Yeast—An Ascomycete

10.11.30

Name ____________________
Date ________ Hour ________

Yeast cells can obtain the energy stored in the bonds of sugar molecules by the process of fermentation. In this process sugar molecules are oxidized anaerobically--that is, in the absence of oxygen. During fermentation, the bonds holding the atoms of carbon, hydrogen, and oxygen together break. As a result, these atoms recombine in different arrangements, forming new compounds. The quantity of usable energy released during fermentation is much less than the energy originally present in the sugar. Some of the original energy is released as heat and some is stored in the bonds of the new compounds that are formed. The remaining energy, which is available to the yeast cells, is temporarily stored in molecules of ATP (adenosine triphosphate). As this stored energy is released, the yeast cells carry on their life processes.

The following equation is a summary of alcoholic fermentation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy}$$

Sugar ($C_6H_{12}O_6$) is the energy source and becomes the reactant in the process. C_2H_5OH (ethanol or ethyl alcohol) is a waste product of the process (Yes, that's the drinking alcohol found in alcoholic beverages!). Carbon dioxide (CO_2) gas is also a waste product. Ethanol and carbon dioxide are products in the reaction. The most important thing the yeast cells get from the process is the energy.

Tell what yeast cells "eat" to obtain energy: ______________ Name the process they use to obtain this energy: ______________ What does the term anaerobically mean? ________________________

Name the chemical yeast cells use to temporarily store energy for use in their life processes: __________, which stands for ______________________ Give the equation for alcoholic fermentation in the space below—label the reactant and two waste products of this process:

In this activity, the amount of carbon dioxide gas produced will be used as an indicator of the rate of fermentation. Since CO_2 is a waste product of the fermentation process, the amount produced is determined by how much fermentation is carried out by the yeast cells.

PROCEDURE: Obtain a screw-top vial. Fill it approximately half-full of tap water and add a measure of yeast (*Saccharomyces cerevisiae*). Cap the vial and lightly shake the contents for ~15 seconds.

Obtain six 16 mm x 150 mm culture tubes and an air-cap for each. Number the tubes 0, 1, 2, 4, 8, and 16. Pour ~1 cm of yeast suspension in each tube. **The amount in each tube MUST be equal—swirl the suspension between each pouring to keep it homogenous.** Extra suspension can be discarded. Rinse the vial and return it to the storage container.

Making serial sugar solutions....Using the balance, mass 16g of sucrose (table sugar) and place it in a 100 mL graduated cylinder.

Add <u>warm</u> water (Fill a beaker from the main lab sink so you will have plenty.) to bring the total volume of the graduate to 100 mL. Cover the top of the graduate with your palm and invert the cylinder until ALL of the granular sugar has gone into solution. Add extra water, if needed, to bring the volume back to 100 mL.

You have made a 16% sucrose solution (16 g sucrose per 100 mL of water)…fill tube number "16" to the **very top** with the 16% sucrose solution.

Next, discard enough of the 16% solution **remaining in the graduate** so it measures 50 mL. (Use a separate beaker to pour-to just in case you pour too much.) The extra sucrose in the beaker can be discarded.

Add warm water to the graduate until it once again reads 100 mL, repeat the shaking process. You have now cut the concentration in half (to 8%) by dilution. Fill the tube marked "8" with this solution.

Once again discard enough of the remaining solution until the graduate reads 50 mL. Add warm water to bring the volume back to 100 mL (don't forget to shake). You now have a 4% solution. Fill tube "4" to the top.

Repeat this process two more times to make your 2% and 1% solutions—fill the appropriate tube each time. Fill tube "0" with the warm tap water.

Beginning with tube 0, place your finger in top of each tube and invert it several times to mix the yeast and sugar solutions.

Cover the tubes with air caps and quickly invert them. **KEEP THE TUBE UPSIDE DOWN FOR THE REST OF THE LAB.** A small volume of air should be trapped in the "top" of each tube. Holding each tube so it is vertical, mark a reference line on the glass at the meniscus. Place the **INVERTED** tubes in a 400 mL beaker.

Slowly add sufficient water to the beaker to cover the base of the air caps. In the table below record the sucrose values in column 1. Allow the tube contents to ferment until the next lab session.

Remove each culture tube from the beaker, hold it vertically, and measure the distance from the original height of the liquid to the current height. Record the height change in column two of the table.

Calculate the volume of CO_2 gas produced in each tube using the formula $V = \pi r^2 h$. Begin by measuring the inside diameter of a spare culture tube with the vernier caliper and record it below. Since the formula to calculate volume requires radius…..divide the diameter by 2.

Diameter =________ Radius =_________

Percent solution (%)	Height change (cm)	Volume of gas produced (cm^3)

Show your volume calculations for the 16% tube in the space below. Prepare a graph of the volume of gas produced vs. the percent sugar solution.

Yeast and the production of alcohol:

Any combination of sugar and water will ferment if yeast (or in a few situations, bacteria) is added to it. Pure sugar will not ferment because it is too concentrated to sustain life. Honey, as made by bees, is a good example of this. You can leave a jar of honey on the table (without refrigeration) because the bees have developed a system of concentrating the sugar to the point that microorganisms cannot live in it.

Most organisms need oxygen to live, but not yeasts. In the presence of oxygen they carry-on aerobic (with oxygen) respiration just as any other organism (you included)...

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{energy}$$

But, when the oxygen gets used-up or the yeasts are placed in an anaerobic (no oxygen) environment, they continue to make energy through the process of alcoholic fermentation...

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy}$$

During the process you can observe the carbon dioxide gas (CO_2) being produced. The alcohol (C_2H_5OH) collects in the fermentation chamber until the level becomes toxic to the yeasts. They die and settle to the bottom of the chamber when the alcohol approaches ~12% (or if they run out of sugar and starve to death). If you think about it, alcohol is a waste product of the fermentation process...so...the yeasts actually die in their own urine when it reaches a toxic level.

Tell why pure sugar will not ferment. ____

Name the reactants in the process of respiration ________________, ____________________

Name the products in the process of respiration ______________, ______________, ____________

Name the reactant in the process of fermentation ________________________

Name the products in the process of fermentation ________________, ______________, __________

Name the gas that yeasts do NOT need to carry-on fermentation ________________

At what percent alcohol do yeasts "pickle" themselves? ______________

The fermentation of "food products" was started by the ancient people of Iraq (Mesopotamia at the time) well over 8 000 years ago. It rapidly spread to Egypt and around the world from there.

Until 1860 fermentation was believed to be a purely chemical process having nothing to do with living organisms. Then, Louis Pasteur showed that fermentation involves a living process carried out by yeasts.

Wine can be made from any fruit with enough sugar in it (or you can add sugar) including: grapes, peaches, bananas, apples (cider), blueberries, carrots, turnips, cherries, grapefruits, watermelons, oranges, onions, pears, pineapples, tomatoes, ...

Once the alcohol is produced, it can then be distilled to increase its concentration. The distilled alcohol can then be further flavored by treating it with additional processes. Several popular alcoholic beverages (some distilled, some not) are listed on the next page. Complete the table using the chart available to you in class so you will have a good idea of how these are produced.

Alcoholic Beverage	Fermented and/or Distilled Material or Process
Beer	
Whisky	
Rum	
Brandy	
Gin	
Vodka	
Tequila	
Sake	
Mead	

Making your own wine....

Obtain a culture jar for your group. Add your name, initials, and date to the jar.

Fill the jar with grape juice to the "roll-over" for the neck. Do not overfill.

Add one vial of yeast to the juice, cover the jar with your thumb, and agitate the contents by inverting it several times.

Pre-stretch a balloon by inflating it at least twice. Stretch the mouth of the balloon over the mouth of the jar—leave the balloon limp. This will serve as a fermentation lock (to keep out oxygen). It will also collect any carbon dioxide gas produced during the fermentation process.

Now you wait—"We shall serve no wine before its time!" Yes, it is that simple.....

Get on the internet and look up an MSDS (materials safety data sheet) for ethanol (CAS 64-17-5). Find section <u>three</u> and summarize the information you find under the section labeled "**Ingestion**"....

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Culturing and observing yeast.....

Label four culture tubes with your initials and number them 1-4. Fill each tube approximately ¾ full of water.

Tube 1: Do not add anything. This will be your control tube.
Tube 2: Add a pinch (use your fingers, it won't hurt you) of yeast. Check-out the odor of the dry yeast.
Tube 3: Add two pinches of sucrose (table sugar) and a pinch of yeast.
Tube 4: Add two pinches of sucrose, a pinch of yeast, and ONE drop of neutral red stain.

Cover each tube with parafilm and, while holding you thumb over the mouth of the tube, invert each tube to mix the contents. Store the tubes overnight.....

Compare tubes 2 and 3 to tube 1. Draw a conclusion based on your observations: ____________________

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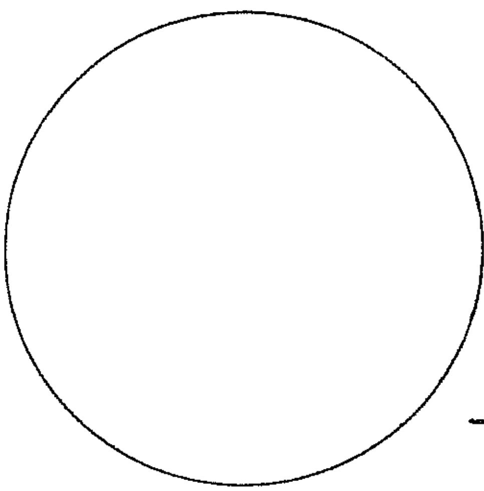
Describe the odor of tube 3: ____

Agitate tube 4 by rolling it between the palms of your hands. Make a wet mount slide of the yeast suspension. Make a drawing in the space provided. Use high power magnification and locate as many of the characteristics as you can (as shown in the diagrams below).

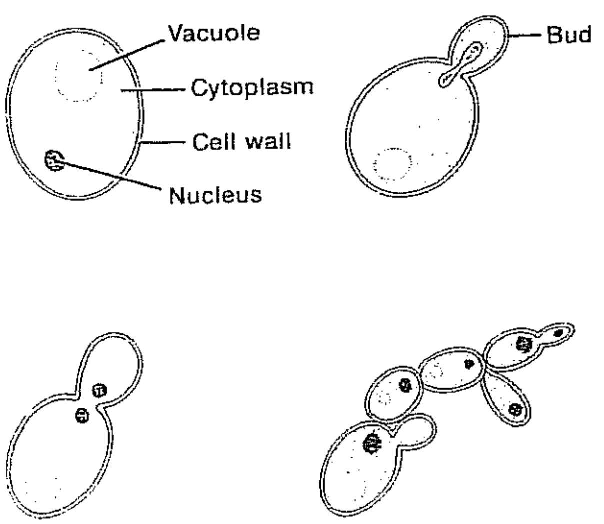


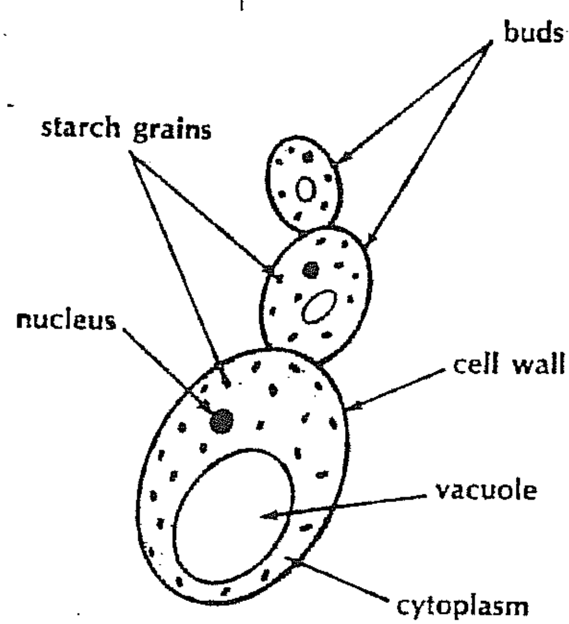
______ x

Magnification ______________

Size ______________ (Show your calculations in the space to the right.)

Verified ________________





Budding Yeast