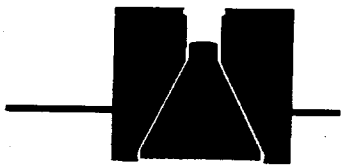

LABORATORY ACTIVITY: TEACHER NOTES

Activity 1: The Effects of Temperature and Copper(II) Ion on the Enzyme Catalase

Major Chemical Concept

Enzymes are proteins that act as catalysts for biochemical reactions. When the temperature is increased, the activity of enzymes also increases. However, a temperature is eventually reached at which the protein enzyme is denatured and is no longer active. Enzymes can be inhibited by many substances including certain metal ions. Catalase is inhibited by the copper(II) ion.

Level

This material is appropriate for first year or advanced high school chemistry classes. It is also appropriate for a biology class.

Time

40-45 minutes

Expected Student Background

Students should understand the role of catalysts in a chemical reaction, the nature of proteins, and protein structure. They must be able to collect data, prepare a graph, interpret the graph, and make predictions.

Safety

Read the Safety Considerations in the Student Version. Materials used in these activities pose few safety problems. Students should not taste any of the chemicals, including the baker's yeast. Care should be taken with the hydrogen peroxide and copper(II) sulfate solutions. Wash with water if any gets on the skin.

Materials (Based on the needs of 10 3-person groups.)*

1 Package of *fresh* baker's yeast (expiration date should be at least six months in the future)

1 250-mL beaker (for preparation of yeast solution)

1 Bottle of distilled water (from grocery store)

1 Small bottle of Ivory Dishwashing Liquid (clear)

1 1-Pint (16 oz.) bottle of *fresh* 3% hydrogen peroxide, (from drug or grocery store; buy only a brand with an expiration date--it should be at least six months in the future)

1.0 M Copper(II) sulfate solution, $CuSO_4$ (*see Advance Preparation by Teacher*)

10 Graduated cylinders, 100-mL (ideally 2-part, plastic base-and-graduated tube assemblies)

10 Graduated cylinders, 10-mL (ideally 2-part, plastic base-and-graduated tube assemblies)

20 16-Ounce Styrofoam cups**

30 pairs of safety glasses plus 1 pair for the teacher

20 sheets of graph paper

10 Stopwatches (preferably). Alternatively 1 wall clock with a second hand (or one watch with a second hand per group)

10 Thermometers, -20° to +110°C

10 Stirring rods

41 Small bottles equipped with medicine droppers and labels for the bottles used

20 containers (10 for hot water; 10 for ice water)

Source of hot water (80°C or hotter)

Source of ice or ice water

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* Scale down the lab equipment in proportion to your reduced requirements if fewer than 10 groups (30 students) are involved.)

** Larger cups or insulated containers will have to be used if 2-part graduated cylinder assemblies are not available.

Advance Preparation By Teacher

Baker's yeast solution: Empty one package of fresh baker's yeast into 60 mL of slightly warm (about 30°C) distilled water in a 250 mL beaker. Stir thoroughly for several minutes in order to dissolve as much of the yeast as possible. Don't worry if some of the yeast does not dissolve. Pour 6 mL of this mixture into each of 10 small labeled bottles equipped with medicine droppers. The yeast mixtures remain active for at least 48 hours (perhaps longer) and can be prepared a day or two prior to their use.

3% Hydrogen peroxide (*It must be fresh!*): Divide contents of purchased bottle among 10 small, clean, labeled bottles.

Dishwashing Liquid: Half fill 10 small, labeled bottles equipped with medicine droppers.

Distilled Water: Fill 10 small, labeled bottles equipped with medicine droppers.

1.0 M Copper(II) sulfate solution: Dissolve 12.5 g copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in 50 mL water. Pour into a bottle equipped with a medicine dropper (This solution is used only by the teacher.)

An electric coffee maker (containing only water) is a convenient source of hot water for the water bath if the water from the hot water faucet is not hot enough.

Pre-Laboratory Discussion

1. Review graphing

   a. Emphasize that the independent variable goes on the x-axis; while the dependent variable goes on the y-axis.

   b. How does one determine which is the dependent variable and the independent variable? ANSWER: The quantity the experimenter changes at will is the independent variable; while the other variable which changes as a result of the change in the independent variable is the dependent variable.

   c. Note that in scientific graphing it is desirable to use as much of the graph paper as possible. Mathematics

teachers frequently urge students to draw numerous small graphs on a single sheet of paper in order to save paper. This is feasible since they have only a small number of points to graph, and they don't need to use the graph for other purposes.

2. Discuss catalysis.

A catalyst is a substance which alters the speed of a chemical reaction, The catalyst enters into the reaction but is regenerated during the course of the reaction. The catalyst is present in the same form and same amount before and after the reaction occurs.

3. Discuss general properties of proteins and enzymes.

Many parts of your body are proteins -- skin, hair, muscle, hormones, and tendons. One class of proteins is called enzymes. Enzymes function as catalysts for biochemical reactions.

Enzymes are large proteins; they are not living organisms, as some people think but are instead molecules functioning in every living cell as catalysts for chemical reactions. The average molecular weight of one amino acid in an enzyme is 110 atomic mass units. The average protein molecule has 300-400 amino acids in it. Hence a typical enzyme would have a molecular mass of around 40,000 atomic mass units We are going to be studying the interaction of a particular enzyme called catalase with hydrogen peroxide.

Catalase has a molecular mass of 250,000 atomic mass units. Note that when enzymes act as catalysts in reactions with simple molecules such as hydrogen peroxide, the molecules on which they are acting are extremely small in comparison with the enzyme itself. The molecular mass of hydrogen peroxide is only 34 atomic mass units. **(Show transparency of protein here.)**

Enzymes can be inactivated by heat and/or chemical conditions. For example, acid often interferes with hydrogen bonding in the active enzyme and disrupts the normal action of the enzyme. (This disruption of the activity of the enzyme is called denaturation.) Another common example of denaturation of an enzyme occurs when an egg white is

heated. This denaturation results in an irreversible change in the egg white. (You can't unfry an egg.)

4. Discuss general principles of reaction kinetics (see *Reaction Rates* module).

5. The success or failure of this activity will be determined by the care which each groups takes to ensure they are getting reliable data.

Laboratory Direction

1. Assign persons to positions A, B, C in three-person groups.

2. Assign each group a temperature at which they will carry out the experiment [in addition to a room temperature (25°C) reaction which all groups carry out first]. Select from these temperatures: 15°, 35°, 45°, 55°, 65°C. In larger classes more than one group would be given the same temperature. A comparison of their data would be interesting.

Several Sources of Error

- Inadequate stirring of bath or failure to rigidly maintain temperature in non-room temperature reactions will lead to scatter of points on the graph.
- Failure to give warning to the person taking the readings so that the actual readings are taken a second or two after the recorded times.

Teacher-Student Interaction

The success of this activity requires a *group* effort. Students must be encouraged to contribute their data, even if it does not seem to *fit* a pattern on the group graph. It is imperative that you move from group to group to be sure that they are doing the activities correctly and safely. Question students about their results as they are conducting the activities.

Teacher Demonstration
Effect Of An Inhibitor On Foam Volume

Just before beginning the post-lab data analysis, begin this demonstration with all students observing. It can proceed during the data analysis.

Use the apparatus and a modification of the procedure of Part 1-A as noted below.

Step 1	Identical
Step 2	Add 19 drops of 1.0 M copper(II) sulfate solution.
Steps 3-8	Identical
Step 9	Record the time that the solutions are mixed and continue with other activities.
Step 10	Wait six minutes and then measure the foam height. How does the foam height compare to that observed by students who did the experiment at 25°C without the copper(II) sulfate added?

Data Analysis -- Anticipated Student Results and Answers

1. 3% Hydrogen peroxide solution, when decomposed by catalase at room temperature, produces a volume of oxygen that is ten times the volume of the solution used. Thus 8.00 mL of 3% H_2O_2 solution can produce no more than 80 mL of oxygen. The total amount of liquid used in the experiment is 10 mL. Therefore, we would expect a total volume of about 90 mL (10 mL + 80 mL). For several reasons the total volume might be as great as 94 mL.

2. Volume of gas (foam) vs. time: The volume of gas (foam) will increase regularly, but may tend to level off after 5 min or so.

3. Temperature vs. volume of gas (foam): The volume of gas (foam) will increase as the temperature increases, but will drop sharply at higher temperatures.

4. Inhibitor [copper(II) ion] and enzyme: The volume of gas (foam) will be much less but may not be zero.

Post-Lab Discussion

1. If student data does not correlate well with the curve of the composite graph, discuss experimental sources of error. Ask students to suggest possible sources of error.

2. Discuss the general relationship between rates of reactions and temperature.

3. Discuss the evidence for enzyme inhibition and gas production.

Answers to Implications and Applications

1. This is normal body temperature. As cells evolved, they adapted to the temperature that would produce maximum product from enzyme action.

2. High body temperature can inactivate protein, including the protein component of enzyme systems.

3. Yes. Class data will generally show a doubling of reaction rate with each 10°C increase in temperature.

4. You would not add meat tenderizer to meat while it was cooking, since the thermal energy would inactivate the enzymes in the meat tenderizer.

5. The best results would be in warm water. This would increase the activity of the enzymes. If the water were too hot, however, the enzymes would become inactivated.

6. Any ion which inhibits the action of an enzyme has the potential to disrupt biochemical processes which are catalyzed by the enzyme.

Simplifications (For lower grade level students)

Catalase in Turnips, Potatoes, and Rutabagas

Purpose

To demonstrate the activity of a typical enzyme.

Safety

Have students wear safety glasses. Be careful when slicing the vegetables in the demonstration.

Materials

3% Hydrogen peroxide (drug store variety) in Beral pipettes
Potatoes, turnips, rutabagas -- fresh, whole; animal tissue, such as liver, works well
Knife
Petrie dishes

Procedure

Slice several potatoes, turnips, and rutabagas into 3-4 smaller pieces. Place each sample in a separate Petrie dish. Allow students to place drops of hydrogen peroxide on the skin side of the vegetable and on the cut inner portion of the vegetable.

This demonstration can be done in two-person groups as a hands-on activity for lower grades. Slices of turnips, rutabagas, Irish potatoes, beets and sweet potatoes can be observed when placed in a Petrie dish, waxed paper or paper plates. Have students determine whether the skin side evolves as much gas as the inner part of the vegetable. Students may want to predict what will happen when raw hamburger meat, cooked hamburger meat, and/or cooked vegetables are tested. A demonstration can allow the students to see whether their predictions were accurate. (Do not allow students to test raw meat because of health considerations.) The raw meat should produce even greater gas evolution than the raw vegetables. The cooked foods should not evolve any gas.

The "Pictures in the Mind" on the bottom of page 13 in ENZY module of SourceBook can be used to give students an idea of the size differential between catalase and hydrogen peroxide.

Possible Extensions (For upper grade level students)

1. Test other simple enzymes. Refer to college biology or biochemistry texts for examples.

2. pH also affects enzymes. Design studies to show the effect of pH on catalase and other enzymes.

3. Other heavy metal ions such as Hg(II) and Fe(II) can be investigated to see how they affect the reaction if at all.

4. For more advanced students the data sheet can be eliminated and the students can generate their own data sheets.

5. For more advanced students reactions rates can be discussed. Have the students compare their graphs with graphs of first and second order reactions.

6. Have students conduct library research and write reports pertaining to enzymes.

TEACHER'S OUTLINE OF ENZYMES, ACTIVITY 1

READ THIS FIRST

All of the information needed for a student to conduct this experiment is "self-contained" in the section of this distributed material labeled "Student Version" (found on white sheets).

However, for a teacher to have comprehensive information about this experiment, he/she must read:

(1) some sections of the module "Enzymes" found in SourceBook
(2) the student version of the experiment found in this distributed material
(3) "Teacher Notes" (on colored paper) also in this distributed material

This outline presents the sequence of readings recommended for the teacher prior to incorporating this experiment into his/her laboratory program.

I. In the SourceBook Module: "Enzymes: Biochemical Catalysts"

Read: Topic Overview, pp. 2-3

II. In Workshop Material -- A substitute procedure for Activity 1 and Demonstration 3 in the Enzymes module.

1. Read in the Teacher Notes p. 7-8 Simplifications (For lower grade level students)

2. Read in the Student Version:
   Introduction
   Purpose
   Safety
   Procedure (down to Procedure A on p. 2)

3. Read in the Teacher Notes: pp. 1-3

4. Read in the Student Version: Procedure A, pp. 2-12

5. Read in the Teacher Notes: p. 4

III. In the SourceBook Module: "Enzymes: Biochemical Catalysts"
Read Assessing Laboratory Learning, p. 10

FRAGMENTS

If your assigned temperature is *below* room temperature (about 25°C), add ice water to the water in your water bath in order to maintain the assigned temperature. If your group's assigned temperature is *above* room temperature, add hot water as needed to maintain the temperature. BE CAREFUL IN WORKING WITH HOT WATER!

On the other hand some enzymes are capable of becoming active again (renaturation). The conditions under which renaturation occurs involves a slow return to the original conditions. Cooling (if heat was the reason for the denaturation) or pH change if that caused denaturation. Slowly returning to the original conditions allows the enzyme to refold returning to the original conditions allows the enzyme to refold and assume the configuration in which it was active.