Fermentation and Distillation

Objectives
- to use yeast and sugar to effect a fermentation
- to purify the alcohol obtained by fermentation through simple distillation
- to compare densities of liquid before and after fermentation (and with commercial distilled spirits)

Equipment/Materials

Materials for fermentation (week 1):
- Dry yeast, 1.0 g (one small envelope of baker’s yeast is more than enough)
- Sugar, 20 g
- water (85 mL)
- modeling clay
- vegetable oil
- 2 empty 6-12 oz plastic bottles (commercial soda or spring water bottles work well)
- 2 plastic bendable straws

Materials for distillation (week 2):
- medicine dropper or spoon
- duct tape
- coffee can
- resealable plastic sandwich bags
- 10 inch (25.4 cm) length of clear vinyl tubing (1/2 inch outer diameter) or garden hose
- sharp knife (Exacto® knife works well)

Introduction

Fermentation

Fermentation is the breakdown of complex molecules to simpler ones through the action of some microorganism, such as yeast. Indeed we normally think of fermentation in the context of beer or wine, but the term also includes the conversion of milk to yogurt or cheese and the making of bread. In all of these examples carbohydrates are being fermented by yeast. In the case of bread, yeast converts carbohydrates into carbon dioxide gas (CO₂). The little pockets of CO₂ make the bread rise and produce the fluffy consistency that we associate with bread. Indeed it is this formation of gas pockets that distinguishes bread from crackers. For fermentations that produce alcohol, such as wine, carbohydrate is converted to ethanol and carbon dioxide:

\[
\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\text{yeast}} 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2
\]

Yeast is naturally present on the skin of grapes, which is why the making of wine dates back before recorded history. Current professional wine makers kill the natural yeast and replace it with commercial yeast so as to better control the fermentation process. This process needs to be
done in the absence of air (specifically oxygen), lest the ethanol be oxidized to acetic acid, which is the chemical we associate with vinegar:

\[
\text{C}_2\text{H}_5\text{OH} \quad \xrightarrow{\text{air oxidation}} \quad \text{HC}_2\text{O}_2\text{H}_3
\]

Fermentation continues until the ethanol concentration is high enough to inhibit yeast growth. This usually happens when the alcohol content is between 10 - 14 %. If you want to produce a beverage that has a greater alcohol content, you must either distill it or fortify it with alcohol that resulted from the distillation of another product of fermentation. Whiskeys, vodka, and gin are examples of distilled spirits. Port wine and liqueurs are examples of fortified wines.

**Distillation**

Distillation is a purification process wherein a substance is heated to its boiling point, the vapor produced upon boiling is allowed to flow away from the boiling liquid, and the vapor is cooled to condense it back to the liquid. A line drawing and a photograph of simple stills are shown below. What is the advantage of converting a liquid to its vapor, moving it to a different place, and reconverting it to the liquid form? The advantage depends upon the differences between a liquid and its vapor. Suppose you have a liquid that contains contaminants, like sea water. You cannot use sea water for a source of drinking because of its high salt content. Drinking sea water has the opposite of the desired consequence of relieving thirst: you wind up more dehydrated than before because your body has to excrete additional water to balance the high levels of salt in the sea water. Now, suppose you boil (or evaporate) the sea water. This vapor phase will contain almost pure water. The nonvolatile salts will not vaporize at 100°C and will be left behind. Thus if you evaporate a container of sea water, the salt will be left behind, clinging to the walls and bottom of the container. If you can capture that water vapor somehow—say by moving it to place where you can cool and condense it—you will have water that is pure enough to drink.

Simple Stills: The big flask on the left (pot flask) is filled with what you want to distill. You heat it up and the vapor travels up to the condenser. The condenser has an inner tube (where your vapor arrives and condenses to a liquid) and an outer jacket that has cold water running through it. Once the liquid condenses (now called the *distillate*), it flows downhill and is collected in the receiving flask.

As an aside: this photograph demonstrates a potentially dangerous situation, in that it is a closed system (i.e., no vent for air)! You should *never* heat a closed system!!! The line drawing has a vented situation which is much safer.
Distillation is a common way of separation and purification of crude oil into its usable components (gasoline, kerosene, asphalt, etc.). As mentioned above, distillation also enables one to produce a beverage or a fuel from a fermentation process that has a higher alcohol content. The fermentation process leaves behind a variety of substances besides ethanol: e.g. yeast, unfermented sugar, residue from the plants that were fermented, water. Distillation will separate the alcohol from all of those contaminants. It also has the effect of concentrating the alcohol. The maximum concentration of alcohol that can be obtained through distillation from a water-based fermentation is 95%. This is because ethanol and water form a mixture (called an azeotrope) that boils at a constant composition. Thus the vapor that forms upon the boiling of any water/alcohol mixture is 95% ethanol and 5% water.

What is meant by “proof”? The proof of an alcoholic beverage is twice the volume percent of ethanol. This means that if you have 80-proof vodka, the solution is 40% ethanol and 60% other things (mostly water) by volume. The word “proof” comes from an old technique of measuring the purity of distilled spirits. A sample of distilled spirits was poured over gunpowder and the mixture was ignited. If the alcohol had a volume percent of 50 or more, one would observe a characteristic blue flame. Thus if it were 50% alcohol, it was 100% proved: 100-proof. Nowadays we use simple density measurements to determine the alcohol percentage.

In this experiment you will ferment a sugar/water mixture using ordinary bakers yeast. You will then do a simple distillation of the fermentation mixture to obtain a purified ethanol. You will determine its proof and perhaps compare it to the proof of a sample of commercially available alcohol.

Procedure

Week 1: Fermentation

Make sure that the two plastic bottles are clean and dry; you will use one for the fermentation itself (fermentation bottle) and the other to ensure that you are excluding air from the system (air exclusion bottle).

Add 85 mL of water to the fermentation bottle. Weigh out 20 g of sugar and add it to the water in the fermentation bottle. Swirl gently until the sugar is thoroughly dissolved.

Pour approximately 100 - 150 mL of water into the air exclusion bottle. Add the vegetable oil until there is a layer of oil on top of the water that is approximately 0.5 - 1 inch (1 - 3 cm) thick.

Place the bottles next to each other and assemble the bendable drinking straws as shown in the figure below. Make sure that the end inserted into the distillation bottle is only 7-8 cm from the top of the bottle (well above the level of where the yeast/water/sugar mixture will be). The straw should be inserted into the air exclusion bottle such that it is well below the oil level, submerged into the water layer, although not touching the bottom of the bottle. You may need to adjust and trim your straws with scissors to get them to the right lengths. Cutting a small slit in one of the
straws enables you to slide one straw over the other. Seal the two straws together with modeling clay or tape. This seal must be airtight.

Weigh out 1.0 g of yeast and add it to the distillation bottle, which contains your sugar and water. Swirl it for about 30 seconds. It will not dissolve.

Place the straws into the bottles, and use the modeling clay or tape to seal the straw into the fermentation bottle. (See the figure below.) Again this needs to be an airtight seal. The other bottle (the one with the oil and water) will be open to the air. Put your fermentation set-up somewhere where it won’t be disturbed and it will neither be exposed to extreme heat or extreme cold. Fermentation needs to proceed for 5 - 7 days.

Fermentation set up. This diagram will look better on the Blackboard web site. Go to “Course Documents”. Look in the Lab Experiments Folder, click on the Fermentation Diagram and you’ll see a better version (although if you print it, it looks just like this one!).

How the air exclusion system works: When the yeast fermentation process begins, carbon dioxide is generated (see the reaction chemistry above). If this process were done in a sealed system, pressure would begin to build up and would eventually blow off our Silly Putty or modeling clay. The air exclusion system allows the gas to be vented to the outside without allowing air back in. The gas moves through the straw and bubbles up through the water and through the oil, but the oil and water prevent any air from entering the system. The oil also prevents the water from evaporating through the course of the week. You may see bubbles forming and moving through the liquid, but you may not. Do not be concerned if you don’t see bubbles; chances are good that fermentation is proceeding nonetheless. Occasionally record your observations of the fermentation set up through the week.

Week 2: Distillation

At some point during the week while fermentation proceeds, you should assemble your distillation apparatus. Clean and dry the coffee can and its plastic lid. Carefully cut a small
circle, just the size of the tubing diameter in the middle of the plastic lid of the coffee can. Insert the tubing into the lid approximately 2-3 cm and no further. Secure the tubing in the lid with duct tape. The tubing will flop over and lie horizontally along the top of the lid, but this is fine as it will need to be horizontal to reach the collection flask anyway. See the figure below for guidance.

The collection flask will consist of a small glass container (e.g. baby food jar or shot glass) surrounded by an ice water bath, which is housed either in a plastic bag or a larger container (e.g. bowl, larger jar, coffee mug). A sealable plastic bag (e.g. Zip-Lock or Glad Lock) full of ice will be placed on the hose to condense the liquid that boils. See the figure below for an example of a set up.

After approximately a week of fermentation, there should be a clear liquid layer on top of the yeast layer in the yeast mixture bottle. Carefully disassemble the fermentation set-up with minimal disruption to the yeast layer. Remove the straw and the clay from around the neck of the bottle. When the mouth of the bottle is clear of residue, carefully decant the clear layer, which contains most of the alcohol, from the yeast layer into the coffee can you will use as a distillation pot.

Obtain 2, 4, and 6 mL increments of the clear liquid and weigh them on your balance. Record the masses and determine the mass/volume ratio (density). Return the weighed liquid to the coffee can, replace the lid with the duct-taped tubing.

Place the coffee can on a stove burner. Insert the collection end of the tube into the collection container (e.g. baby food jar). Fill the surrounding container about ¾ full of ice, and secure the smaller container so that it is not likely to tip over.

Fill the plastic bag with ice (crushed ice works best) and seal it up. Wrap the ice bag around the collection tube; this is your condenser. IMPORTANT: The ice bag must be on the “downhill” side of the tube (see diagram), otherwise the distilled ethanol will not make it into the collection flask—it'll just flow back into the pot. You can use tape or rubber bands to secure the ice bag to the tube. The very end of the tube must be kept free to allow the flow of condensed droplets into the collection container.

Turn the stove burner on low. The coffee can will heat up quickly and the low setting should be sufficient to distill off the alcohol. (You want to avoid the foaming that occasionally accompanies distillations at high temperatures.) The alcohol will distill over a 25 - 40 minute period. Record your observations as the distillation proceeds. Once you have collected 2 - 6 mL of liquid in the collection container, turn off the stove to stop the process. You want to avoid distilling the water in the original pot mixture, as this will contaminate your alcohol sample.

Use your medicine dropper to measure out a specific volume and weigh it on your balance. Record the mass and volume and determine the density. Compare it to the density of the liquid you obtained after fermentation, before distillation.
Optional activity for students 21 years of age or older or students who have parental supervision: Purchase or obtain a small sample of commercial distilled spirits (e.g. whiskey, vodka, grain alcohol, gin, etc.) Use your medicine dropper to measure out a specific volume and weigh it on your balance. Record the mass and volume and determine the density. Compare it to the density of the alcohol you distilled.

Distillation set up.

*Bag of ice should be “downhill” from the top of the hose. This is so that once the ethanol is cooled and condensed, it cannot escape back into the pot (coffee can) but has to flow down into the collecting jar.
1. Briefly describe your fermentation set up. What tools/substances did you use? What problems did you have? Include information about when you deviated from the prescribed procedure. Did it seem to work as designed?

2. What were your observations as the fermentation progressed? Did you actually see evidence of gas evolving? What did it look after the week or so, when you were finished?

3. Briefly describe your distillation set up. What tools/substances did you use? What problems did you have? Include information about when you deviated from the prescribed procedure. Did it seem to work as designed?
4. Data obtained:

Mass of 2 mL increment of fermentation liquid___________
Mass of 4 mL increment of fermentation liquid___________
Mass of 6 mL increment of fermentation liquid___________
Density of 2 mL increment of fermentation liquid___________
Density of 4 mL increment of fermentation liquid___________
Density of 6 mL increment of fermentation liquid___________
Average density of fermentation liquid___________

Volume of distillate collected_______________

Density of distillate___________________

5. Compare the density of the liquid before and after distillation. What did the distillation accomplish?

6. If you accomplished the optional activity, briefly report on your results.