Catalase activity in immobilised yeast - effect of inhibitors

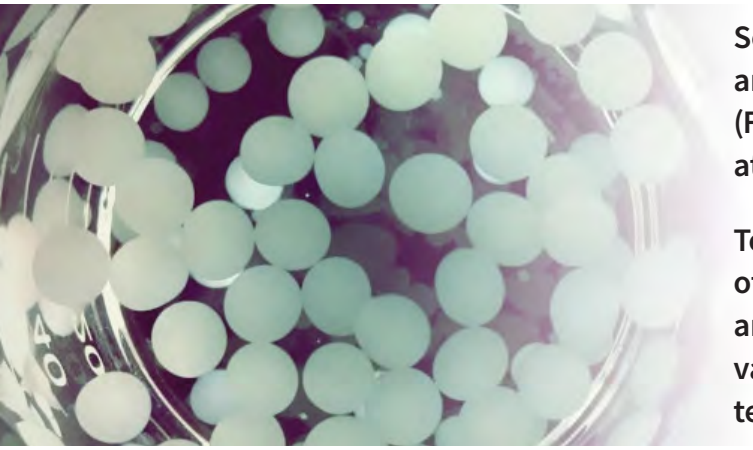

Figure 1 - Immobilised yeast (see [1] for preparation method).

Several schools have indicated to us that they are using catalase activity in immobilised yeast (Figure 1) as the basis for student investigations at both National 5 and Higher.

Teachers and technicians tell us that the simplicity of the experimental system, the low costs involved and the inherent scope for changing a number of variables (principally substrate concentration, temperature and pH) all allow for flexibility.

We have previously published protocols which describe the basic techniques involved in the preparation of immobilised yeast [1, 2]; our protocols are based on the work of Bryer [3].

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 a piece on the National Centre for Biotechnology Education website [4]:

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).

However, we tried a series of protocols in which we added both ethanol and/or methanol to see if 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 [1] and then added copper sulfate to the measuring cylinder to provide a range of different concentrations. We saw no difference in the time taken even at relatively high copper concentrations. It should be noted also 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 >>

	Time taken (/s)				
Copper sulfate concentration (/mol dm^{-3})	Run 1	Run 2	Run 3	Run 4	Mean of Runs 1-4
0.00	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

Table 1 - Time taken for immobilised balls of yeast 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.

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 is presented in Table 1 and plotted graphically in Figure 2. What can be seen is that increasing copper sulfate concentration does indeed lead to catalase inhibition. Such observations open up the possibility of a range of other experiments which 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 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.

Please send any feedback on this article to biology@sserc.scot. <<

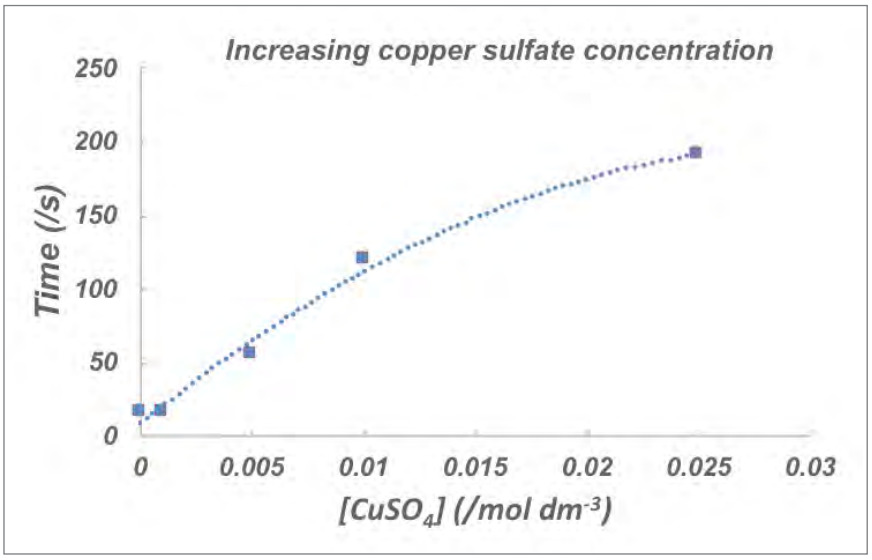

Figure 2 - Data from Table 1.

References

[1] SSERC (2017), Hydrogen peroxide and immobilised yeast, SSERC Bulletin, **258**, 10-13. Available at http://info.sserc.org.uk/images/Bulletins/258/SSERC_S258p10_13.pdf (accessed 14th September 2018).

[2] SSERC (2018), Catalase activity in immobilised yeast – an update, SSERC Bulletin, **264**, 9. Available at https://www.sserc.org.uk/wp-content/uploads/Publications/Bulletins/264/SSERC-bulletin-264webp9.pdf (accessed 14th September 2018).

[3] Bryer. P. (2016), A twist on measuring catalase, *Science Teacher*, **83**, 69-73.

[4] NCBE (2018), Enzymes for Education: Catalase. Available at http://www.ncbe.reading.ac.uk/MATERIALS/Enzymes/catazyme.html (accessed 14th September 2018).