University of Reading

Using

Enzymes in school

National Centre for Biotechnology Education

Enzyme activity units

The internationally-recognised unit of enzyme activity is the Katal (abbreviated to kat). It is defined as the enzyme activity which transforms 1 mole of substrate per second under optimal conditions.

In practice, few enzyme suppliers use the Katal, preferring instead units that reflect the preferences of their typical customers. For example, bakers are familiar with amylase activity expressed in 'SKB units', whereas brewers traditionally refer to amylase activity (which they call diastatic activity) in 'Degrees Lintner'. To obtain these various measures of enzyme activity, different assay methods have been used, and unfortunately there is no means of comparing dissimilar tests without conducting practical tests.

Planning enzyme tests

A solution to this problem is to develop your own tests. This is not as difficult or time-consuming as it might seem, and will almost certainly save you time (and money) in the long run.

Step 1
Define your operating conditions

Identify the pH and temperature likely to occur in your proposed investigation. For example, a simple starch digestion experiment might use a neutral starch suspension in a test tube at room temperature.

Decide how you are going to monitor the course of the reaction. It may be a colour change (like starch and iodine solution); or it may depend upon chemical tests such as glucose test strips.

Specify how long you wish the reaction to take. Sometimes it is useful if a reaction can occur quickly so that it fits comfortably within a practical session. Alternatively, you may want the reaction to proceed relatively slowly so that samples can be taken at convenient intervals to follow its course.

Step 2
Consult the enzyme data sheet

Study the data sheet supplied with the enzyme or the information on the NCBE website. The methods used to measure the enzyme's activity mentioned there may be similar to your proposed practical. For example, the data sheet supplied with *Novozymes Celluclast®* suggests the amount of enzyme needed to break down waste paper to fermentable sugars (*e.g.*, you might be trying to produce glucose from waste paper).

Study the graphs describing the enzyme's activity and stability. Very often the temperature and pH at which a particular enzyme works best are not those at which it is most stable. In practice this means that, for example, you may have to use a protease at less than its optimum temperature or pH (and therefore sacrifice speed) to ensure that it remains stable over the period of the investigation.

Alternatively, a small degree of hydrolysis may be achieved at a high temperature before the enzyme is inactivated. Should either of these approaches prove unsatisfactory, the enzyme dosage can be increased.

Step 3
Produce a plan based on your requirements and information from the data sheet

To start with, it is often easier to use a relatively high enzyme concentration at sub-optimal pH and temperature, then to reduce the amount of enzyme once you know that the reaction is going to proceed within the time you have available.

Step 4
Conduct the practical tests

If 'bucket chemistry' doesn't work, or the results of the reaction aren't very obvious, you may need to modify your procedure.

Continued overleaf...

Pushing enzymes to their limits

Sometimes it is not possible to achieve an optimum environment for enzyme activity in the classroom. Perhaps a water bath is not available, or it is difficult to accurately adjust the pH of the substrate. However, enzymes can and do operate under non-ideal conditions, and it may be useful to bear the following points in mind.

Sometimes lowering the temperature will broaden the effective pH range of an enzyme. For example, *Novozymes Lactozym*® will hydrolyse the lactose in acidic whey from home made soft cheese, at room temperature, even though the enzyme's optimum pH is around 7.0.

Consider high temperature/rapid processing. The Q1O rule states that for every 10 °C rise in temperature, the enzyme will react twice as fast. Of course, this is only true up to a point *i.e.*, until the enzyme is denatured, but substantial catalysis can still be achieved in a short time if lesson timing demands it *e.g.*, washing powder proteases can clear particular types of photographic film in just five minutes at 65 °C.

Increase the substrate concentration. High levels of substrates tend to stabilise enzymes, even under non-ideal conditions. The enzyme optimum and stability curves on *Novozymes* data sheets are often obtained from reactions where the substrate concentration is 10% w/w or less. If you use substantially greater levels of substrate, you may be able to raise the temperature or reduce the enzyme dose. Note: Some enzymes, such as invertase, are *inhibited* by high substrate concentrations.

Please note: This is general advice from the NCBE on the use of enzymes for school investigations. It is not intended as a guide to industrial or research practice.