high).

In previous articles on this topic [1, 3] we have described how carbon dioxide probes can be used experimentally in the classroom. We noted a number of advantages of such probes including the observations that:

- a wide range of different plant materials can be investigated;
- photosynthesis rates can be investigated in 'real situations' e.g. in the field;
- the readings of carbon dioxide concentration are direct and available in 'real time'.

Experimental set-up

The experimental set-up has been described previously [3] and is shown in Figure 2. A tissue culture flask filled with water (to act as a heat sink) is placed in front of the experimental chamber into which the CO_2 sensor is placed.

The light source is a small desk lamp although a range of lamps could be used.

- Leaves from a basil plant (approximately 2.5 g of material [*ca.* 12 leaves]) were placed in a chamber wrapped in aluminium foil to exclude light. Under these conditions carbon dioxide levels rises as respiration takes place in the leaves.
- Data was collected for about 10 min until the concentration of carbon dioxide present increased from its starting level of *ca.* 380 ppm to approximately 580 ppm.

Figure 2 *- Experimental set-up for measuring respiration and photosynthesis rates in plants.*

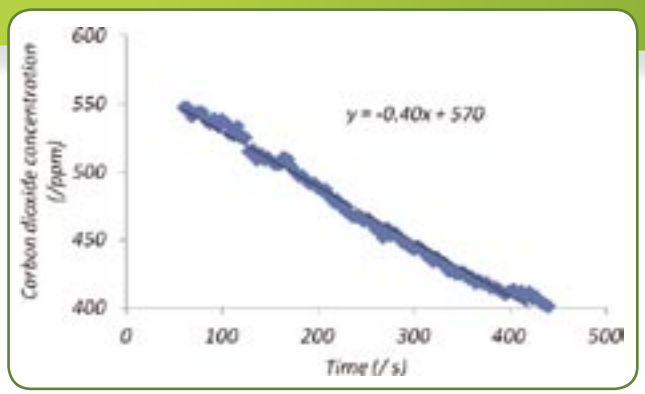


Figure 3 *- The rate of photosynthesis in basil leaves. Data were obtained using a Vernier VR105512 probe. Starting carbon dioxide concentration was measured to be 580 ppm.*

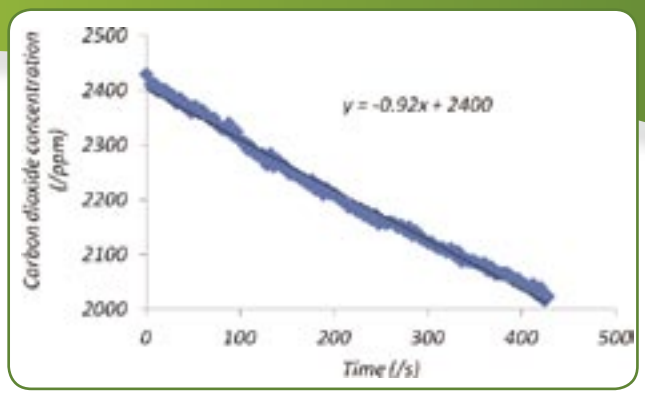


Figure 4 *- The rate of photosynthesis in basil leaves. Data were obtained using a Vernier VR105512 probe. Starting carbon dioxide concentration was measured to be approximately 2400 ppm.*

- The aluminium foil was removed and the lamp switched on and the system allowed to equilibrate (approximately 1 minute).
- Data was collected for a period of approximately 6 minutes (Figure 3) during which time the carbon dioxide concentration fell from *ca.* 550 ppm to *ca.* 400 ppm.

As shown in Figure 3, over the time of observation there is a reasonably linear fall in carbon dioxide concentration allowing the rate of fall to be estimated as -0.4 ppm s^{-1}.

It is possible to repeat the experiment with different starting carbon dioxide concentrations. In order to do this we filled a syringe (5 cm^3) with pure carbon dioxide (in our case this was taken from a cylinder which we had available but there is no reason why carbon dioxide could not be generated chemically - for example using marble chips and dilute acid). Taking care not disturb the experimental set-up shown in Figure 3 we added carbon dioxide from the syringe to the bottle containing the basil leaves. We used a separate syringe to mix the contents and allowed a period of equilibration (1-2 minutes) with the lamp switched off. The lamp was switched on and data on the carbon dioxide concentration recorded (Figure 4). Note the approximately 4-fold increase in starting carbon dioxide concentration compared to the data shown in Figure 3.

Data was collected over a period of some 7 minutes and an estimate of the rate of fall in carbon dioxide concentration (-0.92 ppm s^{-1}) obtained.

Comparing the slopes from Figures 3 and 4 we can conclude that at elevated starting levels of carbon dioxide concentration the rate of photosynthesis is increased; clearly the experiment could be repeated at a variety of different starting carbon dioxide concentrations. In our judgment, the system described above would support those areas of both Higher and Advanced Higher Biology [4, 5] where learners are invited to carry out investigations on limiting factors which affect the rate of photosynthesis. Clearly a carbon dioxide probe set-up would be required in order to do such experiments and the cost of these may be prohibitive for some school departments. Don't forget though that SSERC has a number of probes which can be borrowed!

We have opted to include lines of best fit to the data in Figures 3 and 4. Although we recognise that whilst a reasonable fit is obtained (the R^2 value for the data in Figure 4 is 0.99) a linear plot is somewhat misleading since, as is implied in Figure 1, the rate of photosynthesis is related to the CO_2 concentration present and as this is reduced the rate will fall. Given sufficient time of observation plots such as those in Figures 3 and 4 will appear curved. However, measuring the initial rates allows comparisons between different data sets to be made.

Curriculum links

CfE Higher in Biology [4] - *Sustainability and Interdependence* - learners might *'Carry out experimental investigations on limiting factors in photosynthesis'*.

CfE Advanced Higher in Biology [5]
- *Investigative Biology-2*. Experimentation (c) Experimental design - Design and carry out a simple laboratory true experiment where confounding variables are tightly controlled.

References

[1] Limiting factors in photosynthesis. SSERC Bulletin (2014), **246**, 2-6.
[2] Hall, D.O. and Rao, K.K. (1999) Photosynthesis, 6th Edition, Cambridge University Press, pp. 24-26.
[3] Measuring gaseous carbon dioxide. SSERC Bulletin (2012), **238**, 5-7.
[4] SQA (2012) Higher Biology Course Support Notes - can be downloaded at www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf (accessed July 11th 2014).
[5] SQA (2012) Advanced Higher Biology Course Support Notes - can be downloaded at www.sqa.org.uk/files_ccc/AHCUSNBiology.pdf (accessed July 11th 2014).

Demonstration corner

A CATALYST AT WORK

This reaction can be done as a demonstration but could also be done by pupils as an experiment.

There are many reactions that provide good examples of catalysis but the unique feature of this one is that it is possible to actually see the activated complex appearing and then disappearing.

Hydrogen peroxide oxidises potassium sodium tartrate (Rochelle salt) to carbon dioxide. When solutions of hydrogen peroxide and Rochelle salt are mixed, carbon dioxide is slowly evolved. The reaction can be catalysed by cobalt (II) chloride the addition of which causes the reaction to froth, indicating a large increase in the reaction rate. At the same time the colour of the cobalt (II) chloride turns from pink to green (an activated complex), returning to pink again within a few seconds as the reaction dies down.

This shows that the catalyst actually takes part in the reaction and is returned unchanged when the reaction is complete.

What you will need

- Bunsen burner, tripod, gauze and heat-proof mat.
- One 250 cm^3 beaker.
- One 0-100°C thermometer.
- One 25 cm^3 measuring cylinder.
- One dropping pipette.
- Access to visualiser (optional)
- 5 g of potassium sodium tartrate-4-water (Rochelle salt, potassium sodium 2, 3-dihydroxybutanedioate, $KNaC_4H_4O_6.4H_2O$).

- 0.2 g of cobalt (II) chloride-6-water ($CoCl_2.6H_2O$) **(harmful)**.
- 20 cm^3 of 20 volume (i.e. approximately 6%) hydrogen peroxide solution (H_2O_2 (aq) **(corrosive and irritant)**.
- 65 cm^3 of deionised water.

What you do

Preparation

1) Make a solution of 0.2 g of cobalt chloride-6-water in 5 cm^3 of deionised water.

2) Make a solution of 5 g of Rochelle salt in 60 cm^3 of deionised water in 250 cm^3 beaker.

The demonstration

1) Add 20 cm^3 of 20 volume hydrogen peroxide to the solution of Rochelle salt and heat the mixture to about 75°C over a Bunsen burner.

2) There will be a slow evolution of gas showing that the reaction is proceeding.

3) Stirring the solution makes the evolution of gas more obvious.

4) Now add the cobalt chloride solution to the mixture. Almost immediately the pink solution will turn green and after a few second vigorous evolution of gas starts and the froth will rise almost to the top of the beaker.

5) Within about 30 seconds, the frothing subsides and the pink colour returns.

6) You can get the reaction to repeat by simply adding more hydrogen peroxide. ◀

Safety in microbiology - advice on training requirements

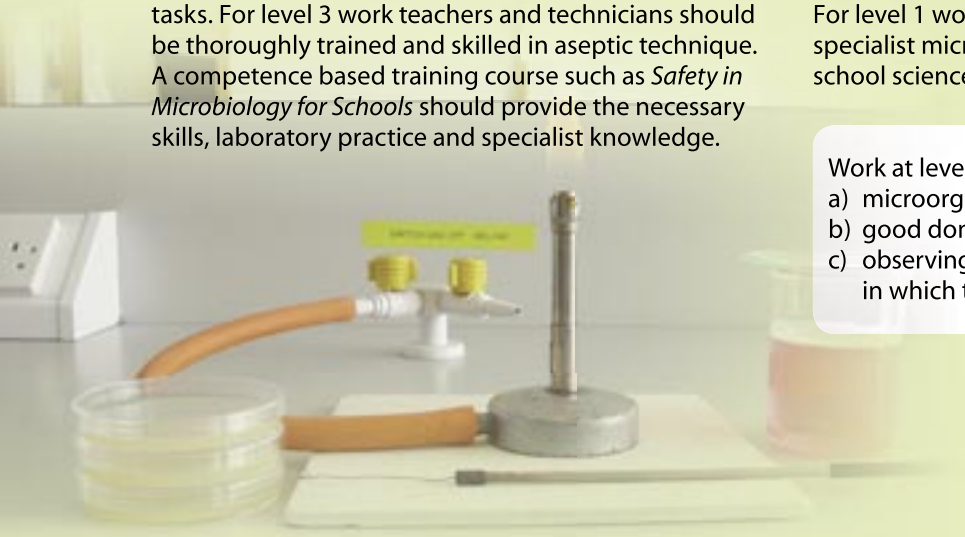
As part of its role as a shared local authority service, SSERC provides advice and guidance on the safe use of microorganisms to all Scottish local authority schools and to member independent schools and colleges. SSERC's advice on safe working with microorganisms comprises:

- a code of practice, *Safety in Microbiology - A Code of Practice for Scottish Schools and Colleges* [1];
- a set of instruction sheets, *Microbiological Techniques* [2];
- a training course, Safety in Microbiology for Schools [3];
- an advice and information consultancy service, by telephone or email [4].

SSERC can only provide advice and guidance on health and safety; it is the responsibility of the employer to decide on health and safety policy and its management including the training of staff. Central to staff training requirements are the three levels of working with microorganisms. The three levels are defined according to the risks which they present and the skills, laboratory practices and specialist knowledge about microorganisms required to control these risks. The level of work with microorganisms that a teacher or technician may undertake will be limited by the training the teacher or technician has undergone.

Most school microbiological laboratory work carried out by learners will be at levels 1 and 2, although students in the senior phase may carry out particular level 3 tasks associated with specific protocols or Advanced Higher Biology Project work.

Staff trained to level 3 are required to prepare for and to support level 2 microbiological laboratory work in schools and to supervise students who carry out level 3 tasks. For level 3 work teachers and technicians should be thoroughly trained and skilled in aseptic technique. A competence based training course such as *Safety in Microbiology for Schools* should provide the necessary skills, laboratory practice and specialist knowledge.



Level 3 tasks required to support microbiological work in schools:

a) order, receipt, labelling and storage of cultures;
b) preparation of sterile media and sterile equipment;
c) preparing sub cultures for class use;
d) sampling from bioreactors;
e) sterilisation and disposal of cultures;
f) sterilisation of used equipment;
g) management of incidents of spillage;
h) staining of incubated plates (e.g. starch agar).

For level 2 work with learners, science teachers may require training and some supervision which can be provided by a knowledgeable biology teacher or technician or by a short in-school training session. The SSERC instruction sheets *Microbiological Techniques* should be a useful resource in such training as will reference to and familiarity with the code of practice *Safety in Microbiology*. Even although it is not an absolute requirement, teachers may prefer and feel more confident in managing level 2 laboratory class work if they are trained to level 3.

Level 2 work does not require the same level of skills as level 3 as it involves:

a) a limited range of microorganisms;
b) a limited range of inoculation and transfer techniques;
c) inoculated cultures remaining unopened;
d) knowledge of how spillages are to be dealt with.

For level 1 work with learners, teachers do not require specialist microbiological training beyond normal good school science laboratory practice.

Work at level 1 involves:

a) microorganisms with little, if any, risk;
b) good domestic hygiene measures;
c) observing microorganisms in the closed containers in which they were grown.

From time to time SSERC receives enquiries on policy decisions for microbiological training of staff. Decisions on training for teachers and technicians are a matter for the employer; SSERC can only offer advice. To operate within the code of practice *Safety in Microbiology* school technicians who are preparing and disposing of materials for level 2 work must be trained to level 3. Although there is no absolute requirement for teachers to be trained to level 3 the advantages of doing so are:

- they can supervise students carrying out some level 3 tasks;
- they can support and supervise colleagues who teach level 2 microbiological work;
- they can assist or lead in establishing good microbiological practice in school;
- they become more confident practitioners as a result of extending their professional learning.

In deciding a policy for the microbiological training of staff an employer will require sufficient staff trained to level 3 to prepare and manage materials for class use, sterilise and dispose of used materials and to manage any incidents of spillage. Although these tasks will largely be carried out by technicians it makes sense and it is good practice to also have teachers trained to level 3 to assist in management decisions related to the school's microbiological practice and to allow teaching where students may be engaged in level 3 tasks or to deal with spillage incidents. It is for the employer to decide upon the number of staff to have trained. Practice varies from one trained technician per school to all school technicians and all biology teachers in a school being trained.

References

[1] Safety in Microbiology - A Code of Practice for Scottish Schools and Colleges (2012), SSERC www.sserc.org.uk.
[2] www.sserc.org.uk/index.php/biology-2/biology-resources/microbiological-techniques265.
[3] www.sserc.org.uk/index.php/cpd-sserc/cpd-courses-sserc.
[4] www.sserc.org.uk/index.php/contact-us.

Health & Safety

Gas masks and Brodie helmets

This could be one to share with colleagues elsewhere in your school. The Health and Safety Executive (HSE) has found that most Second World War gas masks contain asbestos, often in the more dangerous blue form. There is no easy way of determining whether or not a gas mask does contain asbestos, so the following advice is given:

- Children and teachers should not handle gas masks;
- If you have gas masks in school, they should be double bagged and sealed with tape, labelled and securely stored;
- Disposal should be at a local authority licensed site. Alternatively, a licensed contractor can be employed to make the artefact safe for display.
- The majority of World War One "Brodie" helmets have also been found to contain asbestos. They should be treated in the same way as outlined above.
- Replica gas masks and helmets that do not contain asbestos are available.

For more information about asbestos, visit the HSE's website [1].

Reference

[1] www.hse.gov.uk/asbestos/index.htm (accessed June 2014).

The protactinium generator and its rivals

Figure 1 *- latest incarnation of the protactinium generator.*

In Bulletin 218, we last wrote about half life sources [1]. At that time, the protactinium generator was no longer in use in Scottish schools and a direct replacement had not been put on the market. A couple of years ago, the protactinium generator re-entered, blinking, into the sunlight (Figure 1).

Comparable in price to the Cooknell ionisation chamber kit and substantially cheaper than the barium eluting source, it is seen by some schools as a good way to demonstrate half life. We have risk assessed the new incarnation of the protactinium generator and have authorised its use. Though we will continue to do so, there are some things about this source that you should know.

- The protactinium generator has a recommended working life of eight years. At the end of the eight years, it will have to be disposed of. This will incur a cost which could be comparable to the initial purchase price. For some locations in Scotland, it could be considerably greater. If you are buying this source on account of its price this may well be a false economy if you take a long-term view.
- Our CLEAPSS colleagues have had reports of two leaking protactinium generators. One was found to be leaking on delivery. The other had been incorrectly stored on its side.

At this point, it is worth remembering that all half life sources have their good and bad points. The table below summarises the pros and cons of the ones approved by SSERC for use in schools.

Remember, you can't buy the barium eluting source or the protactinium generator without a letter of approval from the Scottish Government. We will help you with this.

Have a look at the Physics area of the CPD section of our website too. We run two courses on using radioactive sources. The fact that these are always well-attended, coupled with the enquiries we receive from people out to buy new sources, makes us very happy indeed.

Rubbish legislation

Of all the sources that you are allowed to have in schools, only the protactinium generator should require a contractor for disposal. This is due to the chemical toxicity of the heavy metal, uranium, rather than a radiological hazard. Well, that's the theory and indeed the intention of the Exemption Order 2011 legislation. Excepting the protactinium generator, it should be possible to put end-of-life sources in the dustbin and dispose of to landfill. Unfortunately, we are in the faintly ludicrous situation whereby most sources are considered too active, according to another piece of legislation, to be taken from bin to coup in a refuse truck. This is despite the fact that it is legal for a lorry carrying 500 smoke alarms, ten of which would be as active as the most active school source, to wend its way around the country. Needless to say, we are working hard to have this situation resolved.

Reference

[1] www.tinyurl.com/sserc-hl.

Half life source	Advantages	Disadvantages
Barium eluting source	It is hard to argue against this being the best method, educationally speaking. The elution yields a radioactive liquid that is quite separate from the main source. The liquid's activity thereafter decays rapidly.	Relatively high initial purchase price. Extra cost of eluent. The elution has to be practised. Requires an annual "bleed-through" test, though this can be incorporated into a standard demonstration.
Protactinium generator	Relatively inexpensive to purchase. Older teachers are familiar with its use.	The only one of the three with the potential to cause serious harm in the event of a mishap, though the chance is small. Small number of reported leakages. High end-of-life costs. Hard-to-understand method of operation (some children think it becomes radioactive only when shaken).
Cooknell ionisation chamber with gas mantles.	Relatively inexpensive. Does not need a GM tube - a voltmeter or datalogger with voltage probe will suffice. No permission required to buy.	A bit of a "black box" (well, a blue box) whose internal workings need to be explained to pupils.

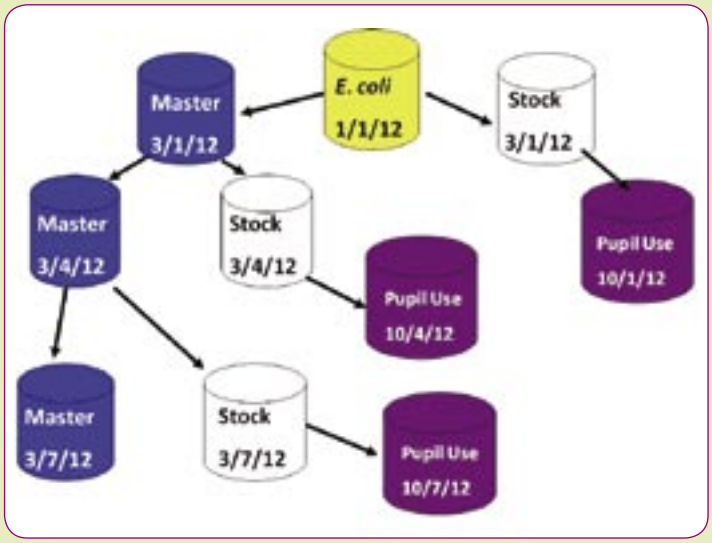
Maintaining microbiological cultures in school

From time to time SSERC receives enquiries about how to best maintain bought in microbiological cultures in schools in line with the SSERC Code of Practice *Safety in Microbiology* [1].

Microbiological cultures should be obtained from an approved supplier. Microbiological cultures used in schools should originate from a national culture collection and be true to type. An approved supplier is one who can trace the provenance of the supplied culture back to a national culture collection. Microbiological cultures from other sources should be avoided or be the subject of a separate risk assessment. SSERC is happy to provide advice in such circumstances. Cultures should be purchased specifically as and when required and stored for the minimum time practicable. Cultures should not be kept for longer than one year from the time of purchase. If cultures have to be kept for longer than one year for specific reasons a separate risk assessment must be carried out.

Purchased cultures should be dated on arrival and placed in a closed container in a refrigerator or cupboard, both of which should be labelled with Biohazard labels. A log must be kept of all cultures showing:

- name of microorganism;
- supplier;
- date of receipt;
- number of sub cultures made;
- date of each sub culture;
- by whom sub cultures were taken;
- date of disposal.



Best practice when maintaining cultures is to sub culture from the bought in culture onto two agar slopes labelled with microorganism, date and initials of operative. Use one these subcultures (stock) as the source of inoculum for preparing materials for class use and dispose of it along with the class materials once the practical work is complete. Keep the other sub culture (master) as a source of inoculum for further class material or for use if contamination is observed. When using this second (master) subculture, again prepare two agar slopes, keeping one as a new 'master' and using the other as 'stock' for preparing class materials (see diagram). Keep the original culture for use as an additional back up if contamination is detected. Sterilise and dispose of all cultures and class materials as soon as is practicable once class work is complete and in any event no longer than one year after purchase.

Reference

[1] *Safety in Microbiology* - A Code of Practice for Scottish Schools and Colleges (2012), SSERC www.sserc.org.uk.

The SSERC Bulletin is published by SSERC · 2 Pitreavie Court · South Pitreavie Business Park · Dunfermline KY11 8UB
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