

# Working With Enzymes



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## 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 workshop 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 discreet 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 as 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”.

## **Monitoring the Progress of Enzyme Reactions**

Reactions involving enzymes can be monitored by following the disappearance of the substrate, the appearance of the reaction products, or both simultaneously. Students can take spot readings then tabulate and graph the results. However, where possible, using a datalogger allows direct comparison of the effect of changes in temperature and pH.

## **Enzyme Safety**

Enzymes are biologically active proteins that can be harmful, so it is prudent to handle all enzymes with care. In particular, avoid inhalation of dust when dealing

with enzyme powders, and wear gloves when handling solutions.

## **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.

### ***Recommendation***

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***

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 (colorimetric 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.

### ***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.

### ***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.

### ***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.

### ***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 neutralize stomach acid in the small intestine.

### ***Pectinase***

Pectinase breaks down pectin, a naturally occurring polysaccharide that acts as a thickening agent in its colloidal form. Mixing pectinase with commercial apple puree causes the pectin to lose its colloidal properties. Without the thickening effect of pectin, the puree becomes a low viscosity slurry.

### ***Rennet and Junket Powder***

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.

### ***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.

### ***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 datalogging colorimeter. Light transmittance rises as the reaction proceeds.

### ***Urease***

This enzyme is found in many plants, fungi and bacteria where it converts urea into an accessible source of nitrogen (ammonia) and carbon dioxide. In some environments, the presence of urease in soil bacteria can be a problem for farmers because it reduces the effectiveness of urea fertilizer by releasing the nitrogen before crop plants can absorb it. Urease is convenient for student pracs because they can follow the reaction by noting the increase in pH as the urea is converted to ammonia.

### **Conclusion**

Enzymes 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.