BIOCHEMISTRY:
Working with Enzymes

Introduction
Enzymes are nature's catalysts. Their role is to speed up reactions that would otherwise be too slow for normal metabolic processes. Being catalysts, they participate in a reaction, but are not consumed. However, their effectiveness can be reduced by changes in temperature and pH. These characteristics make them very interesting to study, but they can be difficult to deal with. This document presents some background information and ideas to help you run successful enzyme pracs.

How Enzymes work
Essentially, enzymes lower the activation energy of a biochemical reaction, thus increasing the rate at which it occurs. There are various mechanisms by which they can do this, but the common factor is for the enzyme to form a complex with the substrate by binding to it in some way. In this situation, the reaction proceeds quickly and the reaction products are released, thus freeing the enzyme to bind to another unit of substrate for the process to be repeated.

Types and Sources of Enzymes
It is tempting to think of enzymes as discrete chemical entities with constant chemical properties. However, this can be misleading because enzymes are proteins that can vary in structure and the way they fulfill their catalytic purpose. For example, amylase is often described as an enzyme that breaks down starch into sugars. However, there are at least three different types of amylase in the human digestive system, and a multitude more in other organisms that metabolise starch. They are collectively referred to as amylase because of their common purpose – the breakdown of starch – but they can be significantly different biochemicals. Most commercial enzymes these days are derived from bacterial or fungal cultures that can be grown in large bioreactors to maximize the yield. Rather than purifying the product to a high degree, commercial enzymes are usually mixed with an inert diluent to minimize variation between batches. The efficacy of enzymes is usually described in terms of their "activity", or the quantity of substrate they can convert in a given time. This is more meaningful than "concentration" when comparing two similar enzymes. For example, it makes no sense to describe two types of amylase at the same concentration as being equivalent if one is ten times more active than the other.

Protein Structure, Stability and Denaturation
The three-dimensional folded structure of enzymes can be disrupted in some circumstances. In many cases, minor structural change occurs when the enzyme binds to the substrate. The enzyme unfolds to some extent in order to form a complex that has a lower energy state. Once the reaction is over, the reaction products leave the site and the enzyme reverts to its original shape until its next encounter with a substrate molecule, when the process repeats.When the three-dimensional shape of a protein is disrupted, it is said to have been denatured. When the structural change is permanent, it is described as irreversible denaturation. This is what happens when a protein is subjected to high temperatures, such as in cooking. However, when the structural change is temporary, it is described a reversible denaturation. This can occur with small shifts in pH or temperature. Denaturation is the reason that enzymes have a particular temperature and pH range at which they function optimally. Once the conditions move outside the optimal range, the enzymes have been denatured to such an extent that they are no longer able to bind to the substrate. As you might expect, mammalian enzymes usually have an optimal operating temperature of 37°C and a pH range that matches the environment where they are situated. For example, the three types of human amylase prefer neutral to acidic conditions, depending on whether they originate from saliva, the oesophagus or the stomach.

Many industrial processes require enzymes that can operate at higher temperatures and extremes of pH, so it is common for commercial enzymes to be selected for their tolerance of extreme conditions. Research efforts are being directed at isolating and culturing microorganisms that live in unusual environments such as hot springs – the so-called “extremophiles”.

What Can Go Wrong During Enzyme Pracs?

Like all biochemical reactions, experiments involving enzymes are prone to problems that can arise from many sources. Here are some examples of problems we have seen:

- **Reagents aged:** As enzymes age, they are subject to deterioration, especially if they are stored inappropriately. For example, if they have been exposed to light or moisture, or left too long at the wrong temperature. However, in many cases, the enzyme is still usable, although some changes to the experimental protocol may be required to accommodate the reduced activity.
- **Incorrect concentrations:** To get a reaction in a suitable time frame, you should ensure that the concentrations of the enzyme and the substrate are correct. Although enzymes are catalysts and therefore able to react many times, overall reaction rates can be increased by using more enzyme because it allows more substrate molecules to participate at any given time.
- **Incorrect reaction conditions:** Since enzymes react in a particular “window” framed by temperature and pH, it is important to control these variables.
- **Changing the source:** Different sources of enzyme or substrate can affect the outcome of enzyme reactions, so carry out a check whenever a new package is opened.

Always check the enzyme reaction ahead of time to ensure it will work as expected for your students. If necessary, adjust the conditions (concentration, temperature, pH) to get a result in a suitable time frame. Enzyme reactions described in texts tend to generalize, so always allow sufficient time to check the results and make any adjustments that might be necessary.

An Introduction to Various Enzyme Reactions

This brief introduction to a number of enzyme reactions provides a quick reference to experiments that have been devised for use by students.

Amylase (clarase and diastase):

Amylase acts on starch to break it down to simple sugars. There are many different forms of commercial amylase, several of which can react at relatively high temperatures. In some cases, the type of starch can also affect the rate of reaction significantly. If you are going to follow the reaction by monitoring the disappearance of starch (colorimentric reaction with iodine), the presence of sugars in the starting materials is not important. However, if you are going to follow the reaction by confirming the appearance of sugars as reaction products (testing for reducing sugars with Benedict’s solution), you should use a form of amylase that is free of sugars so you can be sure that any sugars you detect are true reaction products rather than impurities in the starting materials.

$$\text{Starch} \xrightarrow{\text{Amylase}} \text{Simple sugars}$$

- **Description:** Light coloured powder (clarase). Cream to white powder (diastase).
- **Action:** The enzyme amylase acts on a starch substrate and breaks it into soluble sugars.
- **Storage:** Store in the refrigerator at 4°C.
- **Source:** Amylase is derived from Fungi; *Aspergillus oryzae* (clarase) and *Aspergillus spp* (diastase).
- **Safety:** Enzymes are biologically active proteins. Avoid direct contact or inhalation.

Catalase:

Catalase plays a protective role in cells by mopping up hydrogen peroxide that can arise as a harmful by-product of some biochemical reactions. Hydrogen peroxide is rapidly converted to water and oxygen in the presence of catalase. Monitor the reaction by collecting the oxygen gas that is evolved. When testing natural products that contain catalase (such as potato or liver), you can influence the rate of reaction by varying the particle size of the enzyme source. Smaller particles have higher specific surface area and therefore expose more enzyme to the substrate.

$$\text{Hydrogen peroxide} \xrightarrow{\text{Catalase}} \text{Water + Oxygen}$$

- **Description:** Brown Liquid.
- **Action:** The enzyme Catalase acts upon hydrogen peroxide in cells to produce oxygen and water.
- **Storage:** Store in the refrigerator at 4°C.
- **Source:** Catalase is derived from Fungi, *Aspergillus niger*.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact.

Cellulase:

Plant-eating animals from cattle to termites rely on bacteria in their digestive tracts to break cellulose down into simple sugars with the help of the enzyme cellulase. Cellulase is also behind some of the current efforts to derive alternative "bio-fuels" from plants. Use filter paper as the substrate and observe how it disintegrates in the presence of cellulase. You can also use Benedict's solution to test for the formation of reducing sugars in the reaction mixture.

$$\text{Cellulose (Plant fibre)} \xrightarrow{\text{Cellulase}} \text{Simple sugars}$$

- **Description:** Amber liquid.
- **Action:** The enzyme cellulase acts upon cellulose fibres to break them down into simple sugars.
- **Storage**: Store in the refrigerator at 4°C.
- **Source:** Cellulase is derived from a species of fungi, *Trichoderma reesei*.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Invertase:

Invertase is a type of sucrase. It is known to occur in plants and some micro-organisms, and in school science experiments it can be used as a substitute for the human digestive form of sucrase that is found in the small intestine.

$$\text{Sucrose} \xrightarrow{\text{Invertase}} \text{Glucose and fructose}$$

- **Description:** Amber coloured liquid.
- **Storage:** Store in the refrigerator at 4°C.
- **Source:** Invertase is derived from yeast, *Saccharomyces cerevisiae*.
- **Shelf Life:** When stored correctly, invertase has a nominal shelf life of 12 months.
- **Safety:** Invertase is rated as safe for food contact. Nevertheless, enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Lactase:

Lactase is a mammalian enzyme that digests lactose, a disaccharide found in milk. In humans, lactase is present in infants but for many people, the gene that regulates its expression is switched off in early childhood, leading to lactose intolerance.

$$\text{Lactose} \xrightarrow{\text{Lactase}} \text{Glucose and galactose}$$

- **Description:** Amber coloured liquid.
- **Action:** Lactase hydrolyses lactose into glucose and galactose.
- **Storage:** Store in the refrigerator at 4°C.
- **Source:** Lactase is derived from Yeast, *Kluyveromyces lactis*.
- **Shelf Life:** When stored correctly, it has a nominal shelf life of 12 months.

Lipase:

Lipase breaks fats (lipids or triglycerides) down by cleaving the fatty acid residues off the propylene glycol backbone. A convenient source of fats for this experiment is full cream milk, and the progress of the reaction can be followed by monitoring the change in pH as the concentration of acid rises. Bile salts can play a role in accelerating the reaction.

$$\text{Triglycerides and Lipids} \xrightarrow{\text{Lipase}} \text{Fatty acids}$$

- **Description:** Fine powder, off-white in colour.
- **Storage:** Store in the refrigerator at 4°C.
- **Shelf Life:** When stored correctly, it has a nominal shelf life of 12 months.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Pancreatin:

Pancreatin is a mixture of digestive enzymes. It contains amylase (acts on starch to form sugars), protease (breaks down proteins to amino acids), and lipase (converts fats to fatty acids). The pancreas produces many other digestive enzymes, as well as secretions of sodium bicarbonate that help neutralise stomach acid in the small intestine.

$$\text{Starch} \xrightarrow{\text{Amylase}} \text{Simple Sugars}$$

$$\text{Protein} \xrightarrow{\text{Protease}} \text{Amino acids}$$

$$\text{Fats} \xrightarrow{\text{Lipase}} \text{Fatty acids + Glycerol}$$

- **Description:** Cream coloured powder.
- **Action:** There are three principal enzyme components of pancreatin responsible for
- the digestion of starch, protein and fats.
- **Storage:** Store in the freezer.
- **Source:** Pancreatin is derived from Porcine pancreas.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Pectinase:

Pectinase breaks down pectin, a naturally occurring polysaccharide that acts as a thickening agent in its colloidal form. Pectinase is commonly used to degrade plant materials as mixing pectinase with apple causes the pectin to lose its colloidal properties. By opening glycosidic linkages, pectinase splits polygalacturonic acid into monogalacturonic acid. This causes the cell wall to soften and therefore increases the yield of juice that may be extracted from the fruits.

$$\text{Colloidal Pectin} \xrightarrow{\text{Pectinase}} \text{Non-colloidal Pectin}$$

- **Description:** Brown liquid.
- **Action:** The enzyme Pectinase acts upon Pectin, a complex carbohydrate found in ripe fruit.
- **Storage:** Store in the refrigerator at 4°C.
- **Source:** Pectinase is derived from Fungi, *Aspergillus niger*.
- **Safety:** Enzymes are biologically active proteins. Avoid direct contact or inhalation.

Pepsin:

Pepsin is a digestive enzyme that is found in many organisms. It comes in many different forms, but in every case, its function is to aid digestion by breaking proteins down via hydrolysis into their component amino acids. breaks down proteins into smaller peptides (that is, a protease). Pepsin is produced in the stomach and is one of the main digestive enzymes in the digestive systems of humans.

$$\text{Protein} \xrightarrow{\text{Pepsin}} \text{Amino Acids}$$

- **Description:** Fine powder, off-white in colour.
- **Source:** Derived from Porcine pancreas.
- **Storage:** Store at room temperature (15 – 30°C).
- **Shelf Life:** When stored correctly, this product has a nominal shelf life of 12 months.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Protease:

Protease breaks proteins down into their constituent amino acids. It is used in many industrial processes, and is often present in commercial washing powders. As a laboratory exercise, you can add a protease solution to gelatine and observe the liquefaction that occurs over several hours. Proteases are present in all forms of life and viruses. Different classes of protease perform the same reaction while employing completely different catalytic mechanisms.

$$\text{Protein} \xrightarrow{\text{Protease}} \text{Amino Acids}$$

- **Description:** Brown liquid.
- **Storage:** Store under refrigeration (4 – 5°C).
- **Source:** Protease is derived from a strain of *Bacillus licheniformis.*
- **Shelf Life:** When stored correctly, protease has a nominal shelf life of 12 months.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Rennet:

Rennet is a mammalian digestive compound that contains the enzyme rennin. Rennin coagulates casein in milk, causing it to separate into "curds" (solids) and "whey" (liquid). Junket is a powdered preparation of rennin used for cooking.

- **Source:** Rennet is a pure chymosin solution produced by fermentation on a vegetable substrate with *Aspergillus niger*.
- **Storage:** Keep rennet refrigerated and make up only as much working solution as you need for a prac session.

Trypsin:

Trypsin is another digestive enzyme that converts the milk protein, casein, to amino acids. Treating low fat milk with trypsin causes the white liquid to become clear as the protein is broken down into soluble amino acids. This activity is particularly suited to monitoring with a data-logging colorimeter. Light transmittance rises as the reaction proceeds.

$$\text{Protein (casein)} \xrightarrow{\text{Trypsin}} \text{Amino acids}$$

- **Description:** Off white powder
- **Action:** The enzyme Trypsin acts on a protein in milk called casein and breaks it down into amino acids.
- **Storage:** Store in the refrigerator at 4°C.
- **Source:** Trypsin is derived from Porcine pancreas.
- **Safety:** Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Conclusion

Enzyme experiments can be engaging for students because of their relevance to biological processes. It is also possible to link enzyme reactions with dataloggers in some cases. Being biochemical reactions, enzyme pracs are prone to being affected by many factors, but with an understanding of how they work and good preparation, enzyme pracs can be reliable, safe and lots of fun.