Effervescent Enzymes!

Background

Enzymes, by virtue of the myriad of functions with which they are involved, are necessary for life. Consequently, studying enzymes and the roles which they play continues to be an integral part of many science curricula. In that regard, Curriculum for Excellence (*CfE*) is no exception and one of the Sciences Experiences and Outcomes at Level 4 [1] relates to practical work involving measurement of enzyme properties:

I have taken part in practical activities which involve the use of enzymes and microorganisms to develop my understanding of their properties and their use in industries. **[SCN 4-13b]**

In this short article, we present an experimental protocol that we believe to be both robust and accessible and which, therefore, should prove useful in supporting the delivery of outcome SCN 4-13b. Further experimental details will be made available on the Science 3-18 website [2]. The protocol which follows is adapted from one produced by Roger Delpech of the Haberdashers' Aske's Boys' School - the original article can be downloaded from the SAPS website [3].

The enzyme *catalase* is found in nearly all aerobic cells (animals, plants and microbes). The function of catalase is to protect the cell from the harmful effects of hydrogen peroxide which is generated as a by-product of cell metabolism. Catalase speeds up the breakdown of hydrogen peroxide into molecular oxygen and water.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

The following protocols use a very simple yet elegant method to demonstrate the above reaction. When filter paper discs are added to hydrogen peroxide they initially sink to the bottom of the container. If, prior to their immersion in the hydrogen peroxide solution, the discs have been in contact with a source of catalase (for example, if they have been soaked with suspensions of living cells or in contact with living tissue) oxygen will be produced. Some of this oxygen becomes trapped in the fibres of the filter paper making the discs buoyant and they eventually float to the surface of the solution. The time taken for the discs to rise to the surface can be used as a measure of reaction rate and hence the amount of catalase present.

Experimental

We suggest here two ways of using this technique:

- investigating whether living and non-living materials contain catalase
- investigating the effect of catalase concentration on the rate of reaction

Catalase activity in living and non-living materials

The aim of this activity is to provide a simple method by which students can investigate catalase activity from a number of sources including living (plant and animal) and non-living materials. By pooling class results, students should be able to conclude that (i) catalase is found only in materials which contain live cells, and (ii) the activity of catalase varies depending on the source selected.

- We recommend a control experiment in which a piece of filter paper, which has not been in contact with any of the samples to be tested, is added to a Universal bottle containing 20 cm^3 of 1% hydrogen peroxide solution (IRRITANT). The disc should sink to the bottom and remain there.
- Using a scalpel or a knife make a fresh cut on the surface of plant or animal material to be tested. Using tweezers hold a filter paper disc against the test surface for 10 seconds and then add the disc to the Universal bottle containing 1% hydrogen peroxide solution. The disc will sink to the bottom. However in this case if oxygen is produced, indicating catalase activity, the disc will float to the surface. Students need to be ready to time how long it takes the disc to float to the surface. Alternatively the time taken for a disc to rise a given distance could be used for measurement (this would be particularly useful if catalase levels in a given sample were low).
- Further trials using a variety of plant or animal or non-living surfaces can then be undertaken.
- Students can design a table for results and make comparisons of catalase activity in living / non-living materials and plant / animal tissues. Table 1 shows previously published results for a variety of plant materials [3].

Catalase activity present		Catalase activity absent
apricot	parsnips	apple
banana	potato	citrus fruits
cherry	radish	peaches
broccoli	red cabbage	rhubarb
carrot	turnip	tomato
cucumber	fresh flowers	senescent flowers
onion	leek	

Table 1 *- Catalase activity in some fruit and vegetables (taken from [3]).*

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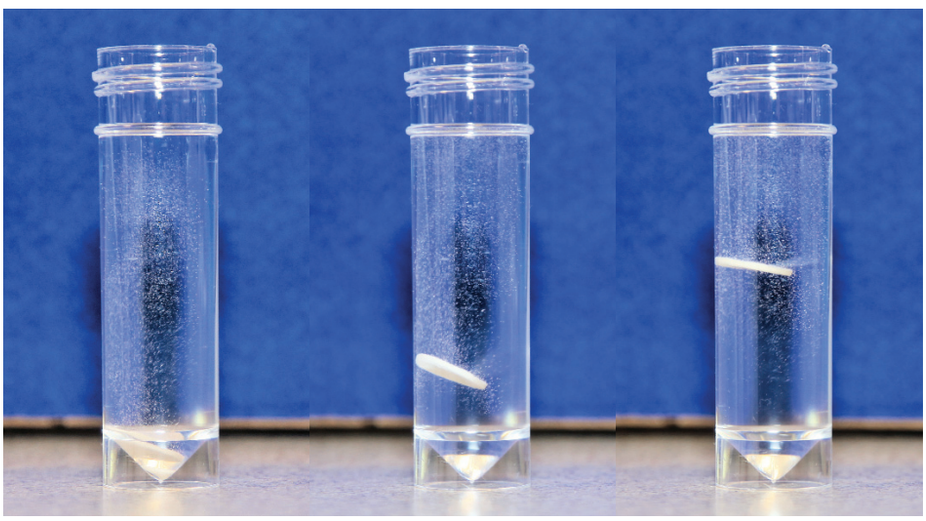

A B C

Figure 1 *- A single drop of a 1% suspension of yeast has been added to a filter disc and then placed in a 1% solution of hydrogen peroxide. Photographs were taken at (approximately) 3 s (A), 8 s (B) and 13 s (C).*

Liver contains a lot of catalase and this can be related to the role of the liver in the removal of toxins from the body. It would be interesting to use a variety of different animal tissues (e.g. kidney, muscle) and make comparisons of catalase levels. However, it is worth noting that the biological significance of catalase is quite complex; for example it appears that some organisms deficient in catalase can survive with little or no harmful consequences [4]. Some additional information on the role of catalase in plants is available [5].

The effect of catalase concentration on the rate of reaction

The aim of this activity is to provide a method by which students can investigate the effect of increasing catalase concentration on the rate of breakdown of hydrogen peroxide. Yeast or algal suspensions are convenient sources of catalase.

Figure 1 shows a set of images when a drop of a yeast suspension has been added to a filter paper disc and placed in hydrogen peroxide solution.

The concentrations of yeasts and algae suspensions can be easily varied. It is reasonable to assume that the greater the concentration of the yeast or algae suspension the more catalase would be present in a fixed volume and so the concentration of yeast or algae can be used as a measure of enzyme concentration. In the protocol that follows we will only refer to suspensions of the alga *Scenedesmus quadricauda.*

For this protocol we recommend that your students carry out a serial dilution from a starting stock of algae. Depending on the year group with which you decide to use this activity the extent of explanation you give will be important; for example with 'Higher' students carrying out the serial dilutions could be done as part of an investigation.

Bearing in mind that you will need only small volumes of material for the assay you will not need to prepare large volumes of each dilution.

We recommend making dilutions to 5 cm^3 although smaller volumes would, in most cases, be entirely adequate. So 0.5 cm^3 of a stock solution is diluted to 5.0 cm^3 with water and 0.5 cm^3 of that solution is further diluted to 5.0 cm^3 etc. (shown schematically in Figure 2)

- A pipette is used to place one drop of the algal suspension on to a filter paper disc - clearly more accurate measurements of the volume added would add rigour to the experiment.

- Using tweezers add the disc to a Universal bottle containing 1% hydrogen peroxide solution. The disc will sink to the bottom. If oxygen is produced, indicating catalase activity, the disc will float to the surface. Students need to be ready to time how long it takes the disc to float to the surface.

- The process is repeated with the range of serial dilutions.

- As a control, the process should also be repeated with a drop of water on a filter paper disc.

- Pupils can record their data in tables and graphs and draw conclusions about enzyme activity against concentration.

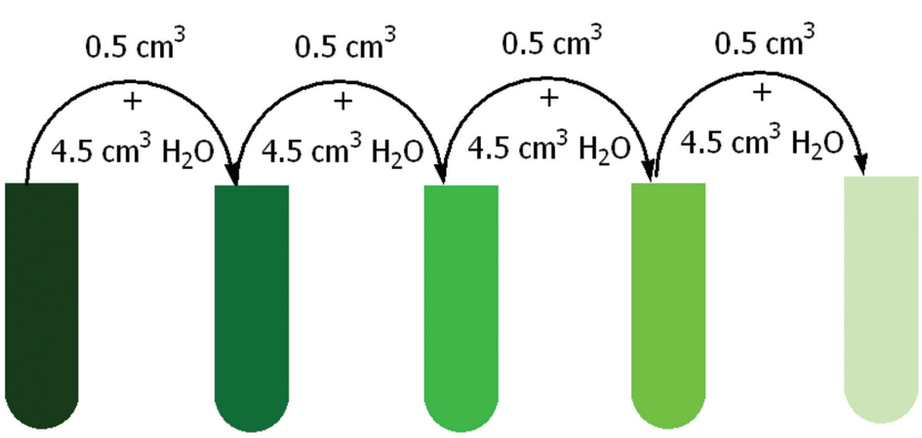

Figure 2 *- Preparation of serial dilutions from a stock solution of Scenedesmus quadricauda*