

What Conditions Influence Enzyme Activity?

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As a biology and chemistry teacher, I am always on the look out for labs that will visually demonstrate concepts that I am trying to teach in the classroom. One of the sticky subjects for my students involves catalysts and enzymes. As a result, a couple of the lab kits that I chose to evaluate center around enzymatic activity.

The Kits

The first lab kit was purchased from Ward Scientific. The kit (*What Influences Enzyme Activity?*) sells for \$56.95 (with a refill kit of \$34.95) and contains enough material for a class of 30 in groups of two. This kit will be extremely useful in helping students understand the conditions necessary for optimum enzyme activity. Variables are changed with regard to temperature, pH and enzyme concentration in order to determine the optimum conditions for enzyme performance.

The second kit was also purchased from Ward Scientific. The *Organelles* kit sells for \$67.00 and likewise contains enough materials for 30 students. One of the activities in this kit demonstrates how a cell organelle would catalyze a reaction using dialysis tubing and the enzyme *catalase* that is found in potatoes. The lab calls for short stretches of dialysis tubing to be stuffed with a potato 'slushie' made by processing a potato with ice shavings. The flimsy potato tubes are then placed in a hydrogen peroxide solution where two different enzymatic reactions will occur causing the tube to expand and the color of the potato to darken.

Background

Catalysts help to control the rate of chemical reactions by either speeding them up or slowing them down. Although they are an important factor in the ability of a chemical reaction to take place, they are unchanged and therefore a small amount of catalyst can be used and reused in a reaction.

Enzymes are protein molecules that act as catalysts of biological systems. They are, however, extremely specific and tend to act on only one substrate (the substance altered by an enzyme) changing it to only one product. This is called the 'lock and key' model and the human body uses hundreds of enzymes to regulate bodily functions. A variety of enzymes are used to catalyze the chemical reactions involved in digestion. Some of the digestive enzymes utilized by the body are:

- lactase – breaks down lactose (milk sugars)
- diastase – digests vegetable starch
- sucrase – digests complex sugars and starches
- maltase – digests disaccharides to monosaccharides (malt sugars)
- pepsin – breaks down proteins into peptides
- peptidase – breaks down small peptide proteins to amino acids
- trypsin – derived from animal pancreas, breaks down proteins
- lipase – breaks down fats found in most dairy products, nuts, oils, and meat

Factors that affect enzyme performance

Some of the factors that influence how an enzyme performs in a given situation include how much of the enzyme is available (enzyme concentration), as well as the temperature and pH conditions the enzyme is working under.

At a constant concentration of enzyme, the reaction rate increases with increasing substrate concentration until all of the available enzyme is joined with substrate. The reaction rate is maxed out and adding more enzyme or substrate will have no further effect on the rate.

Since the lock and key principle is dependant on the shape of the enzyme molecule and its ability to fit together with the molecule of the substrate, anything that affects that shape affects the rate of reaction. Since enzymes are proteins they are highly dependant on changes in temperature and pH.

As the temperature is raised or lowered, the shape of an enzyme is altered slightly. As a result of this change in shape, it can become either more or less effective as it may fit together better or worse with the substrate. This gives the enzyme a range of temperature where it can effectively facilitate the reaction. There is also a maximum temperature, above which the enzyme is denatured, or deactivated.

Like temperature, enzymes are very sensitive to changes in pH. The acidic and basic ions, which have varying concentrations as the pH of a solution changes, react with enzyme molecules. These reactions change the shape of the enzyme much like a change in temperature does, with similar effects on enzyme activity.

The Lab

In this lab, students investigate the activity of the enzyme diastase, and examine the effects of enzyme concentration, temperature, and pH on the ability of diastase to digest starch. Starch is a common nutrient used by many organisms, yet it is insoluble, and cannot be effectively absorbed. It must therefore be broken down to sugars, which are used to produce energy in living systems. Although this reaction would occur on its own, the time it would take to occur spontaneously is so prohibitive that it is not a possibility for organisms. *Diastase* is an enzyme that facilitates this conversion of starch to sugar and will be used in this lab to demonstrate the principles of enzyme activity.

Procedures

To determine the effect of enzyme concentration, students are given a spot plate where they add a drop of diastase to wells containing a starch solution. Every thirty seconds they test for the presence of starch (with an iodine indicator), recording the amount of time it takes for the diastase to convert the starch into glucose. They repeat the experiment with differing amounts of diastase and graph the effectiveness of the varying concentrations.

To determine the effect of temperature on enzyme performance, the diastase is kept at four different temperatures (0-10 degrees C; 20-25 degrees C; 35-45 degrees C and near boiling at 90-100 degrees C) with the help of ice baths and hot plates. Students then test the enzyme effectiveness with iodine indicator.

The final part of the lab will test using the enzyme in solutions in which dilute hydrochloric acid or sodium hydroxide has been added.

Kit Evaluation

The basis of the kit is a good one although for use in the classroom I would change a few things.

The student directions are a bit confusing. I will rewrite these to make them a bit more user friendly. I would also change the order of the experiment as the concentration timing requires the most amount of time and will be the hardest for students to interpret. I would save this for last so that they have had enough time working with the other reagents to get a feel for the process.

Two problems that we encountered with the kit would also have to be addressed. The iodine solution that the company sent is far too concentrated. It will need to be diluted to about the color of strong urine in order for the reactions to show the proper color changes. We also found that the glucose test strips (for whatever reason) did not work. This is easily remedied by purchasing inexpensive diabetic glucose test strips from any pharmacy.

Finally, I would include a more open-ended approach with the students formulating their own hypotheses on how they expect the enzyme to behave under the varying conditions.

How I will use these labs

Using the second kit as a demonstration of how an enzyme works, followed by the adjusted first kit will be an effective tool in helping students gain a better understanding of enzymes. My department has already discussed and added this as a performance assessment in our Biology curriculum for use in our chemistry of macromolecules unit.