Enzymes: What's in your spit?
Teacher Version

In this lab students will investigate a few of the different enzymes from our body. You will learn how these enzymes work and how their activity is dependent on factors such as heat, pH, and concentration.

California Science Content Standards:

- **1. Cell Biology: The fundamental life processes of plants and animals depend on a variety of chemical reactions that occur in specialized areas of the organism's cells.**
- 1b. Students know enzymes are proteins that catalyze biochemical reactions with altering the reaction equilibrium and the activities of enzymes depend on the temperature, ionic conditions, and the pH of the surroundings.
- **9. Physiology: As a result of the coordinated structures and functions of organ systems, the internal environment of the human body remains relatively stable (homeostatic) despite changes in the outside environment.**
- **9f. Students know the individual functions and sites of secretion of digestive enzymes (amylases, proteases, nucleases, lipases), stomach acid, and bile salts.

Prerequisites:

- Good for all students

Complete List of Materials:

- ½ Fresh Pineapple
- Cold Water available
- 100 mL Graduated Cylinder
- 8 Small Cups/group
- Hydrogen Peroxide
- 1 Orange
- Microwave
- Flour (Starch)
- Yeast (Catalase)
- Eye Dropper
- 1 Apple
- Jello
- Sugar
- Iodine
- Vinegar

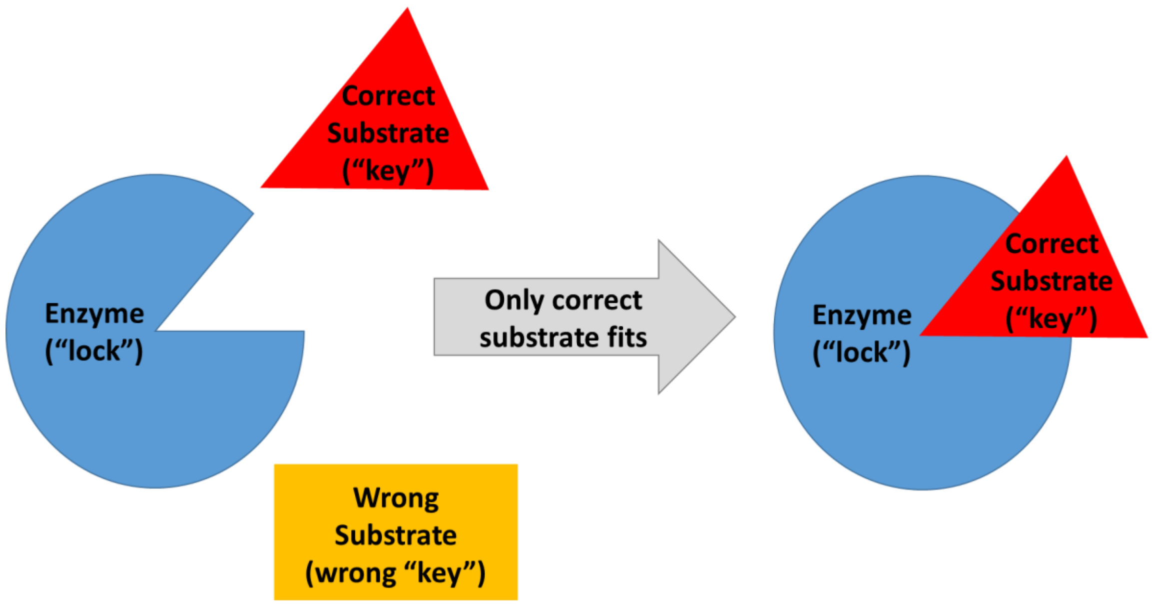
Preparation and Lab Notes:

- If you choose to do Part 1 with the "cooking show" style, you will need to setup up the fruit and Jello an hour before the lab begins. This will give the reaction time to take place, so you can then do the "cooking show" presentation.
- For Part 3 with the Hydrogen Peroxide and Catalase reaction, you will need to prepare the A, B, and C solutions of yeast and water ahead of time.

Key Concepts:

- **Enzymes** are proteins that speed up chemical reactions, and are found in all living organisms because they are important in everyday functions like digestion and blood clotting. They also act like scissors and cut bonds between molecules.
- The process of accelerating chemical reactions is referred to as **catalysis**, and because enzymes take part in a chemical reaction (but are not destroyed in the process), enzymes are referred to as **catalysts**.

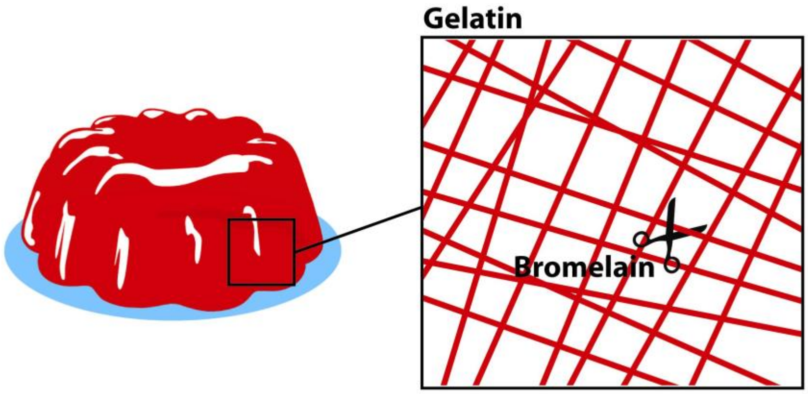
- The shape of each enzyme allows it only to interact with a specific molecule – called a **substrate** (though the enzyme can act with many substrates at the same time) – that makes the enzyme specific for a particular type of chemical reaction. Think of the enzyme as a "lock" and the substrate as the "key". Only that certain "key" (substrate) will fit into that specific "lock" (enzyme).



Helpful link to understand enzymes: **http://www.youtube.com/watch?v=0XjyAkeQJag**

Part 1 – How does Heat affect enzyme activity? (Bromelain)

Gelatin is a protein used in making many of your favorite foods, like gummy bears and Jello! The chemical bonds in gelatin can be broken by an enzyme called **bromelain**, which is found in certain fruits.



We will test different fruits to identify which of them contains the bromelain enzyme, and then investigate the effect of heating on bromelain.

1. Cut 2 equal sized 1 inch by 1 inch pieces of fresh **apple**, **orange**, and **pineapple**.

2. To look at the effects of heat on the enzymes in these fruits, microwave a piece of apple, orange, and pineapple for **1-2 minutes**. Cool these pieces to room temperature by submerging them briefly in cold water.

3. Place the regular and microwaved pieces of fruit on top of the Jello. **What do you think will happen to the Jello in contact with each piece?** *Write your prediction in the table below, under the heading "Prediction".*

4. Let the fruit pieces sit undisturbed for at least **1 hour**. In the meantime, let's move on to **Lab Part 2**…

5. *1 hour later…*Remove each piece of fruit and take a look at the surface of the Jello underneath. **What happened to the Jello in each case?** *Record your observations in the table below, under the heading "Result".*

TEACHER NOTE
Another possible way to do this is in "cooking show" style. Prepare one experiment an hour before the lab begins, so that you can discuss your results with students at the time. Then, compare the experiment you did with the students after the rest of the lab.

Type of Fruit	Prediction	Result
Apple	*Student Answer*	*No Reaction*
Orange	*Student Answer*	*No Reaction*
Pineapple	*Student Answer*	*Dissolves into/Dissolves Jello*
Heated Apple	*Student Answer*	*No Reaction*
Heated Orange	*Student Answer*	*No Reaction*
Heated Pineapple	*Student Answer*	*No Reaction*

Concept Questions:

Q1. Which fruit(s) do you think contain the enzyme Bromelain?

Pineapple contains the enzyme Bromelain, and should break down the Jello.

Q2. It is possible to make Jello with canned pineapple chunks but not fresh pineapple chunks. Why do you think this is so?

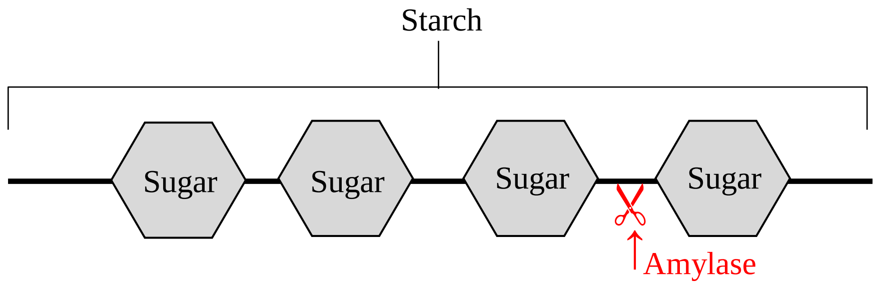
The fresh pineapple chunks still have live enzymes that will try to prevent the jello from forming. Canned pineapple chunks are heated during the canning process; therefore, there are no active enzymes in the canned pineapple.

Q3. How do you think heat affects enzymes? Why is the activity of the enzyme destroyed?

Heat stops the enzyme from functioning, by unraveling (denaturing) it and changing its shape. Therefore, the key no longer fits the lock. (Lock and Key analogy)

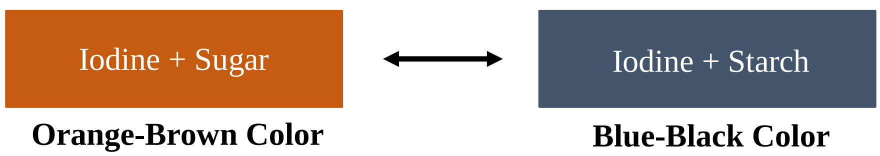
Part 2 – How does pH affect enzyme activity?
(Starch and Amylase)

Starch is a carbohydrate found in many foods, such as wheat, corn, rice, and potatoes. It is a long chain of **sugars** linked together.



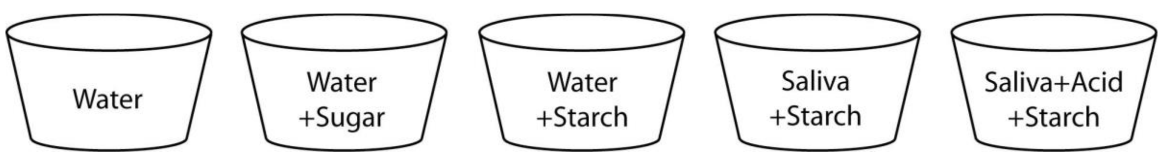
The enzyme **amylase** breaks the bonds linking the glucose molecules in starch. Your saliva contains amylase to break down the starchy foods that you eat.

We can stain with **iodine** to visualize the conversion of starch into sugar. Iodine is normally **orange-brown in color**, but when it binds to starches, it turns a **blue-black color**.



We will test our saliva for the presence of amylase, and investigate how that amylase may be affected by pH…

1. Take **5 cups** and label them as follows:



2. Add **10 mL** of water to each cup.

3. Spit **four times** into each of the cups labeled "**Saliva**" (ew!). Gently swirl the cups to mix the water and saliva together. (The larger the spits, the better!)

4. Add **10 drops of vinegar** to the cup labeled "**Saliva+Acid**". Gently swirl the cup to mix in the vinegar.

5. Add **2 pinches of sugar** to the cup labeled "**Water+Sugar**". Gently swirl the cup to mix in the sugar.

6. Add **2 pinches of starch** to all the cups labeled "**+Starch**". Gently swirl the cup to mix in the starch.

7. Wait about **15-25 minutes**. Meanwhile, let's make some predictions! **What do you think will happen when we stain the solution in each cup with Iodine?** *Write your prediction in the table below under "Predicted Result".*

8. The moment of truth: Add **3 drops of iodine** to each cup and *write your observations below under "Actual Result".* Were your predictions accurate

TEACHER NOTE
The Saliva + Starch reaction can take a while to finish. Initially the color will be Blue-Black, but given time the Amylase will break the starch down into sugar and the color of the solution will slowly get closer the Orange-Brown color of the Iodine.

Cup	Predicted Result	Actual Result
Water	*Student Answer*	*Orange-Brown*
Water + Sugar	*Student Answer*	*Orange-Brown*
Water + Starch	*Student Answer*	*Blue-Black*
Saliva + Starch	*Student Answer*	*Orange-Brown*
Saliva + Acid + Starch	*Student Answer*	*Blue-Black*

Concept Questions:

Q4. When scientists design an experiment, they generally include one or more ***control samples*** *as well as the* ***experimental sample****. The experimental sample is intended to observe the effect you are interested in.*

a. What is/are your experimental sample(s) here? What is each one testing?
The "Saliva + Starch" sample is testing whether saliva contains enzyme that break down starch. The "Saliva + Starch + Acid" sample tests whether the activity of this enzyme is sensitive to acid.

b. The control samples help ensure that your experimental setup is working as expected. What role do the following control samples serve in this experiment?

- *Water+sugar:*
  Makes sure that iodine will remain orange-brown when no starch is present.

- *Water+starch:*
  Makes sure that iodine will turn blue-black if there is starch present.

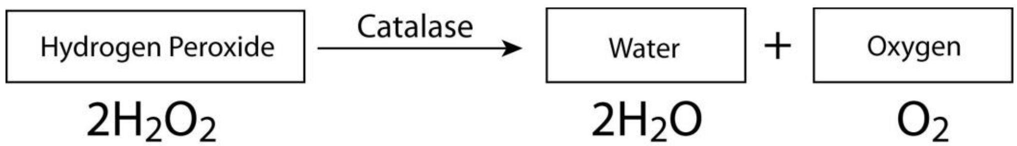
Q5. Vinegar is acidic (it has low pH). Based on your experiment, what can you say about the pH dependence of the amylase enzyme?
The enzyme present in saliva, amylase, is inactivated in acidic conditions, low pH.

Q6. Can you suggest another substance that would inactivate amylase if added to the saliva?
Strong acids or bases will change the pH away from neutral. Hydrochloric acid, HCl or sodium bicarbonate, a base, would change the pH so that the enzyme would not function.

Q7. Amylase works best at a neutral pH of 7. Where in your body are there enzymes which work best at an acidic or low pH?
Your stomach has a low pH of 2, which makes it highly acidic to be able to digest all of the food that goes in. Enzymes present in your stomach will function best near a pH of 2.

Part 3 – How does concentration affect enzyme activity? (Hydrogen Peroxide and Catalase)

Hydrogen peroxide is a molecule made up of hydrogen and oxygen. It can spontaneously decompose into water and oxygen, but this occurs very slowly. The enzyme **catalase** is found in many living cells (such as yeast) and **speeds up** the decomposition of hydrogen peroxide into water and oxygen.



We will investigate the reaction between hydrogen peroxide and catalase…

TEACHER NOTE
Setup solutions A, B, and C before the students arrive. (20 eye-dropper drops = 1 mL)

a) Put 4 pinches of yeast into 26 mL of water. This is solution A. Label cup A.
b) Put 1 mL of solution A into 24 mL of water. This is solution B. Label cup B.
c) Put 5 mL of solution A into 20 mL of water. This is solution C. Label cup C.

Cover the cups so that you cannot see the solutions.

1. Using a graduated cylinder, prepare **3 uncovered cups labeled A, B, and C** with about **15ml of hydrogen peroxide each**.

   Q8. What do you predict will happen when you add the catalase to the hydrogen peroxide?
   The reaction will proceed; catalase will turn hydrogen peroxide into water and oxygen gas. Bubbles will begin to form in the solutions. This is the oxygen gas escaping.

2. Time to start the reaction! Using the **3 covered cups** labeled "**A**", "**B**", and "**C,**" quickly add **15 drops** of each solution to the matching cups. Observe the reaction for a few minutes.

3. ***Write down the order (1st, 2nd, or 3rd)*** *in which you begin to see bubbles on the surface of the hydrogen peroxide. Also note the frequency of bubbles you see forming.*

Catalase Solution	Order of Reaction
A	*1st*
B	*3rd*
C	*2nd*

4. **Rank the catalase solutions** according to the speed and intensity of the reaction:

___*A*___ > __*C*____ > __*B*____

Concept Questions:

Q9. What do you think is different about the catalase solutions?

The rate of reaction is different for each solution because different solutions contain different concentrations of the enzyme catalase.

Q10. What gas is being produced when you add the catalase to the hydrogen peroxide?

Oxygen.

Q11. What are some ways you could confirm the identity of this gas?

Contain the oxygen in the top of an inverted test tube. Then light a match and blow it out. Quickly insert it into the test tube while it is still glowing. If it reignites the gas is oxygen.

Q12. Given that the bacteria that causes gangrene is anaerobic, or cannot tolerate oxygen. How could hydrogen peroxide be used to prevent infection of a deep wound?

This is a more challenging question, so you may want to just discuss it with the students. In the presence of catalase, hydrogen peroxide reacts to form oxygen gas and water. When you pour hydrogen peroxide on a wound, your natural catalase produces oxygen deep in the wound. The oxygen is toxic to anaerobic bacteria and kills them before infection can occur.

***Upon completion of Lab Part 3, check on results of Lab Part 1.**