Catalase activity in immobilised yeast

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Abstract The authors, from the Scottish Schools Education Research Centre (SSERC), describe an experimental system, based on previously published work, in which the catalase activity of immobilised yeast is measured. They have adapted the protocol and developed it in a way that allows its use in investigative work to support national qualifications at both National 5 and Higher in biology in Scotland. Teacher feedback indicates that the protocol will become widely used.

National 5 biology and Higher biology qualifications in Scotland are broadly equivalent to GCSE and AS levels as delivered in other parts of the UK. Recent changes to both National 5 and Higher across the sciences have seen the introduction of a mandatory assessment assignment task that must involve experimental work (Scottish Qualifications Authority (SQA), 2019).

The detailed instructions regarding the assignment are beyond the scope of this article but suffice it to say that schools, colleges and examination centres are increasingly asking us at SSERC (Scottish Schools Education Research Centre) for support in the development of practical activities that are suitable vehicles for the delivery of the assignment. This article has its focus in biology although we frequently receive requests in respect of other science areas. In early 2017, we were approached by SQA with a view to producing exemplar activities that might form the basis of assignments at National 5 in time for the start of the 2017/18 academic year (the corresponding, and similar, changes in respect of 'the assignment' to the Higher biology curriculum took effect at the start of the 2018/19 academic year). In response to this request, we produced two exemplars, details of which were published on the SSERC website (SSERC, 2017). Subsequently, we turned our attention to other activities and decided to work on an enzyme-based practical. We asked several teacher groups about the criteria that they would use in recommending an enzyme-based biology assignment for their pupils. While the responses to such enquiries vary, they broadly fall into one, or more, of the categories listed below. In teachers' judgement, activities should (in no particular order):

- be robust;
- yield clear results;
- involve inexpensive (and readily available) substrates and enzymes;
- be versatile and offer opportunities for investigative work;
- be reliable;
- allow students to extract enzymes from 'living things';
- incorporate an assay that is simple to follow;
- produce results on short timescales;
- have application 'in the real world';
- not require sophisticated equipment;
- allow a range of variables to be studied (so that not all students are 'doing the same experiment').

Given that the SQA assignment activity contributes to the final grade awarded to students, the choice of area is clearly important and practical topics that meet most, if not all, of the above criteria are preferred.

The activity

Several years ago, we published (SSERC, 2010) a protocol (based on previous work (Science and Plants for Schools, 2003)) in which catalase activity is measured. The enzyme catalase is found in nearly all aerobic cells (animals, plants and microbes) and its principal function is to protect against the harmful effects of hydrogen peroxide that may be generated as a result of cell metabolism. Catalase speeds up the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The whole reaction can be carried out on a very small scale. An 'enzyme extract' is adsorbed onto filter paper discs. These discs initially sink in a hydrogen peroxide solution, but then float to the surface as the oxygen that is produced is trapped in the fibres of the paper. The time taken for the disc to rise to the surface is measured and can be related to catalase activity. The 'enzyme extract' might be taken from a variety of sources including fruit and vegetables, although consistency of volume applied can be a problem.

One of the extension activities that we recommend is to test the catalase activity of yeast by adding known (small) volumes of a yeast suspension to filter paper discs and testing the time taken for the discs to rise to the surface. One

of the challenges of this activity is making sure that the number of yeast cells added is done in a consistent fashion and that the cells remain adhered to the filter paper.

We continue to be great advocates of using immobilisation techniques in a variety of experimental systems, most notably using algae (SSERC, 2016). Imagine our surprise and delight (and, it has to be said, just a tad of jealousy) when a recent article (Bryer, 2016) appeared. Bryer immobilised yeast suspensions, thereby producing 'yeast balls', and used these in solutions of hydrogen peroxide to test for catalase activity. In principle, the advantages of such an approach include:

- a consistent numbers of yeast cells can be trapped/immobilised;
- the number of yeast cells in a single yeast ball can, in principle, be estimated using a haemocytometer;
- varying the concentration of the yeast cells in the stock solution is straightforward and immobilised balls with varying numbers of yeast cells can be produced;
- the opportunities for investigations are increased.

The experiments that follow are, with some adaptations, largely based on Bryer's work.

Procedure for making immobilised yeast balls

The basic protocol we adopt is as follows:

1. Prepare a 10% stock solution of dried yeast (typically we use Allison's baker's yeast).
2. Add aliquots (2 cm^3) of the stock yeast solution to a solution of sodium alginate (2%, 2 cm^3) and mix thoroughly.
3. Place the yeast/alginate mixture into a syringe positioned above a solution of 2% $CaCl_2$ (Figure 1).
4. Allow the liquid to flow; the drops form balls of immobilised yeast that are left in the $CaCl_2$ solution for about 5 minutes (Figure 2) and then washed gently under running cold water followed by a final rinse with distilled water. We store the balls in distilled water until use in the experiments.

Methods and results

The experimental set-up for measuring catalase activity is shown in Figure 3. We prepare a stock solution of hydrogen peroxide (1 vol.) and 25 cm^3 of this hydrogen peroxide stock is placed in a measuring cylinder and a single immobilised yeast ball is added. The time taken for the ball to sink and then rise to the surface is recorded (Figure 3).

Provided the ball is removed promptly once it has reached the surface then the change in hydrogen peroxide concentration in the cylinder is minimal and it is easy, therefore, to make repeat measurements with

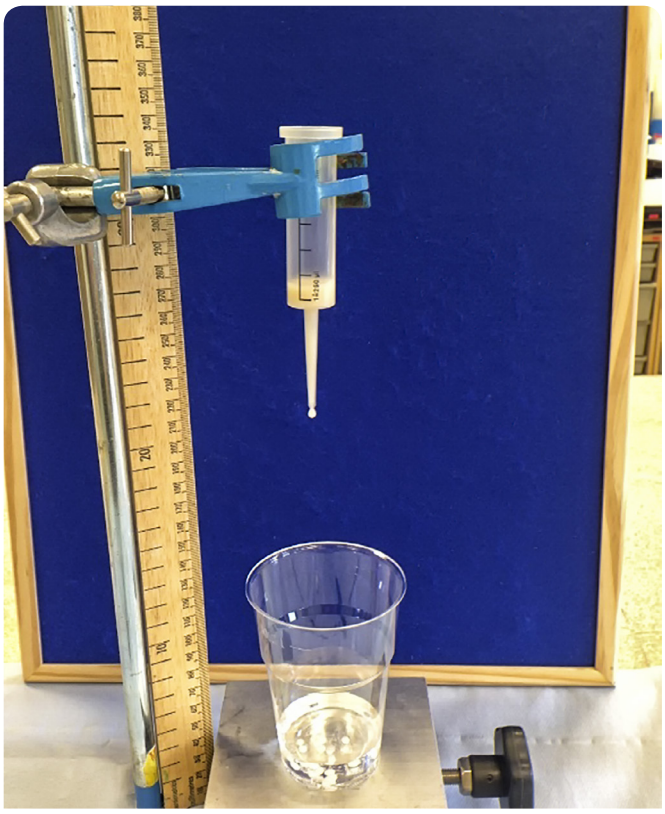

Figure 1 Experimental set-up for producing immobilised yeast balls

Figure 2 Immobilised yeast balls

Table 1 Time taken (five replicates) for immobilised yeast balls to fall and rise in solutions of hydrogen peroxide; for experiments at 4 °C, a stock solution of 1.0 vol. H_2O_2 was refrigerated overnight; balls of immobilised yeast were covered with distilled water and stored in a fridge for 1 hour prior to use

Concentration of H_2O_2/vol.	Temperature/°C	Time/s
1.0	19	9, 9, 10, 11, 11
0.2	19	24, 25, 26, 26, 27
1.0	4	15, 16, 17, 17, 18

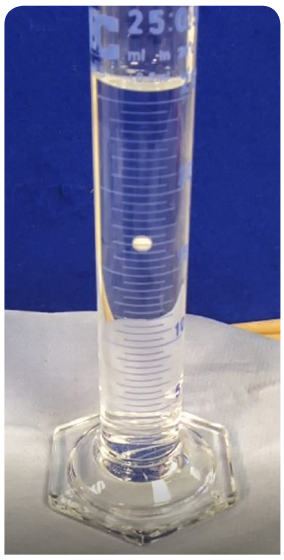

Figure 3 Immobilised yeast ball in hydrogen peroxide solution

fresh balls using the same hydrogen peroxide solution. Typical results for immobilised yeast balls are shown in Table 1.

Lowering the concentration of hydrogen peroxide leads to an increase in the time taken for the fall and rise of the ball and this opens the possibility of investigating the effect of substrate concentration on the rate of reaction.

A decrease in temperature from 19 °C to 4 °C leads to an increase in the time taken for the fall and rise of the ball, again offering opportunities for investigative work. We have not yet tried to study the effect of pH but, in principle, this would be a relatively straightforward set of experiments.

We have tested several different plant-based materials. We made a crude extract by adding approximately 50 g of material to 75 cm^3 of water and homogenising this using a hand-held blender. The resulting suspension was centrifuged in microfuge tubes (approximately 8500 *g*), and portions (2 cm^3) of the supernatant were added to sodium alginate solution as in step 2 above. The resulting immobilised fruit/vegetable balls were then tested for catalase activity. Results are summarised in Table 2.

Table 2 Time taken (mean of five measurements, rounded to the nearest 5 seconds) for immobilised balls of extracts from fruit/vegetables to fall and rise to the surface in a measuring cylinder (25 cm^3) containing 1 vol. (25 cm^3) H_2O_2 at room temperature

Fruit/vegetable	Time / s
Potato	75
Banana	175
Cucumber	75
Blueberry	>180
Peas (frozen)	>180

We also tried some immobilised algae that had been stored under distilled water in the fridge for some 6 months. We allowed the algal balls to come to room temperature and then added them to a measuring cylinder containing 1 vol. H_2O_2; on average the algal balls took around 75 seconds to fall and rise to the surface. Given the length of time we had kept these immobilised algae, we were quite surprised to find that they had retained catalase activity.

We make one observation here in respect of possible control experiments that might be undertaken. In principle, 'pure' catalase could be immobilised and comparisons drawn with the extracts containing catalase described above. Such an approach might yield interesting data. However, we note from previous studies (Cheetham and Bucke, 1989) that immobilised catalase slowly loses activity (over a period of several hours) and this is explained by slow diffusion of the enzyme out of the immobilisation matrix. Other enzymes, such as glucose oxidase, do not appear to diffuse out of the matrix (Cheetham and Bucke, 1989). Such differences cannot easily be explained based on molecular mass (glucose oxidase = 160 kDa and catalase = 232 kDa) since we might expect larger molecules to diffuse out of the matrix more slowly. Clearly, we do not yet have all the answers…

In order to extend the variety and complexity of the immobilised yeast/catalase system, we have been turning our attention to inhibitors that might be used. We were encouraged by the following:

> *Fungal catalase is (noncompetitively) inhibited by ethanol — so ethanol can be used to demonstrate enzyme inhibition (you need roughly 15% ethanol to inhibit the catalase).* (National Centre for Biotechnology Education (NCBE), 2018)

However, we tried a series of protocols in which we added both ethanol and/or methanol to see whether we could detect any inhibition but the results from our studies proved to be inconclusive. Searches of the wider literature indicated that several metal ions, in particular copper, might be inhibitors of catalase.

For our first attempts at demonstrating copper inhibition of catalase we prepared immobilised yeast balls in the standard way and then added copper sulfate to the measuring cylinder to provide a range of different concentrations. We then recorded the time taken for balls to fall and rise. We saw no difference in the time taken even at relatively high copper concentrations. We note that at concentrations of copper sulfate > 0.5 mol dm^{-3}, the addition of copper leads to observable breakdown of the hydrogen peroxide in the absence of added yeast balls. At concentrations of copper sulfate lower than 0.5 mol dm^{-3}, the peroxide appears to be relatively stable but a pupil contemplating such an experiment as part of an assignment might wish to think about appropriate controls.

We decided to 'incubate' immobilised yeast balls with copper sulfate and then measure the time taken for balls to fall and rise in solutions of hydrogen peroxide. The data are presented in Table 3 and plotted graphically in Figure 4. What can be seen is that increasing copper sulfate concentration does indeed lead to catalase

Table 3 Time taken for an immobilised yeast ball to fall and rise in solutions of hydrogen peroxide (1 vol.); approximately 20 immobilised yeast balls were allowed to stand for 16 hours in solutions (50 cm^3) of copper sulfate at the concentrations shown; fresh peroxide solution was used when a change in copper sulfate concentration was made

Copper sulfate concentration / mol dm^{-3}	Time / s				
	Run 1	Run 2	Run 3	Run 4	Mean of runs 1–4
0	16	16	16	16	16
0.001	16	16	15	15	16
0.005	58	47	58	60	56
0.010	125	120	110	119	119
0.025	192	186	190	196	191

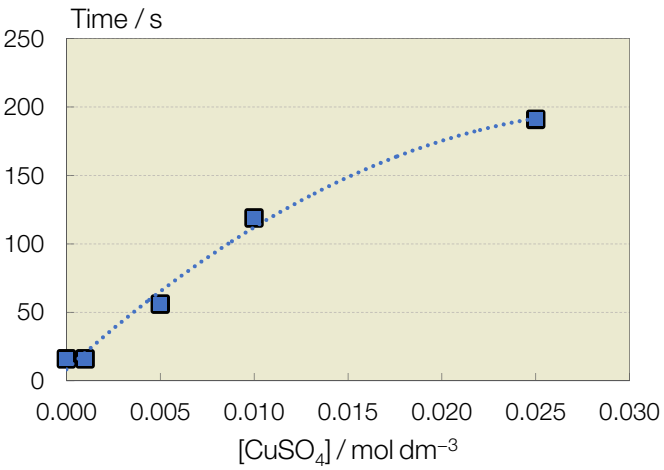

Figure 4 Mean data from Table 3

inhibition. Such observations open the possibility of a range of other experiments that pupils might undertake. For example, we see little inhibition when using zinc sulfate in place of copper sulfate and comparisons might form the basis of an interesting investigation.

Once immobilised yeast balls have been left overnight in the presence of copper sulfate, they have a distinct blue/green colour. Much of the copper can be removed by gently agitating the balls in distilled water; after a few changes of water, the inhibition seen in the presence of copper is reversed.

Teacher feedback

We have been gathering feedback from teachers about how useful the activities outlined here are in providing opportunities for investigative work. We would like to think that the following comments encapsulate widely held views:

- '*Immobilising yeast enzyme experiments has given me great ideas for the National 5 assignments.*'
- '*Enzyme investigations using immobilising techniques – I can use these for assignments.*'
- '*The enzyme lab sessions were really good – we can use these as the basis for investigations.*'

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