

## YEAST AND THE PRODUCTS OF ITS FERMENTATION PROCESS

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### I. OBJECTIVES:

1. To study about the process of fermentation as a source of energy for cells.
2. To relate this to "everyday life" by making "lab brew."
3. To illustrate one of the classic principles in the field of microbiology.

### II. BACKGROUND:

Many microorganisms (**micro** = small), notably yeasts and bacteria, extract energy from their food (glucose) by fermentation. The best-known method of fermentation is alcohol fermentation in which the overall chemical reaction is: (sugar)  $C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH$  (ethyl alcohol). Humans have known about and utilized this fact for many thousands of years.  $CO_2$  liberated by yeast helps our bread to rise, while the liberated alcohol gives it its wonderful smell. The Egyptians and many subsequent civilizations have fermented grains such as barley to break the starch down to malt (maltose), then glucose, and finally alcohol. For at least that long, people have also known that various fruits, especially grapes, could also be fermented to produce alcoholic beverages. In this lab, we will be studying the process of fermentation as performed by the yeast, *Saccharomyces cerevisiae* (**sacchar** = sugar; **myces** = fungus; **Ceres** = goddess of grain; **vis** = to see; **-ia** = state of, condition of, disease).



Figure 1. Yeast

Louis Pasteur was a famous French microbiologist who lived in the 1800s. People involved in the wine industry of that day asked him to research why some bottles of wine were OK while others spoiled and turned into vinegar (**vin** = wine; **aigre** = sour). Pasteur discovered that the whitish bloom on the skins of grapes contained a number of small, oval cells which he identified as yeast (Figure 1). These are the yeast that turned the smashed grapes into wine.



Figure 2. Yeast and *Acetobacter*

In wine that had turned to vinegar, Pasteur also found small, rod-shaped bacteria (Figure 2) (this shape is called **bacillus**) as a "contamination" or "infection" in the wine. These were found to belong to genus *Acetobacter* (**aceto** = vinegar; **bacter** = rod). Pasteur discovered that if the wine was heated

to 63°C and held at that temperature for 30 minutes, the *Acetobacter* would be killed. This process is named in his honor: **pasteurization**. Some people object to treating wine in this way, claiming that the flavor is changed. Today, by law, all wine sold in the United States must have sulfites added to kill anything living in it (and some highly allergic humans).

There is, however, another way to inhibit growth of *Acetobacter*. It has been discovered that while yeast do not need the presence of oxygen ( $O_2$ ) to do fermentation, *Acetobacter* do need  $O_2$  to turn alcohol into vinegar, more specifically acetic acid ( $CH_3COOH$ ). Thus, if  $O_2$  can be eliminated, the *Acetobacter* cannot grow. However, since the process of fermentation is evolving  $CO_2$ , the fermentation vessel cannot be sealed or it will explode. This necessitates the use of an airlock (Figure 3) which allows the  $CO_2$  produced to bubble out through a water barrier which simultaneously prohibits  $O_2$  from entering. We will, therefore, be making use of Pasteur's discoveries to keep our brew from turning into malt vinegar.

For centuries, beer has been flavored by the addition of various bitter herbs, for example, meadowsweet (*Spiraea latifolia*), alehoof (which is another name for Gill-over-the-Ground, *Glechoma hederacea*), and/or alecost or costmary (*Chrysanthemum balsamita*). In the eleventh century, Bavarians started adding hops (*Humulus lupulus*) to the brew to act as a preservative (it helps extend the "shelf-life" of the beer) and to flavor the beer, as a replacement for the bitter herbs previously used. This practice was borrowed by the British in the sixteenth century. Modern breweries start with a cooked "mash" of sprouted barley which is fermented by a special strain of yeast. Added to this is a water-extract or "tea" from hops. For homebrewing, cans of barley malt with hops extract added can be purchased to add to sugar water to make beer. This is what we will use in this experiment.

Hops (*Humulus lupulus*) is in the family Cannabinaceae. Its native habitat includes damp areas where it can be found twining tightly around willow (and other) trees. Because of this, the Greek philosopher

Pliny called it *lupus salictarius* which means "willow wolf." Interestingly, apparently it always twines in a clockwise direction. "Humulus" is a Medieval latinization of an Anglo-Saxon word, "humule." In the U. S. today, most hops are grown in Washington, Oregon, California, and Idaho.

Like a certain other member of the Cannabinaceae, the part of the hops plant that is used is the female **strobilus** (a part of the female plant surrounding the flowers). This has also been used medicinally for centuries. The primary constituents responsible for the medicinal properties and bitter flavor are two chemicals called humulone and lupulone. These are unstable in the presence of light and air, thus dried hops rapidly lose their flavor and medicinal effectiveness (and must be used for brewing within a relatively short time after

harvest). Hops is a well-known sedative and has long been used as a sleep aid. Animal and human research has shown that, indeed, it is a CNS depressant. Hops is also used herbally as a diuretic and antibiotic.

Within the yeast cells, the actual chemical reactions that turn sugar into alcohol are catalyzed by a number of **enzymes** (**en** = in; **zym** = yeast) – biological catalysts that help other chemicals to react. Although numerous enzymes are found in living organisms, each one with its own specialized function, the very first such chemicals to be studied were those involved in the process of fermentation. Since they were found in yeast, they were called "enzymes." We now know of many more enzymes, most of which are not found in yeast, yet the name is still used.

### III. MATERIALS NEEDED FOR FIVE GALLON BATCH:

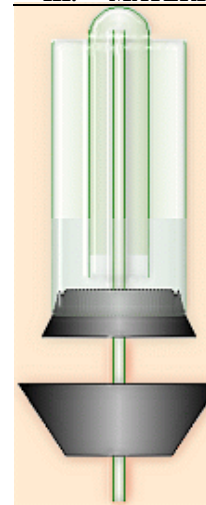


Figure 3. Airlock

nothing happens – it's dead – try again.

B. In a pot, place a few inches of water and the can containing the malt extract (opened and with label removed). Heat the can of malt until it can be poured.

C. Into a separate, large pot, measure 1 1/3 C malt and dissolve in 1/2 gal. (= 8 C) of water.

\*\*\*\*If unhopped malt is used, first heat this water, then put in the hops and let it steep to make "tea" (technically, this is called an **infusion**). Optionally, pour the hops tea/infusion through a strainer to remove most of the hops pieces before adding the warmed malt extract.

D. Add the (6 C) sugar and stir to dissolve.

E. Slowly heat to boiling but do not let it boil over. Stir slowly to prevent burning.

one 5-gal. jug (or five 1-gal. jugs)

airlock(s) (Figure 3) made from rubber stoppers and glass tubing

hot pads, measuring cup(s), 1-gal. pot, hot plate, wooden spoon

1 1/3 C hops-flavored malt extract OR unhopped malt + a packet of dried hops (available from a beer/wine supply store)

6 C white sucrose (2 C brown sugar may be substituted for 2 C of the white for darker beer. Dextrose/glucose or maltose may also be substituted for part/all of the sucrose or the ratio of malt extract to sugar may be increased. Sugar may totally be replaced by malt extract. We have made a delicious dark beer with 1/2 can of dark malt + about 2 C of glucose.)

1 pkg (or 1 T) live yeast, preferably brewer's or ale yeast, but regular baking yeast or wine yeast will work with a slightly different final product

9 or 10 CLEAN 2-liter pop bottles with plastic lids – if you have some, please bring them in when we are ready to bottle the brew

### IV. PROCEDURE:

A. Add 1 pkg (or 1 T) yeast to 1/2 C water at about 37° C (warm but not hot) with 1 tsp sugar. Let sit for about 15 min. It should, then, be bubbly or frothy. If

nothing happens – it's dead – try again.

F. For a 5-gal. jug: add 2 gal. cold water to the jug, then add the hot sugar solution with a funnel. (For 1-gal. jugs: pour in about 6 1/2 C cold water and 1/5 of the sugar solution – just divide it up as you go along.)

\*\*\*\*Avoid running the hot solution down the sides of the jug or it may crack from the heat. Pour it down a funnel directly into the cold water.

G. Fill the jug to the "hip" (about 4 1/2 gal. in 5 or 3/4 gal. in 1) with cold water. This sugar solution is called the "wort."

H. If the yeast solution is bubbly, add it to the jug (for 1-gal. jugs, divide it among them). This is called "**pitching the wort**," with "pitching" referring to setting up something (like pitching a tent), not throwing something (like pitching a ball) – the wort is being set up to ferment. If it's not bubbly, mix up some new as per step #1. It is very important to add all the cold water first – if the solution is too hot when the yeast is added it will kill the yeast.

I. Place an airlock on top of your bottle and fill with water to the correct level. This will allow CO<sub>2</sub> from the fermentation process to escape without permitting entrance of outside air that contains O<sub>2</sub> needed by bacteria that could cause the beer to spoil or turn to vinegar. Label the bottle with the lab section, date, type of malt, (hops,) sugar, and yeast.

J. After 1 to 2 days (next lab period), the beer should have a good head on it (called the “high kreusenens” stage). After about 4 days, the foam should subside, at which point, fill the jug up to the neck with water. In pharmaceutical jargon, one would say, “**Q. s.** to the neck,” where “**q. s.**” stands for “*quantum sufficit*” in Latin which means as much as suffices, as much as is necessary. Replace the airlock. Note: you may be able to do this step as soon as the next lab period.

K. After about 8 to 10 days or so (may take longer if the room is cooler), bubbling should have decreased to a very slow rate. About two to three weeks after the brew was made, it will be ready for bottling. In each of the 1-qt. bottles, add 1 tsp. sugar using a funnel (= 2 tsp in a 2-liter bottle or ½ tsp in a pint bottle). Carefully **decant** (carefully pour off the liquid or **supernatant** [**super** = over, above; **natant** = swimming]), leaving the sediment on the bottom) or siphon the beer into a large, clean beaker or bucket, avoiding the sediment on the bottom. From that container, pour the beer into the bottles, leaving about 1½ inch of head-room in the bottles. If the last bottle is just a tiny bit low, q. s. with water.

L. Cap the bottles and check for leaks. Store in a 60 to 70° F location for a minimum of 10 to 15 days to allow residual yeast to

#### V. DATA:

Take notes on any verbal information provided by your instructor and on what your class did. Record any deviations from the written protocol (did you use a different type of sugar?). Make sure to include all observations and data. Draw pictures, especially of any new equipment (airlock on

#### VI. CONCLUSIONS:

In your discussion, you should include any of the following that apply.

1. As time and interest allow, look up and read about how breweries make beer. Summarize your findings and cite your source(s).

2. As time and interest allow, look up and read about how wine is made. Summarize your findings and cite your source(s).

3. As time and interest allow, visit one of the local breweries or wineries for a factory tour. Tell about your visit and what you

ferment the added sugar. This forms the carbonation in the beer. Note that if you bottle the beer too soon and/or add too much sugar and/or get too much of the yeast sediment into the bottles, too much CO<sub>2</sub> will be formed and the bottle(s) may/will explode.

M. Swirl the sediment in the jug to loosen and pour into designated container. Immediately and thoroughly wash out the jug, making sure to scrub off any yeast stuck to it, and place in rack to dry. The yeast mixture is difficult to impossible to remove once it has dried.

N. While current University policy does not allow us to let you take any of this lab brew home, if you later decide to make some on your own, after the beer has aged at least two weeks, to taste, refrigerate upright for at least 12 hours. Do not shake or disturb. When you open it, decant it into a pitcher (or into glasses, but you must pour it all out and not stop midway), avoiding the yeast sediment at the bottom. Again, rinse out the bottle as soon as possible, before the left-over yeast dries. The flavor improves with age. Do not let the bottles freeze or get too warm or they may explode. It is a good idea to store the bottles in a location that will not be damaged if one does explode – it does occasionally happen.

Note that the left-over yeast, especially the strain used by commercial breweries, is processed to remove some of the bitter flavor, killed, and purified, then dried and sold as brewer’s yeast in the health-food stores or used as a supplement in livestock feed. It is an excellent source of the B vitamins and other nutrients.

bottle, bottle-capper), and/or take notes where needed. Optionally, a “souvenir” yeast packet or piece of malt label (only if the can is empty and the label no longer needed) may be included.

learned.

4. As time and interest allow, study the formation of CO<sub>2</sub> by yeast by baking a batch of bread at home. How easy or hard was it to make? How did it smell while baking? What do you think makes it smell that way – what chemical is being evaporated/released? How did it taste? How well did the yeast do its job?

5. In step #1, why do you think it is important that the water used to dissolve the yeast be warm but not too hot?

### OPTIONAL AT-HOME EXPERIMENT: BREAD

#### III. MATERIALS NEEDED:

½ C honey  
2 T or 2 pkg yeast  
2 C warm (not hot) H<sub>2</sub>O  
whole wheat flour (approx. 6 C or so)  
¼ C oil (safflower is highest in vitamin E, olive has a good taste, others will work, too)  
big mixing bowl  
measuring cup(s) and spoons  
wooden spoon (and/or electric mixer)  
2 loaf pans or cookie sheet (coated with oil and dusted with whole wheat flour)



#### IV. PROCEDURE:

A. In a large bowl, mix the honey and warm water. If the water is too hot, it will kill the yeast. Warm tap water is OK.

B. Sprinkle in the yeast and gently stir to dissolve. Wait about 10 min to make sure the yeast is alive: the mixture should start to get frothy/foamy.

C. Add the oil and 2 C of flour. Beat approx. 1000 strokes by hand or for a while with the electric mixer to develop the gluten, a protein that makes the structure of the bread that holds in the CO<sub>2</sub>.

D. Gradually, add approx. another 4 C of flour. The dough should stick together but not be too stiff. Optionally, you may add raisins, sunflower seeds, chopped nuts, cinnamon, or chopped onion and various herbs such as basil and sage, or whatever sounds like a good bread ingredient. You may also substitute soy, rye, oat, or other flour or meal for 1 to 2 C of the whole wheat flour.

E. Knead the dough about 10 min until it becomes elastic.

F. Coat lightly with oil and place in an oiled bowl. Cover with a damp (clean) cloth or kitchen towel (not terry cloth). Allow to rise in a warm place (a gas oven that’s turned off is good) for about one hour until doubled in size.

G. If not already done, coat the loaf pans/cookie sheet with oil and dust with flour.

H. “Punch down” the dough (deflate it somewhat), and form into two loaves. Place into the loaf pans or form round loaves on the cookie sheet.

I. Cover lightly with the towel and let rise until double again (maybe another hour although it shouldn’t take as long this time).

J. Bake at 350° F for about one half hour or until done (check it after ½ to ¾ hr). Signs of doneness are a) if the sides have shrunk away from the pan and the crust is browned and b) when you turn it out of the pan and tap on the bottom, it sounds “hollow.” If not, put it back in the pan and back into the oven for a while. Time may vary depending on a number of factors.

K. Turn out onto a wire rack to cool. To keep for more than a day or two, store in refrigerator in a plastic bag. May be frozen.

Variations: You may add from 1 to 2 C up to not more than half the total of a different kind(s) of flour. Unbleached white flour will make a lighter-colored, higher-rising bread but lower in vitamins and fiber. Rye bread can be made by adding up to half rye flour and some caraway seeds. A couple tablespoons of unsweetened cocoa and instant coffee will make it dark like pumpernickel (using molasses in place of honey will help darken it, too). A cup of soy flour plus a cup of wheat germ will increase the protein content of the bread. Instead of loaves, you could braid the bread by forming three strands and braiding them (bake on cookie sheet). Dinner rolls could be made by forming into small balls and baking on cookie sheet or in muffin tins (will not take as long to bake).

#### V. DATA

Take notes on what you tried.

#### VI. CONCLUSIONS:

Make any comments and/or suggestions you can think of. In the past, a Biology lab tradition has been lab brew accompanied by popcorn seasoned with Fankhauser popcorn seasoning. Hopefully, we can still sample the popcorn.

**FANKHAUSER POPCORN SEASONING:**

4 T brewer's yeast  
 2 T powdered kelp  
 1 T salt

Note: Larger batches may be made by using the same proportions of ingredients (like 4 C + 2 C + 1 C, etc.).

Mix thoroughly (in blender if yeast is in flakes). Store in tightly-sealed jar.

**TO MAKE A BATCH OF POPCORN:**

1. Place about 2 T (walnut-sized piece) of coconut oil into a heavy pot and place the pot on the largest burner on the stove. Turn the burner on high.

2. When the oil is melted and hot (a corn kernel will sizzle), add 400 mL corn kernels and cover. Shake until all the corn is popped.

3. Pour the popped corn into a grocery sack (with top turned over twice to stiffen it).

4. While shaking the bag to stir and mix, slowly drizzle on 30 to 40 mL of a 1:1 butter/oil mixture (provides butter flavor with

less cholesterol). Then, dust with 30 to 40 mL of seasoning mixture.

In this popcorn, the brewer's yeast provides a number of the B vitamins. Kelp provides iodine, needed by your thyroid gland. The kelp, brewer's yeast, and popcorn are complementary protein sources, thus form a complete protein when combined. By mixing the butter 50:50 with vegetable oil, it is possible to have the good buttery taste, yet reduce the amount of cholesterol (cholesterol is found only in animal products, thus would be in the butter, but not vegetable oil).