Enzyme activities for BGE

The Biology Team at SSERC has decided to have another look at some activities which we think might be suitable for the study of enzymes at BGE, or National 4 Biology, where learners are required to take part in practical activities which illustrate the, "…properties of enzymes and their use in industries" [1].

We believe that the experiments described are particularly useful for enzyme study with younger pupils for the following reasons:

- They provide a strikingly visual illustration of the degradation of starch by enzymes.
- They show that in the presence of barley grains starch is broken is down thus allowing learners to conclude that the enzymes responsible are present in living cells.
- They clearly demonstrate the effects of temperature and pH on enzyme activity.

The method uses diastase solution added to starch-agar in Petri dishes to demonstrate the breakdown of starch by diastase. The term 'diastase' could be misleading. 'Diastase' usually refers to a mixture of enzymes found in germinating barley seeds, principally α-amylase and β-amylase, which bring about the hydrolysis of starch to fermentable sugars. So, diastase is not the name of a specific enzyme; it could refer to any one of a group of plant amylase enzymes. However, it is reasonable for learners to describe diastase as plant amylase. For these experiments we used Alpha-amylase (*Termamyl*®) purchsed from NCBE [2].

A well is cut in the starch-agar using a cork borer. The well is filled with enzyme solution and left at room temperature. After 24 hours the dish is flooded with iodine solution which becomes blue-black except in the area around the well where the enzyme solution has diffused through the gel and has broken down the starch (Figure 1).

The technique can be used in variety of ways to demonstrate some properties of enzymes. Here we suggest three:

1) The effect of diastase solution is compared to the effect of germinating barley seeds and boiled germinating barley seeds.
2) A series of plates placed in different temperatures for 24 hours is used to investigate the effect of temperature on the activity of diastase.

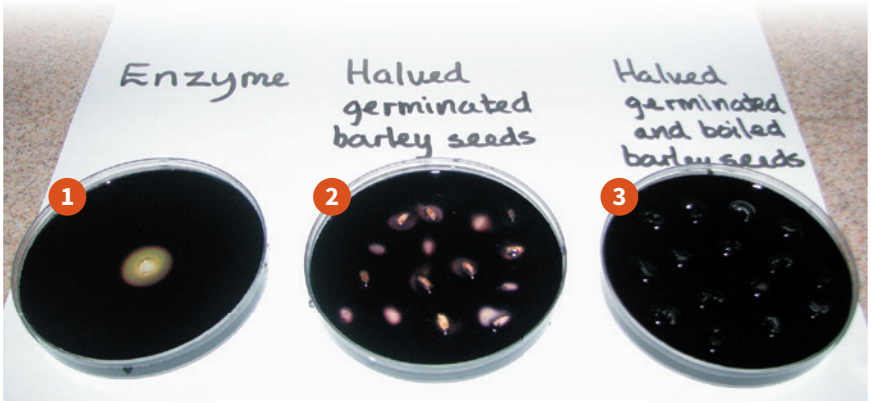

Figure 2

Figure 1 - The left hand well acts as a control by using distilled water instead of enzyme solution.

3) Diastase solution in a variety of pH buffers is used to investigate the effect of pH on the activity of diastase.

Activity 1 - Do barley seeds contain enzymes?

The aim of this activity is to provide a simple method by which pupils can conclude that diastase breaks down starch and that living cells present in germinating barley seeds produce the same substance (Figure 2). The seeds are soaked for 48 hours. Some of the seeds are boiled (for about 10 minutes). The seeds are halved and placed on top of the starch-agar. In this set-up you could include a plate containing boiled enzyme.

Plates 1 and 2 contain diastase and barley grains respectively. When flooded with iodine, after 24 hours incubation, areas of clearing can be seen around the well containing diastase and the barley grains. There are no areas of clearing on Plate 3 where the cells of the barley have been killed by boiling, and the enzyme is no longer active. This can later be related to an understanding of denaturation of enzymes by high temperatures. >>

Figure 3 - 1) Barley mush. 2) Germinating barley seeds. 3) Soaked and boiled barley seeds. 4) Distilled water.

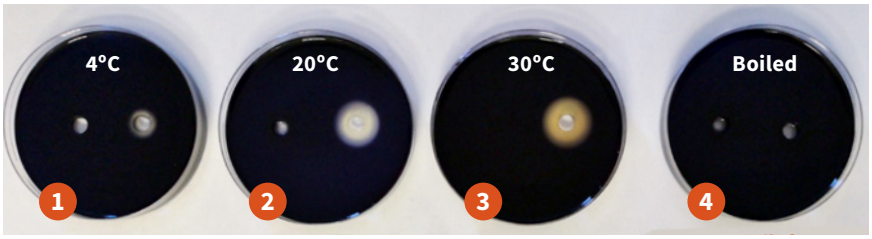

Figure 4 - 1) Fridge 4°C. 2) Room temperature 20°C. 3) Oven 30°C. 4) The 4th plate, containing water and boiled enzyme solution, is set up and left at room temperature.

The set-up in Figure 3 includes 'barley mush' created by grinding up several soaked barley seeds in distilled water and filtering the 'mush'. This might form the context for learners investigating the use of barley in the brewing industry and the role of 'malting' barley.

Activity 2 - The effect of temperature on the activity of diastase

Starch-agar plates each containing enzyme solution in the right hand well and distilled water, as a control, in the left hand well are set up and left for 24 hours at various temperatures, then flooded with iodine solution as illustrated in Figure 4.

The advantage of using this method to investigate enzyme activity is that, once the plates have been flooded with iodine solution, they can be rinsed and set out on a white surface in order starting with the one stored at the lowest temperature. Learners can easily see and compare the size of the clear zones. This provides an immediate and visual illustration of the relationship between enzyme activity and temperature. The effect of very low, or very high, temperature is evident. The fact that there is an 'optimum' temperature for the activity of an enzyme and that enzymes are denatured above a certain temperature can be introduced.

Setting the plates up as illustrated above also gives scope for discussion of experimental design. The use of controls, dependent and independent variables, method of measuring and recording results, and drawing conclusions can be discussed.

The appearance of plates after storage for 24 hours at different temperatures, followed by flooding with iodine solution can be recorded on diagrams, or by using digital/phone cameras, to provide a visual record of the results.

Quantitative data can be gathered by measuring the diameter of the clear zone at each temperature. Diameter of clear zone (mm) can be graphed against temperature (0°C) and used to draw conclusions about the effect of temperature on enzyme activity.

Activity 3 - The effect of pH on the activity of diastase

Diastase solution in a range of buffer solutions is set up in starch agar wells and stored for 24 hours at room temperature.

The enzyme at each pH will produce areas of clearing of different size. The appearance of the plate on flooding with iodine solution after storage at room temperature for 24 hours, allows pupils to conclude that enzyme activity is influenced by pH of the surrounding solution (Figure 5).

Protocols, a Teacher's Guide and a Technical Guide for the activities described here can be found on the SSERC website [3].

The protocols described here are based on activities described in *The Science of Life*, Strathclyde Biology Group, 1970. <<

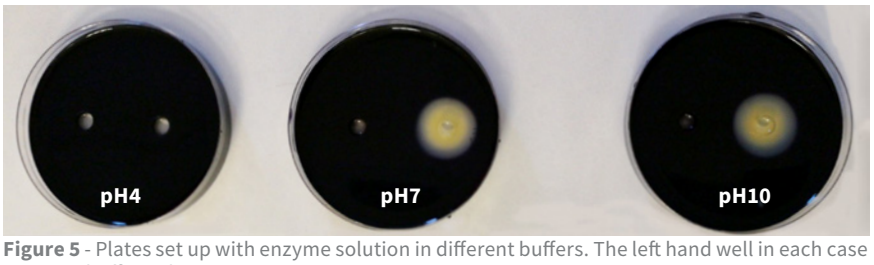

Figure 5 - Plates set up with enzyme solution in different buffers. The left hand well in each case contains buffer only.

References

[1] National 4 Biology, https://www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_N4_Sciences_Biology.pdf.
Sciences Experiences and Outcomes, https://education.gov.scot/Documents/sciences-eo.pdf.

[2] http://www.ncbe.reading.ac.uk/MATERIALS/PDF/NCBEpricelist.pdf.

[3] https://www.sserc.org.uk/subject-areas/biology/biology-national-4/properties-of-enzymes-and-use-in-industries2/.