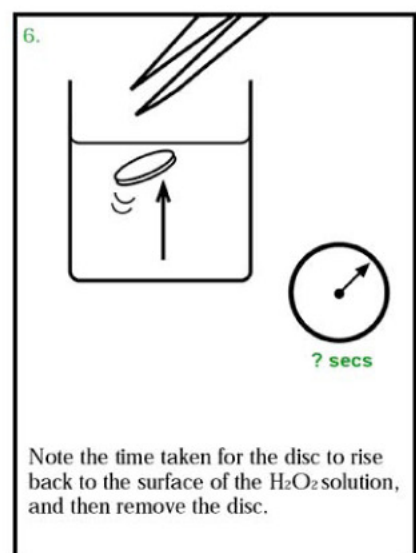
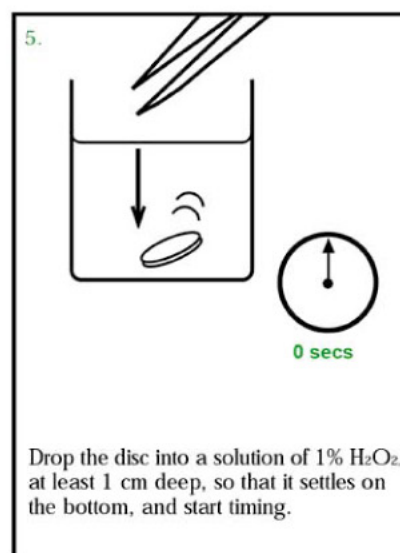
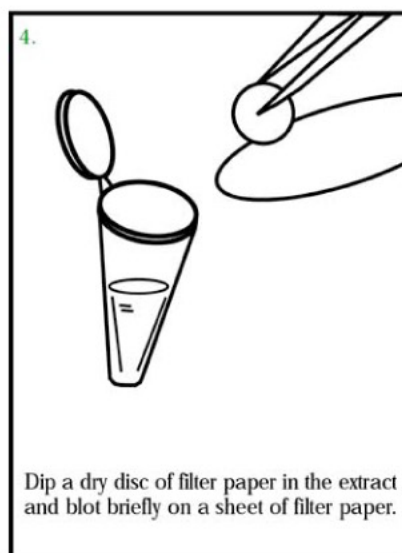
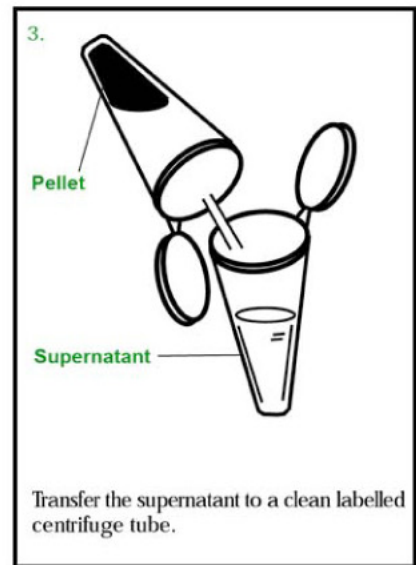
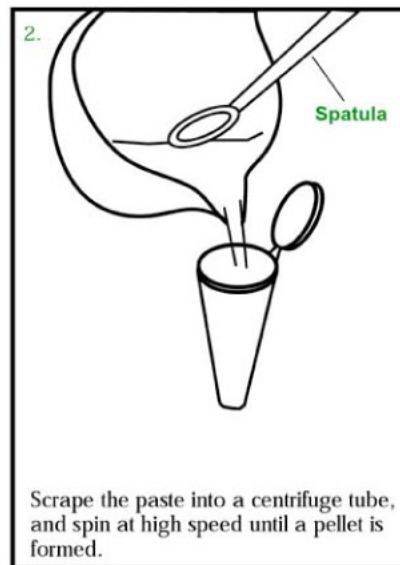
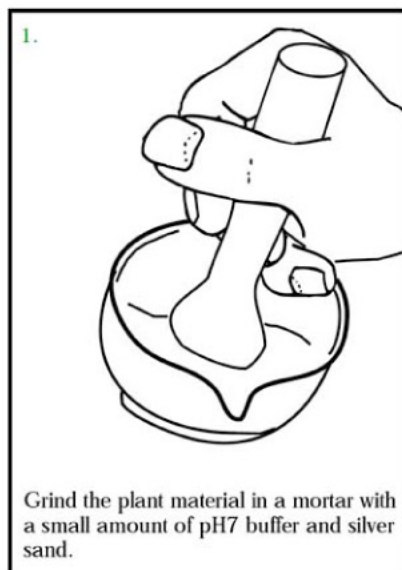


Microscale Investigations with Catalase

Catalase (EC 1.11.1.6) is a widespread enzyme, found in nearly all aerobic cells (animals, plants and microbes). It serves to protect the cell from toxic effects of hydrogen peroxide, generated as a by-product of cell metabolism. It does this by catalysing the decomposition of hydrogen peroxide into molecular oxygen and water.



This protocol uses a deceptively simple, yet very accurate method to measure the rate of reaction by collecting the oxygen evolved as a product of the reaction. The whole reaction can be carried out on a very small scale, in a centrifuge tube. An enzyme extract is adsorbed to filter paper discs. These discs initially sink in a hydrogen peroxide solution, but then float to the surface as the oxygen produced gets trapped in the paper fibres. The time taken for the discs to rise is measured.



It is important to have a clear view of what is happening, so this is best done on the laboratory bench, looking sideways. Occasionally the disc settles at the side, or gets caught on the wall of the vessel - you should remove these discs and repeat the process.

Further notes for teachers and students

The six steps on the Student Sheet provide an outline method that can form the basis of a protocol for investigations with catalase. The comments given here should help you to adapt this method for the particular investigation being undertaken (see below for some suggestions).

Different plant materials contain very different amounts of catalase activity (see table on page 6). In making the extract, try to add only the minimum amount of buffer to make the extract and never use a greater mass of buffer than of plant matter. The critical thing is to get a little clear supernatant in the centrifuge tube (only a tiny volume is used for each test). With a 'watery' tissue you may not need to add any buffer! A very crude method of collecting the enzyme is to hold a disc of dry filter paper in contact with the freshly cut surface of a fruit or vegetable.

The volume of 1% H₂O₂ required depends on the reaction vessel being used (e.g. 5 cm³ in a small glass specimen tube, or 2 cm³ in a large Comboplate[®]* well). Using a depth of <2 cm allows more precise timing. Comboplates[®] have proven very useful for such investigations, as they are so stable, and provide a series of reaction vessels.

One great advantage of this system is that you can easily do a series of repetitions, simply by removing the used disc from the vessel and then starting again with another disc. There is no need to change the H₂O₂ solution until you have used more than 10 discs in it. There seems to be surprisingly little diffusion of the adsorbed enzyme from the disc fibres into the solution. If reaction times are longer than 2 minutes, you might consider using a more concentrated H₂O₂ solution, and if they are less than 5 seconds, then it might be worth using a less concentrated H₂O₂ solution.

Whilst the method is quick with a microcentrifuge, if you do not have one, you can carry out the technique satisfactorily with a normal centrifuge, but larger amounts of plant material would have to be used. Also, an advantage of using small samples of tissue, is that you can take a series of samples across, for example, a diseased vegetable, and investigate any differences in catalase activity.

If the enzyme extract is going to be kept for some hours, then it would be better to store the tube in a beaker of crushed ice, but remember to warm it up again before use. It will not keep in a fridge overnight!

* Comboplates[®] are plastic microplates with numerous wells in two sizes, available from: EDU-LAB, Karoo Close, Bexwell Business Park, Bexwell, Norfolk PE38 9GA (Tel: +44 (0)1366 385777).

Ideas for investigations

Some suggested investigations with seeds and seedlings

- What is the time-course of catalase activity in whole seeds during the germination process?
- Do hypogeal cotyledons have more catalase than epigeal cotyledons - during imbibition and germination, or after their emergence from the soil?
- Do endospermic seeds have more catalase than non-endospermic ones?
- Do any of the following stresses affect the catalase levels of seedlings?
 - flooding (e.g. by extended periods of immersion in water)
 - heat / cold shock (e.g. give heat / cold treatments to imbibed seeds for at least 1 hour)
 - salinity (try watering with sodium chloride solution)
 - shortage of water
 - infection of seedlings with damping-off fungus
- Does hormone treatment (e.g. with auxins, gibberellins, ABA) affect catalase levels?

Some suggested investigations with larger vegetables and organs

- How does catalase activity vary across or along the length of the organ?
- How does catalase activity vary from a healthy to a diseased region?

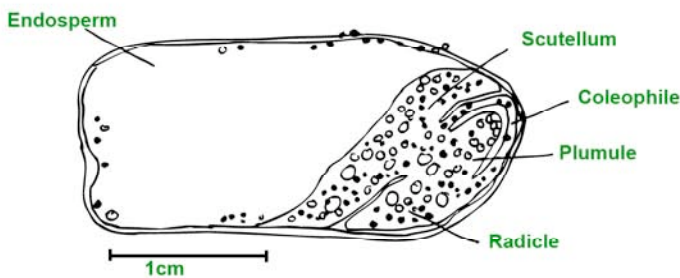
References

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- Delpuch R. (2000a) Can you distinguish between ... the living and the dead? *Osmosis SAPS Newsletter* 17
- Delpuch R. (2000b) Investigating the phenomenon of programmed cell death in maize (*Zea mays*) seeds *Journal of Biological Education* 35 (1) 41-44
- Lane N. (2002) *Oxygen – the molecule that made the world* Oxford University Press, Oxford (A superb review of the roles of oxygen, free radicals etc. in the evolution of life and the ageing process – a must for the sixth form library)

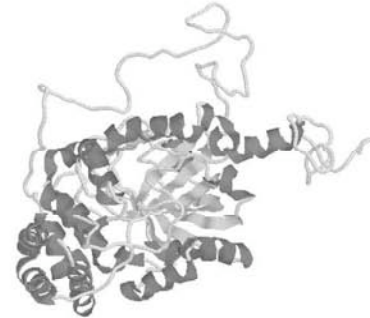
Background information (catalase)

In the mature plant, the catalase protein has a quaternary structure, in that it consists of four similar polypeptide chains (subunits), forming a tetramer (1 subunit = 65 kD). Each polypeptide subunit contains a haem prosthetic group at the active site. Different catalase isozymes can be identified within a particular species, the number of them ranging from 1 in lentil cotyledons to 12 in mustard cotyledons.

You can reveal catalase activity in imbibed and germinating seed tissues quite simply by placing a sliced seed under the surface of a 1% H_2O_2 solution, with the cut surface uppermost. Use a hand lens, or binocular microscope, to detect the production of bubbles of oxygen. Most revealing are sections of endospermic seeds, as the endosperm has undergone programmed cell death (Delpech R 2000a; Delpech R 2000b). The metabolically active embryo and cotyledons should bubble furiously, but the endosperm remains bubble free, except at the borders of the seed, where (in monocotyledons) the living aleurone layer is active. Remember that the oxygen may be trapped for longer in tissues that have smaller air spaces. You can apply this same method to other plant tissues.



Drawing of section of maize seed showing bubbling areas when submerged in 1% H_2O_2 solution



Ramsol image of catalase subunit

The wide occurrence of catalase across the living world means that there are many catalase sequences in the bioinformatics websites that allow information to be collected, and employed to create molecular models, such as the one shown above.

Table to show some fruits and vegetables, with and without significant catalase activity

Catalase activity present			Catalase activity absent
apricots	broccoli	parsnip	apples
bananas	carrots	potato	citrus fruits
cherries	cucumber	radishes	peaches
fresh flowers	leeks	redcabbage	rhubarb
	onions	turnips	tomato
			senescent flowers