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ENZYME ACTIVITY

I. OBJECTIVES:

1. To study the functions of various enzymes.
2. To study the effects of temperature and pH on enzyme activity.

II. BACKGROUND:

Enzymes are biological **catalysts**, that is, molecules which aid chemical reactions to happen without being consumed in those reactions. For example, the enzyme **ptyalin** in saliva causes starch to break down into maltose, but nothing happens to the ptyalin in the reaction, and once the maltose has formed, the ptyalin is free to cause more starch to react. Because enzymes are types of protein molecules, they are affected by the sorts of things that can affect proteins, for example, temperature, pH, or concentration of the enzyme and/or its **substrate** (the substance upon which it acts). The first enzymes (**en** = in; **zym** = yeast, leaven) to be studied were those in yeast that are involved in fermentation of sugar to alcohol.

Digestion of foods begins in the mouth. The ptyalin in saliva belongs to the general category of **amylases** (**amyl** = starch, **-ase** = enzyme ending), enzymes which break starch down into maltose. Later, the enzyme maltase in the small intestine can convert the maltose to glucose which then is absorbed and burned for "fuel" in one's body. The effect of this **salivary amylase** on starch can be demonstrated by mixing saliva with a starch solution. However, since the saliva, the starch solution, and any resulting maltose solution are all clear, we need a means by which to show that the starch has, indeed, disappeared and that maltose has formed. Iodine (which is a brownish-orange solution) combines with starch to form a purplish-blue complex, thus if iodine is added to a starch solution, the solution should turn bluish and then fade in color as the starch is converted to maltose. Benedict's reagent (which is a light blue solution) reacts with most sugars, including maltose, to form a yellow-orange product, thus if maltose has formed as a result of the action of the salivary amylase, the presence of this maltose can be shown by testing the solution with Benedict's reagent.

Bromelain, an enzyme found in pineapple (plant family Bromeliaceae), digests protein to smaller polypeptides, thus some people take this enzyme as a digestive aid. Gelatin is a protein extracted from animal tissue by prolonged boiling (and thus, is not consumed by many vegetarians). Gelatin works by forming a "network" that holds water molecules, thereby causing a mixture of the tow to "gel." When gelatin has been digested by the activity of enzymes such as bromelain, it will lose its ability to gel, and a gelatin-water mixture will turn into a liquid. Enzymes such as bromelain (as with many other proteins) are denatured by heat thereby losing their effectiveness. For this reason, canned pineapple can be used in gelatin-based products, while fresh pineapple cannot. Also, most enzymes have an optimum temperature at which they function best. We will be studying the effects of the enzyme bromelain on gelatin and on egg albumin (another protein) and the effects of temperature on the ability of this enzyme to function.

Various chemical reactions in our bodies result in byproducts chemically similar to **peroxide**, H_2O_2, but this chemical is very reactive, and therefore toxic (it reacts with lots of other "good" chemicals that make up the structures in our cells, thereby changing these chemicals, thus the structure and/or functioning of those cell parts). To help protect our cells (and those of many other organisms) from this damage, we (and other organisms) have a type of enzyme called **catalase** that breaks down H_2O_2 by the reaction $2\ H_2O_2 \rightarrow 2\ H_2O + O_2\uparrow$. When this reaction is performed in a test tube, the evolution of O_2 (fizzing) can be used as an indication of enzyme activity (like when you put peroxide on a cut). Catalase, as with other enzymes has an optimum pH at which it functions best (for example, the pepsin in one's stomach functions best in an acidic environment). Part of this experiment will be to determine the pH range in which catalase functions best. Frequently, catalase activity is demonstrated by extracting the catalase from liver (purchased from a grocery store). Because peroxide-forming reactions also occur in plants, most plant cells also contain catalase enzymes, thus a rather interesting variation on this experiment can be done by using red cabbage. The purplish-red pigments in red cabbage are collectively known as **anthocyanins** (**anthe** = flower; **cyano** = blue) and are sensitive to pH. While these pigments exhibit the familiar purplish color at a neutral pH, when placed in acidic or basic (alkaline) solutions, they change colors, thus serving as pH **indicators**. This enables the correlation of pH, as indicated by a color change in the cabbage juice, to the level of activity of cabbage catalase.

III. MATERIALS NEEDED:

PART A	*PART B*	*PART C*
5 test tubes, 10 × 100 size	Jell-O® or other gelatin	blender
wax marking pencil	10 test tubes, 16 × 150 size	red cabbage
saliva	wax marking pencil	small strainer
1% starch solution (fresh)	refrigerator	250 mL beaker
1% maltose solution	fresh pineapple	7 test tubes, 16 × 150 size
Gram's iodine	stove and/or microwave	white vinegar (acetic acid, CH_3COOH)
Benedict's reagent	knife and cutting board	dilute ammonia (1:10), NH_4OH
37°C incubator	hard-boiled egg	3% peroxide, H_2O_2
vortex	blender	vortex
Bunsen burner and goggles	small strainer(s)	
test tube holder	250-mL beaker(s)	
10 mL grad cylinder	incubator	

IV. SAFETY CONSIDERATIONS:

A. We will be using **Bunsen burners** to heat test tubes. Refer to the separate protocols on safe use of Bunsen burners and safe heating of test tubes. Watch carefully as your instructor demonstrates these techniques. **Goggles** should be worn by everyone while test tubes are being heated. Long hair should be tied back and loose clothing tucked in or kept under control in some other way while there are lit burners nearby.

B. Benedict's solution is just "strong" enough that it *probably* won't hurt to get it on your skin, but if you do, the safest thing is to immediately wash it off with lots of water.

C. Iodine, in any appreciable amount, should not be heated because it will evaporate and form toxic iodine fumes in the air. All containers of iodine solution should be kept tightly capped to prevent evaporation.

D. Federal regulations prohibit drain disposal of anything that's not pH 7. Thus, for Part C, do not pour your chemicals down the drain. Rather, pour them into the designated beaker, so your instructor can make sure that they're properly neutralized.

V. PROCEDURE:

EFFECTS OF SALIVARY AMYLASE ON STARCH:

A. These solutions were probably made up by the lab staff ahead of time. If these are not already available (your instructor will know), as a class, make Gram's iodine and Benedict's reagents according to the following recipes (goggles should be worn while weighing and/or mixing any chemicals):

BENEDICT'S SOLUTION

A. 173 gm crystalline sodium citrate
100 gm anhydrous sodium carbonate ($NaCO_3$)
800 mL dH_2O
Mix and filter if needed.

B. 17.3 gm copper sulfate ($CuSO_4$)
100 mL dH_2O
Mix together and add to Part A.
Add dH_2O to bring volume to 1 liter.

GRAM'S IODINE SOLUTION

300 mL cool dH_2O
2 gm potassium iodide (KI)
1 gm iodine crystals (I_2)
Mix and let iodine dissolve,
— OR —
30 mL 0.1 N iodine solution
q.s. to 100 mL with dH_2O
(0.1 N = 12.7 gm I_2 + 20.0 gm KI per liter)
KEEP TIGHTLY CAPPED!

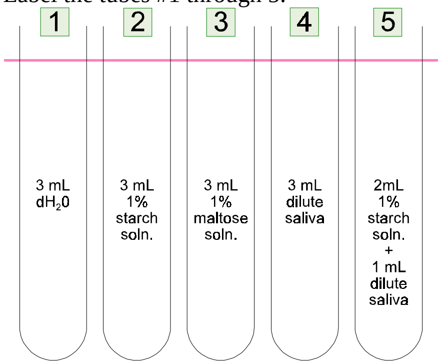
Note that these directions are for your reference and that these solutions will be mixed up before you come to lab. Neither of these are very good things to get on your hands – if you do, rinse thoroughly with water. Note that iodine evaporates easily from solution, releasing poisonous, violet I_2 gas into the air. **Do not heat iodine solutions** because this greatly speeds up the release of I_2 into the air. Also, keep the lid on all iodine solutions when not in use.

If 1% starch and 1% maltose solutions are not already made, these can be made by the class by adding 1 gm of that substance to 99 mL of dH_2O. The starch solution will need to be heated (stove or microwave) to boiling to dissolve the starch.

B. For this part of the experiment, you will be working in groups of about five students. A volunteer from your group should obtain a 10 mL graduated cylinder and use it to collect 1 mL of saliva.

C. 4 mL of dH_2O should be added to your volunteer's saliva (thus, a total of 5 mL). Temporarily, remove the plastic bottom from the cylinder, and use a **vortex** to mix the saliva and water, as demonstrated by your instructor. (Optionally, as time, interest, and volume of saliva allow, you could compare saliva from different people or different dilutions: full strength, 1:1 dilution, etc.)

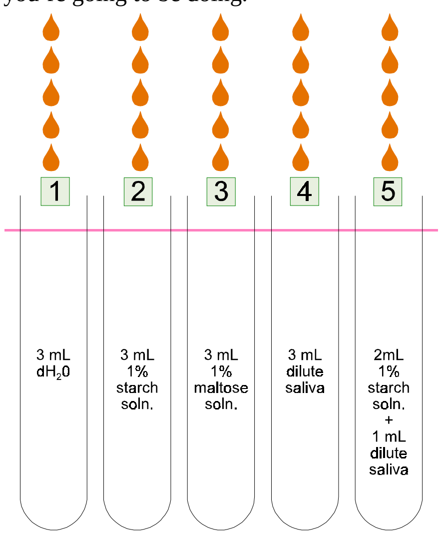
D. Obtain five 10 × 100 test tubes (“small” ones) and a rack in which to place them. Label the tubes #1 through 5.



E. Add the following, specified solutions to the appropriate test tube:

#1	3 mL dH_2O
#2	3 mL 1% starch solution
#3	3 mL 1% maltose solution
#4	3 mL diluted saliva
#5	2 mL 1% starch solution + 1 mL diluted saliva

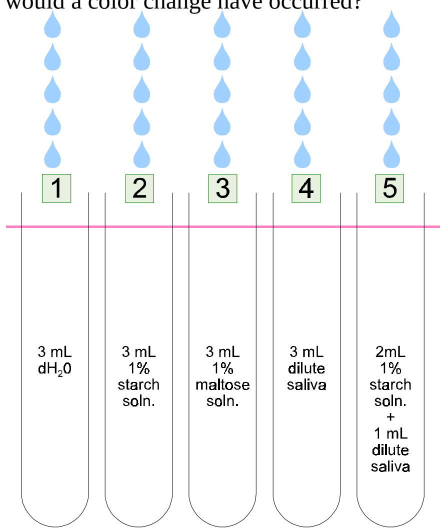
Note: plan things such that after mixing #5, you will be able to proceed through the next several steps in a fairly short period of time so you don’t miss seeing any reaction that occurs – know and understand, ahead of time, what you’re going to be doing.



F. Add 5 drops of Gram’s iodine to each of the test tubes, vortex each of them, and record what happens. Does the iodine stay brownish, or does it change color? If a color change is observed in any of the tubes, what does that signify – for what substance is iodine an **indicator**?

G. Set the rack of tubes in the **incubator**, and incubate at 37°C for 30 min. (or until tube #5 changes color). Check periodically, watching for a color change in tube #5.

H. At the end of that time, note any color changes that have occurred. Pay particular attention to tube #5. If a color change has occurred there, what does that mean – why would a color change have occurred?



I. While, in general, it’s not a good idea to heat iodine solutions, the amount in these tubes is low enough to not be toxic. After your instructor demonstrates safe test-tube-heating techniques, one at a time, carefully heat each tube over a Bunsen burner until quite warm, but not yet boiling. Use a Pasteur pipet to add 10 drops of Benedict’s reagent. Continue to heat the tube for a short while, but do not let it start to boil. Watch for and record any color changes that do occur. If a color changes is observed in any of the tubes, what does that signify – for what substance is Benedict’s an indicator? If there is a color change in tube #5, what chemical reaction does that indicate took place – what substance is in there now that wasn’t there before, and how did it get there?

J. When you’re done, thoroughly clean all glassware (scrub the inside bottoms of the test tubes with a brush and soapy water). Using a gentle stream of warm water, insert the hose all the way into each test tube to **thoroughly** rinse out all the soapy water and “left-overs”. Place wet glassware in the “dish” racks to dry – not back on shelves or in drawers.

EFFECTS OF TEMPERATURE ON ENZYME ACTIVITY OF BROMELAIN:

A. If not already available, <u>the period before the enzyme lab is scheduled</u>, as a class, mix up Jell-O® (any flavor) according to package directions and pour into 16 × 150 test tubes about 5 cm (2 in.) deep (Pour all the Jell-O, even though it will make more tubes than what you’ll need). Try to get the same amount of Jell-O in all of the tubes. Place caps on test tubes and refrigerate until use.

B. The first day of the enzyme lab (while your saliva is in the incubator), AS A CLASS, obtain about a 1 in. thick slice off a pineapple. PLEASE SAVE THE REST OF THE PINEAPPLE FOR THE OTHER LAB SECTIONS THAT NEED TO USE IT, TOO!!! Cut your class’ pineapple slice into chunks small enough to be easily handled by a blender. Blend with at least an equal amount of water (may need slightly more water, but don’t get it too dilute), then strain through strainer(s) into beaker(s). Discard the pulp in the “chicken bucket,” and rinse the strainer(s) immediately so left-over pulp doesn’t dry onto them. As a class, you want to end up with two beakers with about the same amount of pineapple juice in each.

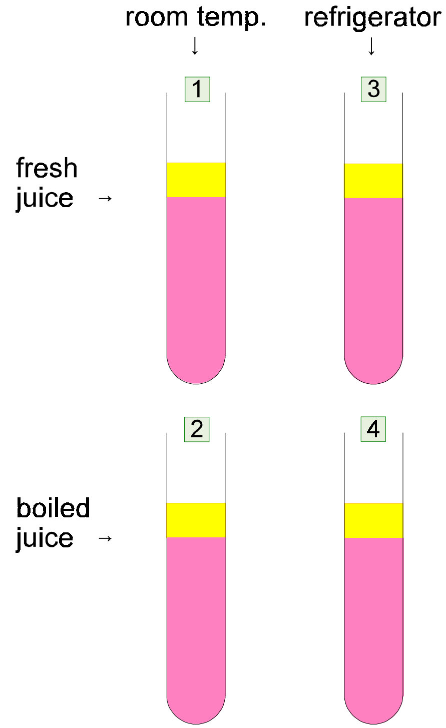
C. Half of the juice (in a marked beaker) should remain at room temperature, while the other half (in a second marked beaker) should be microwaved for several minutes until boiling (watch it doesn’t boil over!).

D. Your group should obtain four of the Jell-O tubes. Number the test tubes “1" through “4,” place a mark on the side of each tube to indicate the top of the Jell-O, then weigh these tubes (without caps), recording the weights in your lab notebook. Add 2 mL of the appropriate pineapple juice (see chart below) to each of the tubes. Mark the side of the tube to correspond to the top of the liquid and re-weigh the tubes, then replace the caps. Caution: Note that these caps are NOT water-tight, so don’t tip over a tube to examine it!

tube #	tube #	fruit
#1	#3	fresh pineapple juice
#2	#4	cooked pineapple juice

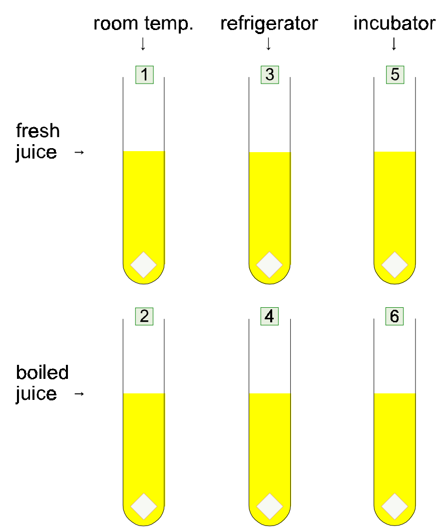
E. Space in our incubators and refrigerator is limited. Thus, your instructor will obtain and label one large beaker for your class for each of the incubator, room temperature, and refrigerator. Place your tubes #1 and 2 in the “room temperature” beaker and tubes #3 and 4 in the “refrigerator” beaker. Why would it not work well to make a third set of Jell-O tubes to keep in the incubator?

F. Your group should obtain six more 16 × 150 test tubes (and caps) and number them (1 - 6). Obtain six cubes of egg white, approximately 1 cm in size, and place one into each tube.



G. Place 5 mL of the appropriate juice into each of the 6 test tubes as follows:

tube	tube	tube	fruit
#1	#3	#5	fresh pineapple juice
#2	#4	#6	cooked juice



H. Place lids on the tubes. Tubes #1 and 2 should be placed in the "room temperature" beaker, tubes #3 and 4 in the "refrigerator" beaker, and tubes #5 and 6 in the "37°C incubator" beaker.

I. When all Jell-O and egg tubes from your class have been prepared, as a class, put the three labeled beakers in the appropriate locations until the next class period.

J. The next class period, obtain all your tubes. Make note of the condition of the Jell-O or egg white in each tube and any other significant changes.

K. The test tubes containing the Jell-O should be re-weighed, again without lids, to see if the total weight has changed. Also examine the tubes to see if your top mark still corresponds to the top of the liquid. Then, if necessary, use forceps or a teasing needle to remove any mold that has formed on top of the liquid and pour off the liquid. Then, re-weigh the tubes. Make a new mark on the side to indicate the new level of the solid Jell-O and use a meterstick to measure the difference in height.

L. In your lab notebook, you should now have recorded the difference in height of the solid Jell-O for each tube. Also, from the "before" and "after" weights, calculate the change in weight of the solid Jell-O portion and the liquid "juice" portion.

L. When you are done with the experiment, thoroughly wash out all test tubes and lids with hot soapy water, rinse thoroughly, and place upside down in a rack to dry. Please remove any pieces of egg white, etc. from the sink and dispose of them properly in the trash.

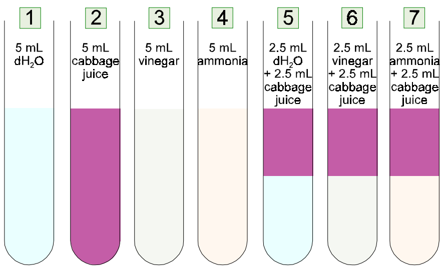
EFFECTS OF pH ON CATALASE ACTIVITY:

A. As a class, from a head of purple cabbage, peel off several leaves. Tear the leaves into chunks, then place these in a blender with about an equal amount of dH_2O and blend until liquified. Strain through a small strainer into a (marked) 250 mL beaker. Save the juice, but discard the pulp in the regular garbage – it's too smelly for Dr. Fankhauser to take home for the chickens. Note the color of the juice.

B. In your groups, obtain seven 16 × 150 test tubes and label them 1 through 7. Use separate 10 mL graduated cylinders to obtain each type of solution as you prepare the following contents for each of the tubes:

tube #	contents
#1	5 mL dH_2O
#2	5 mL cabbage juice
#3	5 mL vinegar
#4	5 mL ammonia (1:10 dilution)
#5	2.5 mL dH_2O + 2.5 mL cabbage juice
#6	2.5 mL vinegar + 2.5 mL juice
#7	2.5 mL ammonia + 2.5 mL juice

C. Use a vortex to thoroughly mix these solutions, and record any color changes and/or reactions that occur as they are mixed.



D. Use pH paper to test the pH of each solution. Correlate pH with color of solution. How does acid affect the color? How does base affect the color? If any of the solutions turn green, what might be causing the green color (Hint: if the anthocyanins would turn clear, what other **pigment** is in leaves that might show up?)?

E. One at a time, measure 5 mL of 3% H_2O_2 (peroxide) with a clean, small graduated cylinder and add to each of the tubes. Do these one at a time so you can watch carefully to observe any changes and record any color changes and/or reactions that take place. If a reaction occurs, also note whether it is a strong, moderate, or weak reaction.

V. DATA:

PART A

Set up a chart similar to the following:

tube #	reaction with iodine	reaction with Benedict's
1		
2		
3		
4		
5		

PART B

Set up charts similar to the following:

Jell-O tubes				
weight	before		after	
tube #	wt w/o juice	wt w/ juice	wt w/ juice	wt w/o juice
1				
2				
3				
4				

Jell-O tubes				
	room temp.		refrigerator	
juice	Δ wt	Δ ht	Δ wt	Δ ht
fresh	(1)		(3)	
cooked	(2)		(4)	

Egg tubes			
juice	refrigerator	room temp.	37°C
fresh	(3)	(1)	(5)
cooked	(4)	(2)	(6)

PART C

Set up a chart similar to the following:

	alone		with H_2O_2	
tube #	pH	color	reaction	color
1				
2				
3				
4				
5				
6				
7				

VI. CONCLUSIONS:

PART A

1. How does the presence of saliva affect the starch content of a solution?

2. How does the presence of saliva affect the sugar content of a solution?

3. Why did we incubate the tubes for the salivary amylase experiment at 37°C?

4. What might be some further tests you could perform on the effects of saliva on starch?

PART B

1. Why would it not work to test the effects of bromelain on Jell-O by incubating tubes of Jell-O at 37°C?

2. What do the gelatin in Jell-O and albumin have in common that would be affected by bromelain?

3. What influence does temperature have, both in terms of boiling and in terms of incubation temperature, on the activity of bromelain. What differences did you notice in the activity of these two enzymes?

PART C

1. How does the color of the cabbage juice correlate to the pH of the solution in which it is dissolved?

2. How does the pH of a solution correlate to the amount of catalase activity ("strength" of the reaction) in that solution?