Name _____ **Instructor** _____ **Lab Section**_____

Objectives: To gain a better understanding of:	**Background material may be found in**
• Cellular Respiration	• Chapter: 3.11 - 3.14
• The Structure of Proteins	• Chapter: 5.14 - 5.15
• The Structure and Function of Enzymes	• Chapter: 6.1-6.4, 6.13
	Biology: Concepts and Connections, 8th ed.

All organisms require certain organic molecules for structural materials and as a source of energy to carry out life processes. Organisms that are unable to manufacture these materials themselves (via photosynthesis and other cellular processes) must be supplied with these molecules, which we collectively call "food". Organic molecules composing food include **fats, carbohydrates**, **nucleic acids** and **proteins**.

Potential chemical energy stored in the bonds of food molecules can be harvested and stored in ATP in one of two ways: (1) **Aerobic Respiration or Cellular Respiration** - involving the release of energy with the use of oxygen, and (2) **Anaerobic Respiration or Fermentation** - where energy is released in the absence of oxygen.

Today we will examine respiration in yeast as it relates to bread and wine making in particular and cellular metabolism in general. Secondly, we will look at enzymes and factors affecting their activity.

CELLULAR RESPIRATION

AEROBIC RESPIRATION

BREAD

The process of making bread demonstrates the process of cell respiration. The living yeast in the bread dough play a critical role in the preparation of leavened bread as described below. Unleavened bread does not contain yeast and therefore does not rise. This is how crackers are made. We will perform a demonstration of bread making and this bread will be available for you to eat near the end of the lab. The recipe we will use is given below.

FIRST, GET 4½ CUPS FLOUR IN THE SMALL BOWL.
This is all the flour you will need for the entire process.

IN THE LARGE BOWL MIX:	**AFTER MIXING:**
1 teaspoon sugar	**Add 1 package yeast.**
1 ½ cups flour **from the small bowl**	Then, whip with wooden spoon for 2 minutes until frothy.
1 ½ cups hot water (130 F)	Add and mix in **1 ½ teaspoon** salt
¼ cup olive oil	Add and mix in **2 cups** flour (from small bowl) a little at a time until mixture comes away from bowl

Scrape bowl and place the dough on a well floured bread board. Add flour to your hands and knead the dough vigorously for at least 10 minutes, adding flour constantly to board and hands to reduce stickiness. Use scraper to get dough off board while kneading. Lightly oil the bread pan to coat sides and bottom. Form the dough into a loaf, put in pan and lightly oil top. Cover it with a towel and place under the heat lamp for 45 minutes.

Make a very shallow cut into the top of the loaf to allow for even rising and bake at **400 °F** for 45 minutes, or until golden brown. Your instructor will assist you at the oven.

In the making of this and many other breads, the active "ingredient" is the single-celled organism known as yeast. Yeast cells are capable of both cellular respiration and fermentation; the type of pathway taken depends on the availability of oxygen. In bread making, it is the cellular respiration of yeast which is all important. Yeast cells feed on the sugars in the dough, metabolizing them to release energy, as well as producing the by-products carbon dioxide and water (see below).

$$1\ C_6H_{12}O_6 + 6\ O_2 \text{ ----------> } 6\ CO_2 + 6\ H_2O + 32\text{-}38\ ATP$$

Glucose Oxygen (gas) Carbon Dioxide (gas) Water "Energy"

It is the carbon dioxide trapped in the dough that causes the dough to "rise," giving it volume. Additionally, this expansion causes stretching of the dough, which gives it structure and texture. Lastly, other by-products of yeast metabolism along with the yeast cells themselves modify and give the breads their characteristic flavor.

QUESTIONS

1. With regard to the function of the yeast in bread making, why is water added to the yeast?

2. What would happen to the bread if boiling water were added to the yeast? If cold water were used?

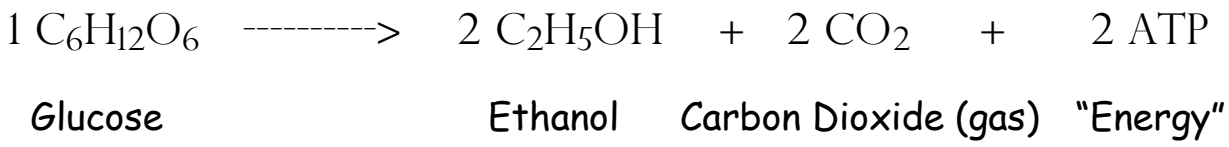
3. After baking, why doesn't the bread continue to rise?

YEAST

Examine the yeast set-up under the microscope. Make a rough sketch of what you see. Do you see any yeast cells dividing?

FERMENTATION

Wine production is the result of fermentation by yeast cells, more commonly known as alcoholic fermentation. Crushed fruit (usually grapes) containing large quantities of sugar and yeast (found living naturally on the skin of the grapes themselves) are sealed in an air-tight container. Yeast cells then feed on the sugar, using it as a source of energy for their growth, maintenance, and reproduction. In the absence of oxygen, the breakdown of sugar is via the anaerobic pathway, in which ethyl alcohol and carbon dioxide are the end products along with energy (see below).



After the alcohol concentration reaches about 12%, it is enough to kill the yeast cells (i.e. alcohol becomes a lethal "pollutant" in the yeast's environment) and fermentation stops. Thus, the alcohol content of most wines is close to 12%.

Your instructor will briefly discuss the fermentation demonstration set up in the front of the lab and the wine brewing in the container.

QUESTIONS

1. Given the choice, would it be more efficient (in terms of amount of ATP produced per glucose molecule consumed) for a yeast cell to use fermentation or cellular respiration? Explain.

2. Cellular respiration produces 32-38 ATPs worth of energy from each glucose molecule, whereas fermentation produces only 2 ATPs. Which molecule that results from fermentation contains most of the energy that was released from glucose?

3. With regard to question #2 above, what does this tell you about the amount of calories you consume when drinking alcohol (even clear drinks like vodka)?

PROTEINS

As we have discussed, proteins are chains of amino acids which are connected together by peptide bonds to form their primary structure. Secondary and tertiary chemical interactions cause these strands of amino acids to form helical and sheet-like arrangements that give each specific protein a particular three-dimensional shape. A protein may consist of only a few to thousands of different amino acids in length. It is the overall three dimensional shape which is determined by the primary, secondary, tertiary and potentially quaternary structure of a protein that gives each protein its specific function in a living organism. The diverse structures of proteins allow for their critical and varied functional roles in cells. Some cellular functions of proteins are listed below:

1. **ENZYMES (ATP synthase, lactase, phosphofructokinase, creatine kinase, pepsin, etc.)**
2. **STRUCTURAL PROTEINS (collagen, keratin, tubulin, fibrin, elastin, etc.)**
3. **CELL RECEPTORS (T cell receptor, histamine receptor, testosterone receptor, etc.)**
4. **HORMONES (insulin, glucagon, LH, FSH, growth hormone, TSH, etc.)**
5. **TRANSPORT PROTEINS (hemoglobin)**
6. **DEFENSIVE PROTEINS (antibodies)**
7. **MEMBRANE CHANNELS (sodium channel, potassium channel, etc.)**
8. **And many other varied functions…**

When a protein or other macromolecule loses its specific three dimensional molecular shape, it will no longer retain the same natural function. A change in the three dimensional molecular shape of a protein or other macromolecule that affects its function is called **denaturation**.

A series of experiments to demonstrate the effects of denaturation are given below.

DENATURATION OF THE PROTEIN IN EGG WHITE

PROCEDURE

1. Obtain three test tubes and label them Tube A, Tube B, and Tube C. Fill tubes A and B about 1/4 full of distilled water. Fill test tube C about 1/4 full of the acid from the dropper bottle.

2. To each tube, add about 1/2 eye dropper full of egg white.

3. Place one test tube containing egg white/water into the 80 degree water bath for 5 minutes. Note the color and structural change of the egg white with time. Compare it to the egg white in the other tube with egg white/water. "Cooking" of the egg protein actually involves the unwinding of the protein molecules in response to heat.

QUESTIONS

1. Explain what is meant by the term "denaturation" as it applies to proteins.

2. Once it has occurred, can denaturation be undone? That is, can you "uncook" an egg?

Gently swirl the tube containing acid and egg white. DO NOT place your finger over the open end of the tube while mixing!!! Compare this egg white with that in the untreated egg white/water tube.

QUESTIONS

1. How are the egg whites in tubes A, B, and C similar or different? Using your knowledge of protein denaturation, explain these results.

2. Keeping in mind the results of the egg white experiments, explain why it is so important for us to maintain a relatively constant body temperature and pH.

ENZYMES

Living cells have the ability to perform a large number of chemical reactions at a very rapid rate. This is made possible by enzymes, which are proteins that catalyze or speed up the rate of chemical reactions without being used up themselves in the process. Without enzymes, the myriad of reactions occurring within each cell would proceed too slowly to sustain life.

Each specific chemical reaction in the cell, whether it involves the synthesis or breakdown of chemicals, is catalyzed by a specific enzyme. For a reaction to occur, the substrate molecule (i.e. the molecule upon which the enzyme acts) needs to fit into a precisely shaped slot (called the active site) on the surface of the three dimensional enzyme specific for that substrate. Thus, each enzyme can catalyze only one specific type of reaction, and each type of reaction can be catalyzed by only one specific type of enzyme.

From this, it follows that the shape of the active site must be maintained for the enzyme to function as a catalyst. However, there are factors (e.g. temperature, pH, etc.) which can alter the three dimensional structure of the enzyme, thus altering the shape of the active site and the ability of the enzyme to function. When the alteration of the active site is sufficient to render the enzyme completely inactive, we say that the enzyme has been denatured.

THE EFFECT OF TEMPERATURE ON THE ENZYME RENNIN

Enzymatic reactions are affected by temperature. In cold environments reactions proceed very slowly, while in a warm environment reactions proceed very rapidly. With each 10°C rise in temperature, an enzymatic reaction will proceed about twice as fast. However, if the temperature is raised too high, the enzyme, being a protein, will become denatured and won't work as a catalyst anymore. In this exercise we will observe the effect of temperature on an enzymatic reaction.

In mammals, one of the first steps in the digestion of milk is the denaturing of milk protein so it becomes solid. Milk protein is denatured in the stomach, not by heat in this case, but by the enzyme rennin (sometimes called rennet). Keep in mind that it is the rennin that we are studying. If it works then milk will be denatured and become solid. If the rennin is too cold to work or if it becomes overheated and denatured, the milk will not become solid. The following experiment will demonstrate how temperature can affect the functioning of rennin.

PROCEDURE

1. Add 5 ml of **whole** milk to each of 3 of the smaller test tubes.

2. Place one tube into the **0° C ice bath**, a second tube into the **43° C water bath**, and a third into the **80° C hot water bath**.

3. Let the tubes stand for **5 minutes** to allow the milk in each to come to bath temperature.

4. After the 5 minutes, bring the rennin solution at your bench to the water baths and add **5 drops** of rennin solution from the container to each of the test tubes.

5. Gently shake the tubes to mix the enzyme with the milk. Then place each back into its bath and allow them to stand for **10 more minutes.**

6. After the 10 minutes, look at the milk in each tube and **indicate on the chart on the next page** whether the milk has curdled (+) (has the milk protein been denatured by the rennin), or not (-).

7. **If your milk did not curdle** (-), take the sample and place it in a temperature bath where the milk curdled. After 10 minutes in the bath, record your results (+ or -) in the "2nd INCUBATION" column on the chart. Has the enzyme from the non-curdled specimen been irreversibly denatured or not?

QUESTION

Explain your results **in terms of temperature** and its effect on the **shape** and **activity** of enzymes.

Shape of the Substrate Molecule and Enzyme Activity

PROCEDURE

1. Add 5 ml of soybean "milk" to a test tube and 5 ml of Mocha Mix (non-dairy creamer) to another test tube. Label each tube with **S for soybean or M for Mocha Mix.**

2. Place the tubes into the **43° C water bath.**

3. Let the tubes stand for **5 minutes** to allow the contents to come to bath temperature.

4. After the 5 minutes, add **5 drops** of the rennin solution to each tube. Gently shake to mix the enzyme with the milk. Then place them back into the bath and let them stand for **10 more minutes.**

5. After 10 more minutes, look at the contents of the tubes and indicate in the table below whether the soybean protein and/or the mocha mix was denatured (+) or not (-).

TYPE OF PROTEIN	TEMPERATURE	RESULT (+ OR -) = CURDLING	
		1ST INCUBATION	2ND INCUBATION
MILK	0° C		
MILK	43° C		
MILK	80° C		
SOYBEAN "MILK"	43° C		
MOCHA MIX	43° C		

QUESTION

1. Did the soybean "milk" curdle? Why or why not? Check the ingredients on the Soymilk carton.

2. Did the Mocha Mix curdle? Why or why not? Check the ingredients on Mocha Mix carton.

3. Explain the above result in terms of what you know about the importance of the shape of the substrate molecule in relation to the shape of the enzyme.

Clean-up:

_____ **Washand dry all bowls and utensils. Place on cart (front of room).**

_____ **Do not wash bread pans. Wipe with a dry paper towel and place on cart (front of room).**

_____ **Washand dry plastic cutting boards and knives and return to counter by stove.**

_____ **Scrape all dough and flour off of the wooden cutting boards with utensil provided.**

_____ **Clean all test tubes (8 total) using the applicator sticks and test tube brushes by sinks. Acid in test tubes can be washed down the sink. If you have marked on your test tube with a sharpie (marker), you do not need to remove these marks. Place clean test tubes upside down in test tube racks, and return to side counter. If you do not have 8 test tubes, check water baths and ice baths.**

_____ **Rinse 10ml graduated cylinder and return to tray on side counter.**

_____ **Return all supplies to correct trays on the side counter.**

LABORATORY NOTES

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