Figure 1 - Experimental set-up for producing immobilised yeast balls.

Hydrogen peroxide and

Have you ever had that thought which broadly goes 'Now why didn't I think of that'? That was the response of at least one member of the SSERC Biology team when we read an article recently published in the journal *Science Teacher* [1]. The article offers a thought-provoking and, as we hope to show here, a fun approach to measuring catalase levels in yeast.

Background

Several years ago we published [2] a protocol (based on previous work by Roger Delpech [3]) in which catalase activity is measured. 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:

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

Briefly the whole reaction can be carried out on a very small scale - in SSERC we tend to use Universal bottles but, as they say, other containers are available. An 'enzyme extract' is adsorbed on to 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, vegetables etc. although consistency of volume applied is a problem.

One of the extension activities which 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. Yeast concentration can be varied by making a serial dilution of the yeast suspension. One of the challenges of this extension 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. Despite these challenges we are aware that student investigations have successfully yielded semi-quantitative and in some cases quantitative results.

In SSERC, we continue to be great advocates of using immobilisation techniques in a variety of experimental systems most notably using algae [4] and adopting an assay from the National Centre for Biotechnology Education [5] for immobilising lactase.

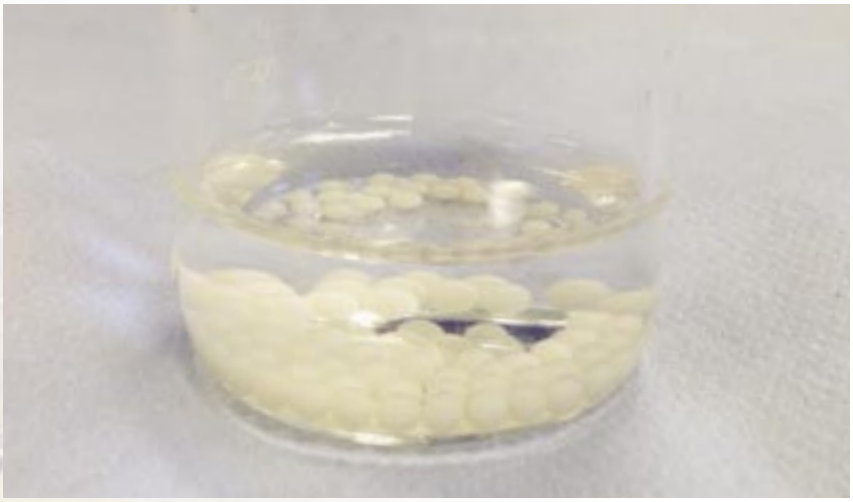

Figure 2 - Immobilised yeast balls.

immobilised yeast

Imagine our surprise/delight (and just a tad of jealousy) when the paper [1] by Bryer appeared. What the author has done is to immobilise yeast suspensions thereby producing 'yeast balls' and she uses these in solutions of hydrogen peroxide to test for catalase activity. In principle the advantages of such an approach include:

- consistent numbers of yeast cells can be trapped/immobilised;
- the number of yeast cells in a single yeast ball can 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 which follow are, then, largely based on Bryer's work [1] but we have adapted them to use the same apparatus as we would utilise for producing immobilised algae [6]. In due course our detailed protocols will appear on the SSERC website (www.sserc.org.uk).

Procedure

The basic protocol which we have adopted will be familiar to those of you who routinely immobilise algae for use in photosynthesis/respiration experiments follows below.

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).

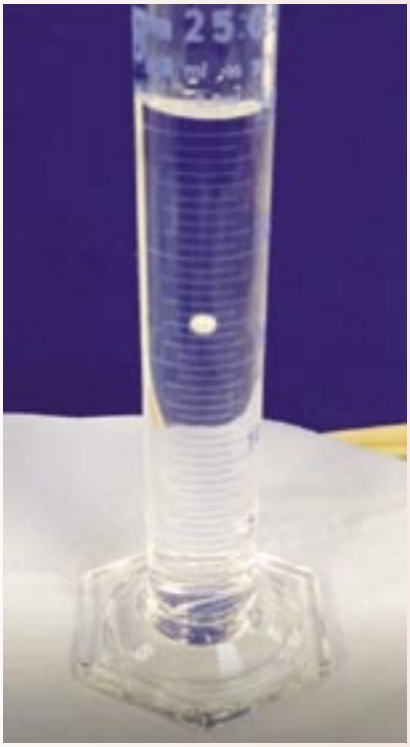

Figure 3 *- Immobilised yeast ball in hydrogen peroxide solution.*

4) Allow the liquid to flow, the drops form balls of immobilised yeast which 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 used in the experiments which follow.

Results

The experimental set-up for measuring catalase activity is shown in Figure 3. A stock solution of hydrogen peroxide (10 vol) is prepared (see hydrogen peroxide entry in Hazardous Chemicals Database for instructions on doing this safely) and stored in the fridge when not in use. Dilutions of this hydrogen peroxide stock are prepared and added to a measuring cylinder (for convenience we use one with a 25 cm^3 capacity).

A single immobilised ball of yeast is placed in the cylinder and the time taken for the ball to sink to the bottom 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 fresh balls using the same hydrogen peroxide solution. Typical results for immobilised yeast balls are shown in Table 1.

As one might have predicted, lowering the concentration of hydrogen peroxide leads to an increase in the time taken for the fall and rise of the ball.

Concentration of H_2O_2 in measuring cylinder	Temperature*	Time taken to fall and then rise to the surface (s)
1 vol	19°C	9, 9, 10, 11, 11
0.2 vol	19°C	24, 25, 26, 26, 27
1 vol	4°C	15, 16, 17, 17, 18

Table 1 *- Time taken for immbolised balls of yeast to fall and rise in solutions of hydrogen peroxide.*

** For experiments at 4°C a stock solution of 10 vol H_2O_2 was left in a fridge overnight and diluted to the final concentration using distilled water also stored at 4°C. Balls of immobilised yeast were covered with distilled water and stored in a fridge at 4°C for one hour prior to use.*

This observation opens up the possibility of investigating the [substrate] 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 and this offers up the intriguing possibility of an investigation to measure the effect of temperature on catalase activity. We have not yet tried to study the effect of pH but in principle this would be a relatively straightforward set of experiments.

Material (g)	Volume of water added (cm^3)	Time taken to fall and then rise to the surface (s) [Mean of 5 measurements, rounded to nearest 5 seconds]
Potato	75	75
Banana	75	175
Cucumber	75	75
Blueberry	50	> 180
Frozen peas	100	> 180

***Table 2** - Time taken for immobilised balls of immobilised extracts from fruit/vegetable 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.*

In an attempt to extend the range of materials which are immobilsed and tested for catalase activity we have tested a number of different materials. For the list which follows we made a crude extract by adding a given mass of the material with some distilled water and subjected the mixture to homogenisation 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.

***Figure 4** - Innocent Smoothies™.*

We also tried out some immobilised algae which 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 when we found that they retained catalase activity.

We were very excited when one of the Biology team spotted the list of ingredients on 'Innocent Smoothies™' (Figure 4). So, for example the 'Invigorate' bottle lists:

- 4 pressed apples
- 18 white grapes
- 1.5 mashed bananas
- 0.5 crushed mango
- 0.5 crushed kiwi
- some squeezed cucumber juice
- a dash of spirulina extract
- some milled flax seeds
- a squeeze of lime juice
- a dash of safflower extract
- a dash of matcha green tea infusion
- some milled wheatgrass
- some vitamins (B1, B2, B3, B6 and E)

Having centrifuged a sample and immobilised the supernatant we discovered that there was little or no catalase activity. Given the list of ingredients this was a bit of a surprise until we read the 'small print' only to discover that such smoothies are gently pastuerised as part of the manufacturing process and presumably all catalase is inactivated at that point.

Conclusions

We think the methods described here, based on the work of Bryer [1], offer lots of opportunities for student investigations. Much of our work is at the 'proof of concept' stage and we will undertake further experiments over the coming months and hope to report on those in future issues of the Bulletin. We make one observation here in respect of possible control experiments which 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 studies by Cheetham and Bucke [7] 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 e.g. glucose oxidase do not appear to diffuse out of the matrix [7]. Such differences cannot easily be explained on the basis of 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...

References

[1] Bryer. P. (2016), A twist on measuring catalase, *Science Teacher*, **83**, 69-73.
[2] SSERC (2010), Effervescent Enzymes, *SSERC Bulletin*, **234**, 9-10.
[3] Delpech, R. (2003) Microscale investigations with catalase. Available at http://www.saps.org.uk/secondary/teaching-resources/293-student-sheet-24-microscale-investigations-with-catalase (accessed 13th November 2016).
[4] SSERC (2012), Immobilised algae and respiration, *SSERC Bulletin*, **238**, 11-12. Available at http://www.sserc.org.uk/images/Bulletins/238/Immobilised_algae_and_respiration.pdf (accessed 13th November 2016).
[5] Madden, D. (2015), Better milk for cats: immobilised lactase used to make lactose-reduced milk. Available at http://www.ncbe.reading.ac.uk/MATERIALS/Enzymes/PDF/Catmilk1.5_UK_eng.pdf (accessed 13th November 2016).
[6] Immobilised algae - laboratory protocol. Available at http://www.sserc.org.uk/index.php/biology-2/biology-resources/biology-national-4/n5-cell-biology/3400-photosynthesis (accessed 13th November 2016).
[7] Cheetham, P.S.J. and Bucke, C. (1989), Simple visual demonstrations of the catalytic activity of immobilized cells and enzymes. In *Practical Biochemistry for Colleges* (E.J. Wood ed.), pp 7-8, Pergamon Press, Oxford.

Evelyn Lee

Evelyn Lee joined the SSERC technician team on a part time, temporary basis in January.

Having worked at SSERC on various occasions in the past Evelyn is very happy to be returning to a place she knows and loves!

Evelyn's first job as a technician was at Stewart's Melville College in Edinburgh where she spent several happy years in the physics department. While at Stewart's Melville, Evelyn was seconded to SSERC and re-joined the staff when she was ready to return to work after starting her family. Leaving again to have another baby and spend some extended time with her young children Evelyn is delighted to be back "I have now been lucky enough to be welcomed back for a third time. It would seem they can't get rid of me!"