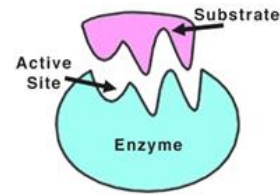


CATALASE LAB: CATALYTIC ACTIVITY OF ENZYMES

(Adapted from CIBT & Joslin/Boulay)



INTRODUCTION

Enzymes are biological molecules that **catalyze** (*speed up*) chemical reactions. You could call enzymes the “**builders & do-ers**” in the cell since, without them, life could not occur. Every cell makes hundreds of different enzymes to carry out the reactions necessary for life. Fortunately for the cell, enzymes are not used up when they catalyze a reaction; instead, they can be used over & over again.

The **DNA** in each cell encodes all the information needed to make its many different enzymes. Enzymes are relatively **LARGE** molecules of **protein**. They are produced when cells need them.

The molecule (or molecules) on which an enzyme acts is called its **substrate**. Enzymes are said to be very “**specific**” in that they recognize only one substrate (or a few closely related substrates) and convert it into a specific **product(s)**. You could say that each enzyme only performs one type of job. Each enzyme is specific because it is folded into a particular **3-D shape**. Within the folds of each enzyme is the **active site**, the place where the substrate fits and where the chemical reaction takes place.

Enzymes work **very quickly**, often catalyzing thousands of reactions per second. The rate at which an enzyme works is influenced by many factors including **temperature & pH**. Enzymes have a temperature and pH at which they work the best, and if an enzyme is exposed to extremes of heat or pH it won't work at all! The interactions that hold the protein in its particular shape become disrupted under extreme conditions, and the 3-D structure unfolds. In this case, the enzyme is said to be **denatured**. Other important factors that influence enzyme activity are the **concentration of substrate** and the **concentration of the enzyme**. Up to a point, the more substrate that is present, the faster the reaction. However, when the substrate concentration is so high that an enzyme is working as fast as it can, further increases of substrate concentration will have no effect on the rate of product formation.

BACKGROUND INFORMATION

The **enzyme** that you will study in this lab is called **catalase**. Its job is to break down its **substrate hydrogen peroxide** (H_2O_2), which is a naturally occurring poisonous by-product. Without catalase, H_2O_2 could kill the cell. The reaction catalyzed by catalase is:



The products remaining after catalase does its job are **oxygen gas** (O_2) & H_2O , two very non-poisonous molecules. In the home & hospital, hydrogen peroxide is used as an **antiseptic** to clean out wounds. Have you ever noticed that when hydrogen peroxide is swabbed on a cut it bubbles? This is because enzymes in the cut from your body (and from infecting bacteria) catalyze the rapid degradation of H_2O_2 into water & oxygen. The bubbles being released are O_2 bubbles.

Catalases are very common. They are found in almost all cells that grow in oxygen, including **potato tubers**. In this experiment, a blender is used to grind up a potato in water to release the catalase from the potato cells. The ground up potato is **filtered** through cheesecloth to separate potato skin and cell debris from the liquid, which contains most of the cell's enzymes - including catalase.

To actually measure catalase activity, small paper disks are dipped into potato cell **extract**. When this **enzyme-containing disk** is placed in a solution of hydrogen peroxide, the enzyme begins to work. As the **catalysis** occurs, oxygen gas is produced - and its bubbles become trapped in the **fibers** of the disk. When there are enough O₂ bubbles, they lift the paper disk to the surface. The speed with which the oxygen is produced depends **BOTH** upon how much enzyme is present & on the concentration of hydrogen peroxide (i.e., the **substrate**).

Teacher Demonstration of CONTROLS

Copy the following table into your lab book.

*****THINK***** about the importance of each control.

CONTROL #	Liquid in Well	Paper Disk Soaked In	Time for Disk to Float (sec)
1	3 ml 1% H ₂ O ₂	dH ₂ O	
2	3 ml dH ₂ O	100% Catalase	
3	3 ml 1% H ₂ O ₂	100% Boiled Catalase	

INQUIRY TIME: What other factors do you think can affect the rate of catalase's reaction?

Variables to choose from: **enzyme concentration, pH, substrate concentration, temperature, or salinity** ***We are doing enzyme concentration***

Open: AP Enzyme Concentration Protocol and copy the protocol into your lab book after the above table.