

The Breakdown of Starch by Diastase

Teacher Notes

The following protocols are based on activities described in *The Science of Life*, Strathclyde Biology Group (1970).

These simple enzyme activities can be used to develop understanding of enzyme properties at various levels in the curriculum. The activities are based on the action of diastase on starch.

diastase



NB The term 'diastase' could be misleading. 'Diastase' usually refers to a mixture of amylase enzymes found in germinating barley. These enzymes change starch to maltose. So 'diastase' is not the name of a specific enzyme, it could refer to any one of a group of amylase enzymes. However, it is reasonable for pupils to describe diastase as plant amylase.

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

- They provide a strikingly visual illustration that diastase catalyses the degradation of starch.
- They show that barley grains can break down starch by enzyme action and allow the pupils to conclude that the enzyme diastase actually occurs in living cells.
- They clearly demonstrate the effects of temperature and pH on diastase activity.

The method uses diastase solution added to starch agar in Petri dishes to demonstrate the breakdown of starch by diastase. Starch reacts with iodine to form a blue-black colour. Maltose, the product of the reaction, maltose does not react with iodine.

A well is cut in the starch-agar using a cork borer. The well is filled with diastase 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 diastase has diffused through the gel and has broken down the starch.



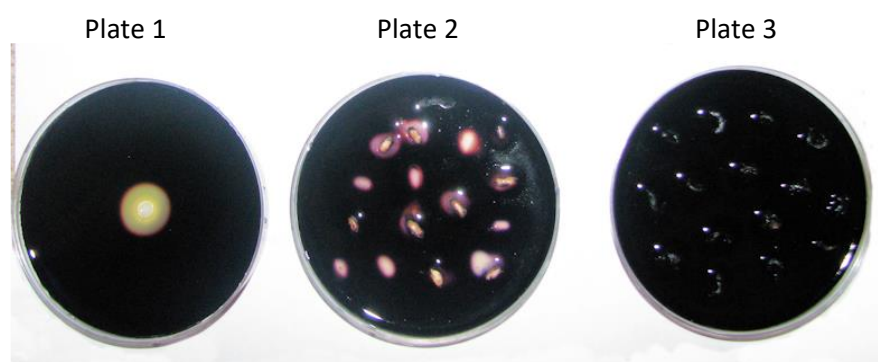
The results of investigations carried out using this method can easily be recorded by pupils using digital / phone cameras or on simple diagrams representing Petri dishes showing which areas turn blue-black and which remain clear when iodine solution is added.

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, demonstrating that diastase is denatured by boiling.
2. A series of starch-agar plates placed in a variety of situations is used to investigate the effect of temperature on the activity of diastase.
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. 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 present. This can later be related to an understanding of denaturation of enzymes by high temperatures.



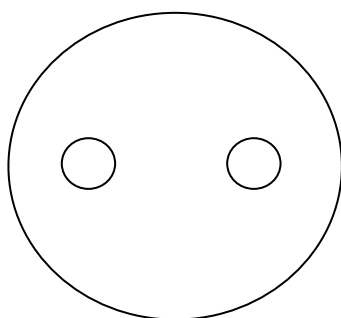
diastase solution

*halved germinating
barley seeds*

*germinating barley seeds-
halved and boiled*

Having understood that the clear zones evident when the plates have been flooded with iodine solution are an indication of enzyme activity, pupils can then set up the following activities which use the diameter of the clear zones in a series of plates as a measure of enzyme activity in different conditions.

Activity 2. The Effect of Temperature on Diastase



water

enzyme solution

Three plates are set up and stored for 24 hours as follows:

1. Fridge; 3⁰ C
2. Room temperature; 20⁰ C
3. Oven set at 35⁰ C

A fourth plate containing boiled enzyme solution is set up and left at room temperature.

Of course it is possible to include a larger number of plates in the range.

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. Pupils 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 temperature and 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.

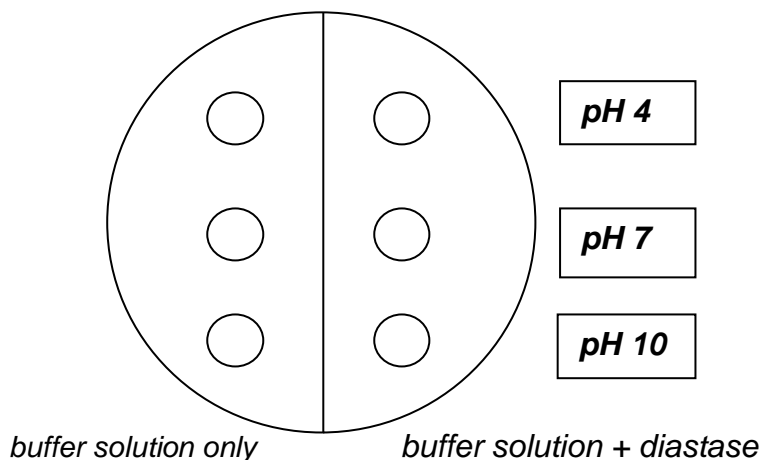
Setting the plates up as illustrated above also gives scope for discussion of experimental design. The use controls, dependent and independent variables, method of measuring and recording results, and drawing conclusions can be discussed.

The appearance of plates after storage for 24 hours at different temperatures, followed by flooding with iodine solution can be recorded on diagrams like the one above, or by using digital / phone cameras, providing 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 (°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

In this activity diastase in a range of buffer solutions is set up in starch agar as illustrated below 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 for 24 hours, allows pupils to conclude that enzyme activity is influenced by the surrounding pH.

Again pupils can discuss the reason for the inclusion of wells containing only buffer solutions. Alternative ways of using this basic design to investigate pH can be explored.

Further activities

- The method suggested above for illustrating the effect of temperature and pH on enzyme activity could be used to illustrate the effect of enzyme concentration on activity. This can be done using a variety of diastase concentrations set up in wells cut in one starch-agar plate, or by using a series of plates with wells containing different enzyme concentrations.
- It is possible to grind up germinating barley grains with water to create a 'mush' containing diastase which can be placed in wells and used in various experiments. Again pupils could explore experimental design using this method. For example, a fourth plate could be added to Activity 1 as a starting point for considering possible investigations.
- Other experimental methods of investigating enzyme properties can be explored.
- Experiments using enzymes other than diastase can illustrate that the pH optimum is different for different enzymes.
- Pupils could research the use of germinating barley seeds in the brewing industry.
- The action of diastase (a plant amylase) can be compared to the role of amylase in human digestion.
- Pupils could research the ubiquitous nature of enzymes and their importance to sustaining life.