

Fractional Distillation of Biosynthesized Ethanol (#2)

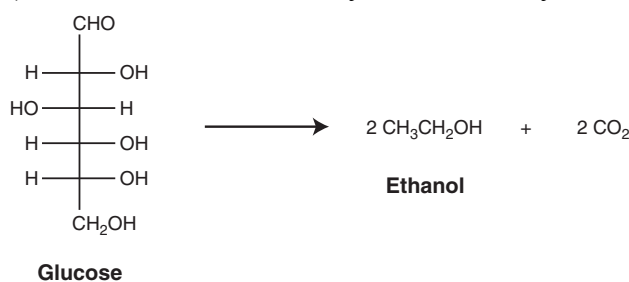
Reference

Williamson, K.L.; Minard, R.; Masters, K.M. *Macroscale and Microscale Organic Experiments*, 5th Ed. Houghton Mifflin Co.: Boston, 2007, p 774.

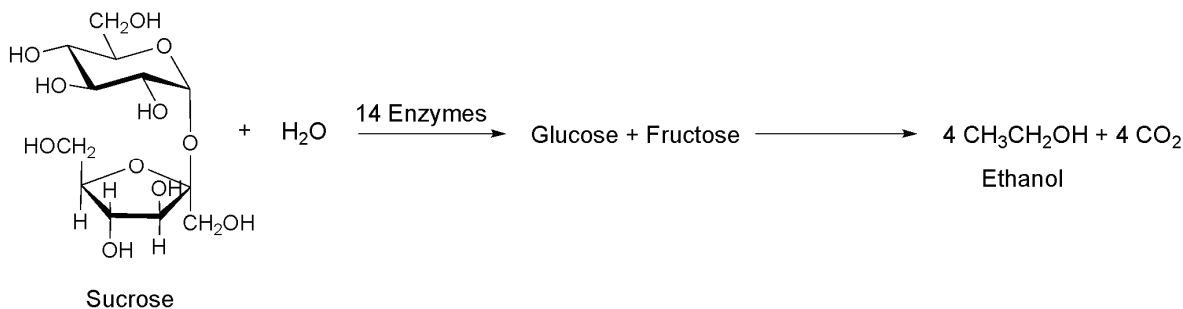
This experiment is a modification of the biosynthesis of ethanol from sucrose. The fermentation reaction takes one week, so you will mix the reagents one week ahead of time. The actual experiment will be performed the following week.

Background

Human beings have been preparing fermented beverages for more than 5000 years. The history of fermentation, whereby sugar (sucrose) is converted to ethanol by the action of yeast, is also a history of chemistry. The word "gas" was coined by van Helmont in 1610 to describe the bubbles produced in fermentation. Leeuwenhoek observed yeast cells with his microscope in 1680. Joseph Black in 1750 showed carbon dioxide is the product of fermentation. Lavoisier in 1789 showed that the fermentation of sugar gives ethanol and carbon dioxide. Gay-Lussac in 1815 showed that fermentation of one mole of glucose gives 2 moles of ethanol and 2 moles of carbon dioxide.



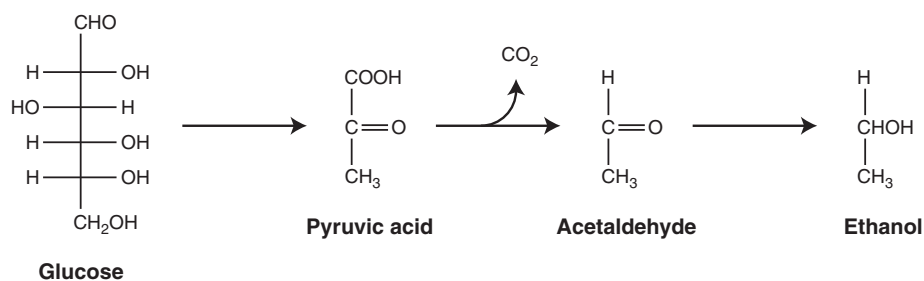
Pasteur in 1857 described fermentation as the action of a living organism. Edward



Buchner made a cell free extract of yeast that caused the conversion of sugar (sucrose) to alcohol observing that the extract contained catalysts. In 1905 Harden discovered that inorganic phosphate increased the rate of fermentation and was consumed.

A baker makes use of fermentation by taking advantage of the gas released to leaven the bread. Baker's yeast is used to convert sucrose, table sugar, into ethanol and carbon dioxide with the aid of 14 enzymes as catalysts present in yeast. Sucrose (table sugar) is a disaccharide that is split into two simple sugars glucose and fructose by enzymes. Thirty one kilocalories of heat are released per mole of glucose consumed in the sequence of anaerobic (no oxygen present) reactions. Fructose is converted to ethanol in the same way as glucose.

Glucose is converted via a number of reactions involving enzymes to form pyruvic acid. A decarboxylase converts pyruvic acid to acetaldehyde in fermentation. Yeast alcohol dehydrogenase, a highly studied enzyme, catalyzes the reduction of acetaldehyde to ethanol. The fermentation reaction must be protected from exposure to oxygen because under aerobic conditions acetobacteria can convert ethanol to acetic acid (vinegar). So the fermentation reaction must occur in the absence of oxygen (anaerobic) to prevent the conversion of sugar into vinegar.



Methodology

In this experiment the flask will be sealed with a balloon to provide an anaerobic environment which will also allow for the expansion of volume required due to the carbon dioxide formed during the fermentation process. Yeast will be used to ferment sugar to an ethanol/water mixture. A fractional distillation will be performed on the mixture and the amount of ethanol will be determined by simple gas chromatography. Below is an explanation of fractional distillation and gas chromatography.

Fractional Distillation

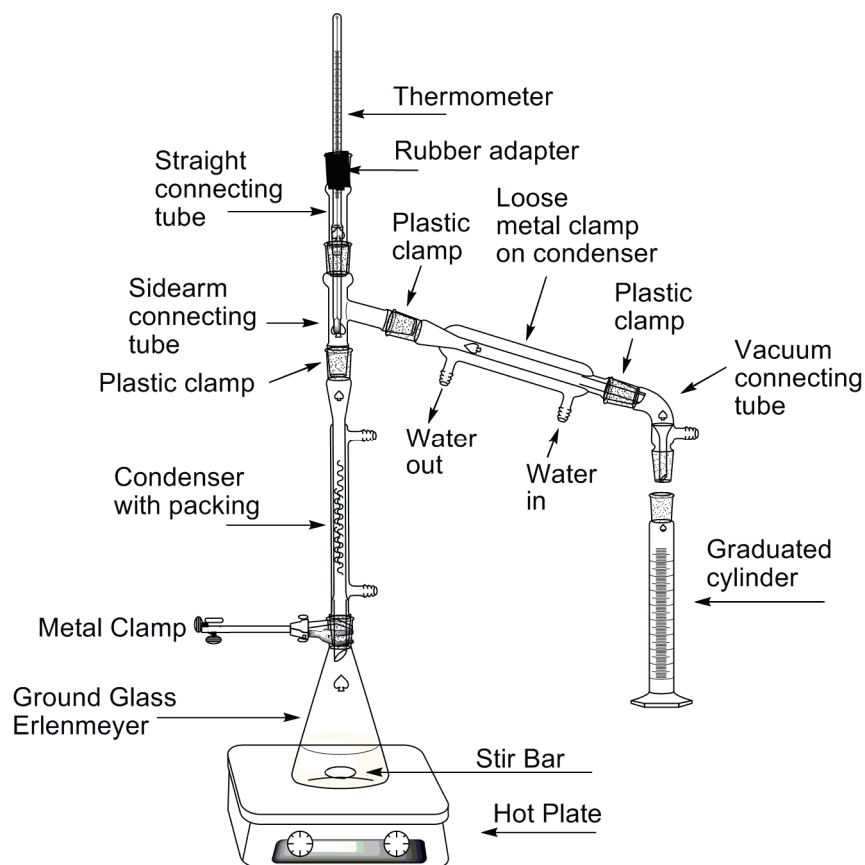
Organic compounds have specific boiling points regardless of whether they are pure or mixed with other compounds. The separation of one compound from another can be accomplished by a process called **distillation**. There are several types of distillation. **Simple distillation** is used to separate a liquid boiling below 150°C from a nonvolatile impurity or from another liquid that has a boiling point 25°C higher than the first liquid. **Fractional distillation** is used to separate liquid mixtures that are miscible in each other and have boiling points within 25°C of each other. **Steam distillation** is used in the isolation of oils, tars, and liquids that are insoluble or slightly soluble in water. In all distillations the mixture of organic compounds to be separated, called the **distilland**, is heated in a round-bottom flask and the compound with the lower boiling point gradually leaves the mixture as a vapor. Much of the vapor condenses on the walls of the distillation apparatus and drips back down the round bottom flask. This ring of vapors condensing on the walls of the apparatus is known as a **reflux ring**. In the process heat is released from the vapor to the walls of the apparatus and to the thermometer bulb, registering the temperature. Eventually vapor reaches the water-cooled condenser and is condensed back into a liquid state (**distillate**) and collects in a receiving vessel. For efficient separation of the organic compounds it is best to have a slow rate of collection from the condenser. When most of the lower boiling compound has distilled from the round bottom flask, the amount of vapor in the flask diminishes. As a result very little vapor condenses on the thermometer bulb, causing a drop in temperature. If heat is applied beyond the distillation point of the first compound, the higher boiling organic compound, or the **second fraction** can be distilled.

In an **azeotrope** the mixture of two compounds, A and B, behaves differently than A and

B as pure compounds and a mixture of A and B codistills. The mixture of A and B is called an azeotrope. In a minimum boiling azeotrope, a mixture of A and B codistill at a boiling point that is lower than the boiling point of either A or B alone. In the case of ethanol (bp 78°C) and water (bp 100°C), a mixture of 95% ethanol and 5% water distills at a boiling point that is less than that of ethanol. As a result it is not possible to distill 100% pure ethanol.

Fractional distillation utilizes a packed column before the still head to give an increased surface area for repeated vaporization-condensation cycles. The temperature of the packed column closer to the round bottom flask is at a higher temperature than the end closer to the still head. This results in the vapor with higher water content to condense, then re-vaporize with a lower water content as the vapors climb the fractionating column. This cycle continues until the vapors reach the still head, having an ideal concentration of 95% ethanol.

The apparatus for fractional distillation is shown below. The only difference between the apparatus for fractional and simple distillations is the packed column.



As mentioned, the vapors have to fill the boiling flask first, then continue through the system. For this reason the size of the boiling flask is critical. The flask should be one-third to one-half full to minimize the amount of vapor lost to the dead volume of the flask. Consider two flasks, one 25 mL, and another 50 mL, both filled with 12 mL of distilland. The 50 mL flask will contain about three times more vapor than the 25 mL; therefore, less distillate will be collected if the larger flask is used. It is also necessary to add a stir bar to the flask to reduce bumping.

Bumping occurs when superheated vapors are quickly emitted from the solution. A stir bar, when it is stirring, breaks up large vapor bubbles by emitting a steady stream of small bubbles and should always be used when distilling or refluxing. It is important to have the stir bar in the flask before heat is applied to the system since a violent eruption may occur if it is added to a hot solution. Assembling the apparatus is tricky, but with practice, as with everything, it gets easier. *Instead of assembling the apparatus then trying to clamp it to a ring stand, build it on the lab bench one piece at a time using plastic clamps as shown in the diagram (you will use three plastic clamps).* Do not insert the straight connecting tube into the apparatus yet. Once the **ground glass Erlenmeyer flask** is full of distilland and a stir bar, clamp it to the ring stand using a metal clamp and start the stirrer. Add the plastic clamped apparatus (packed column, sidearm connecting tube, condenser and vacuum connecting tube) you have made to the ground glass Erlenmeyer flask that contains your distilland. Lastly, insert the straight connecting tube with your thermometer and rubber adapter into the sidearm connecting tube. You may use a very loose metal clamp on the condenser to stabilize your apparatus. If you need help assembling the apparatus, ask your TA.

The rubber thermometer holder should be placed over the straight connecting tube with a drop of glycerin applied at the hole of the holder. Gently but firmly insert the thermometer with a twisting motion. Hold the thermometer close to the rubber holder to avoid snapping it. The thermometer should be centered in the still head no closer to one side than the other, with the entire bulb below a line extending from the bottom of the sidearm (see diagram). Cooling water needs to be flowing through the condenser before heat is added. It flows from the lower end of the condenser to the upper, to condense the vapors into distillate.

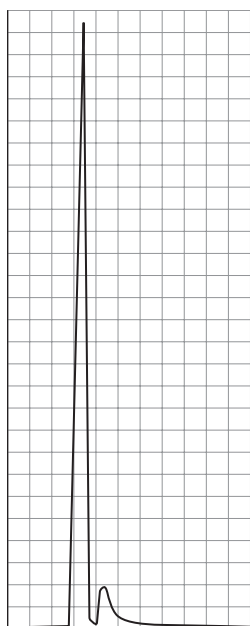
Once the apparatus is assembled and you are sure there are no loose connections turn the hot plate on and start heating the solution. Once boiling starts, reduce the heat and adjust until the distillate drips at a rate of about one drop per second. This is an open system; the vacuum adapter is open to the atmosphere. You should never heat a closed system, as it may explode or fly apart. Distillation is complete when either the upper limit of the expected boiling range is reached or there is little distilland remaining in the flask. **Do not ingest the product of this experiment.**

Gas Chromatography

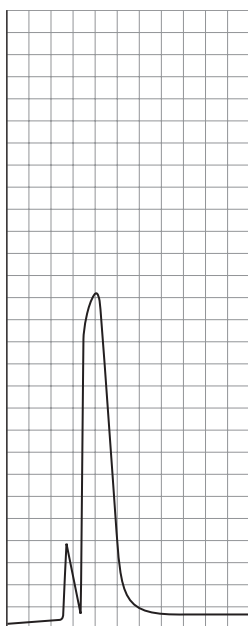
A gas chromatograph (gc) separates a mixture via partitioning between a liquid stationary phase and a gaseous mobile phase called the carrier gas. While the sample molecules are in the gas phase they travel through the column at the speed of the mobile phase whereas they remain immobile while in the stationary phase. The amount of time it takes the sample to pass through the column is called the retention time. If a component spends most of its time in the gas phase it will have a short retention time whereas a long retention time will be the result of a component in the liquid phase for most of its time.

The time a component spends in one phase versus the other is dependent on several factors. The temperature of the oven, the characteristics of the column and the rate of flow of the carrier gas are factors that determine time spent in a certain phase. These factors can be controlled by the operator of the instrument. The sample is introduced into the injector port via syringe, where it is vaporized. The vaporized sample is then swept into the column by an inert carrier gas where it is partitioned between the mobile phase and the liquid stationary phase. The column is a long capillary tube with a thin film of the liquid stationary phase adsorbed or chemically bonded to the inner wall. The column is coiled and mounted inside an oven so the

temperature may be controlled. The liquid stationary phase is a high boiling liquid that has some affinity for the components of the mixture which enables separation. After the compounds desorb from the liquid stationary phase and exit the column they are swept by the carrier gas to the detector which generates a signal that is proportional to the amount of compound present. This signal is sent to a recorder, which produces a chromatogram. The chromatograph of 95% ethanol (95% C₂H₅OH; 5% H₂O) is shown below. Notice the large peak due to ethanol and the smaller peak due to the water. The ethanol elutes first due to its lower boiling point of 78°C and water elutes next due to its higher boiling point of 100°C.



95% Ethanol



Student sample of fermentation

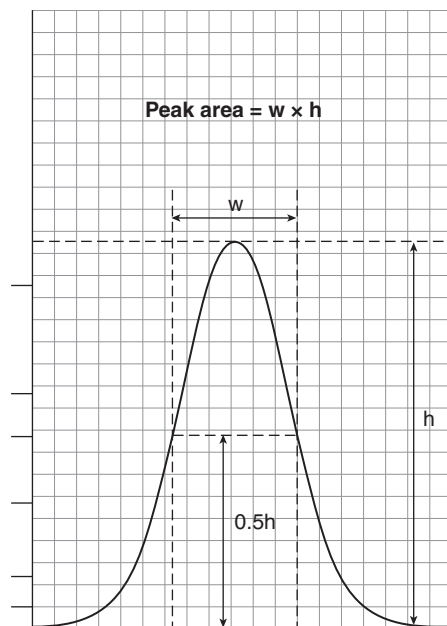


Diagram of peak area analysis

Gas chromatography can be used qualitatively by comparing retention times. It can be used quantitatively by using an integrator, which is limited to more expensive instruments. One manual method involves calculating the areas of the peaks using the height of the peak from the baseline (measured in millimeters) multiplied by the width at half-height. The drawback to this method lies in the symmetry of the peaks. A gc of a student sample of the filtrate from the fermentation has been taken for you and is shown above. Notice that the fermentation mixture is approximately 7.4% $[(6.5 \times 1) / (6.5 \times 1) + (27 \times 3) \times 100]$ ethanol and 92.6% water. The equations below will help you calculate the total volume of ethanol in your distillate.

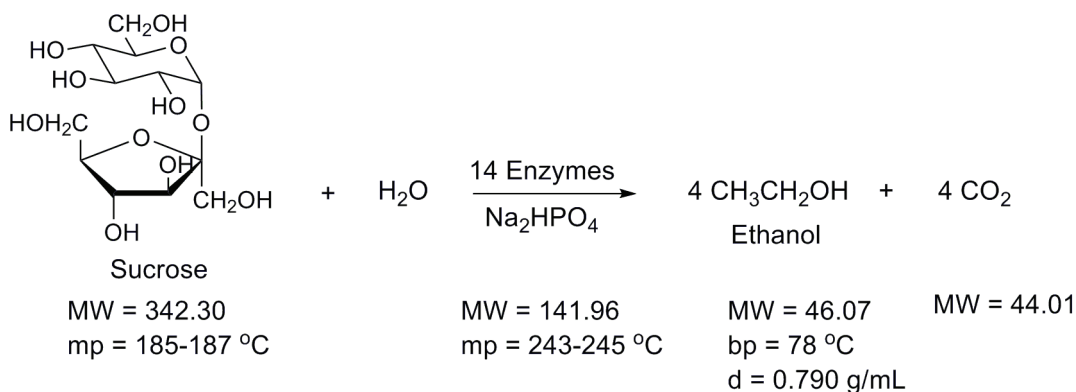
$$\text{Area} = \text{Height} \times \text{Width at half-height} \quad \% \text{ EtOH in fraction} = \frac{\text{Area of EtOH peak}}{\text{Area of EtOH peak} + \text{Area of H}_2\text{O peak}} \times 100$$

$$\text{Volume of EtOH distilled} = V_{\text{fraction 1}} \times ((\% \text{ EtOH in fraction 1}) / 100) + V_{\text{fraction 2}} \times ((\% \text{ EtOH in fraction 2}) / 100)$$

$$\text{Total EtOH in 100 mL of fermentation mixture} = 2 \times \text{Volume of EtOH distilled}$$

Reaction and Properties

The only reagent in this experiment is the sucrose. The yeast contains enzymes, which are catalysts, not reagents that are consumed. The Na_2HPO_4 is also not consumed.

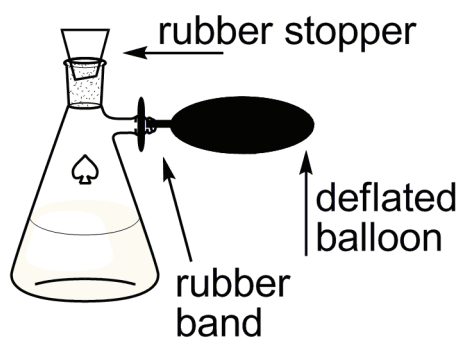


Mechanism

The mechanism involves several enzymes and is very complicated. Therefore, it will not be discussed. **There is no mechanism to put in your prelab report.**

Experimental Procedure

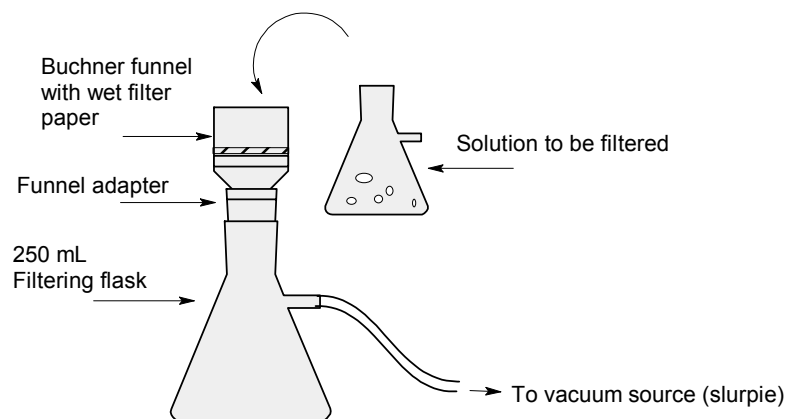
Part 1. You will be working in pairs for this experiment. *Check to make sure that the balance reads “g” not “N” on the readout. Treat the balance with care and do not move it or bump it. It is very sensitive.* Place a large empty weigh boat on the balance, and press the tare button (“O/T”) to tare the balance to zero. The mass should read “0.00 g.” **Remove the weigh boat from the balance** and add a scoop of sugar to it with your spatula. Place the weigh boat back on the balance and wait for the reading. Repeat the process until it reads “16 g.” **Record the exact mass in your notebook** and transfer the sugar to a 125 mL filtering flask. Add 90 mL of warm (body temperature; not hot) water to the flask. Stopper it and



shake to dissolve the sugar. Add 1 g of dry brewing yeast and 0.13 g of disodium hydrogen phosphate. Stretch the balloon by blowing it up and allowing it to empty. Stopper the flask tightly with a rubber stopper that does not contain a hole and cover the filter nozzle with the balloon. Secure the balloon tightly with a rubber band as shown in the figure. Make sure the flask is sealed well or the fermentation will not work. Swirl the mixture for five minutes to suspend the yeast (it will not dissolve) and initiate the fermentation. Store the flask in your drawer until the next lab period.

Part 2. A successful fermentation mixture will have a strong yeast smell. If the mixture smells like vinegar, the fermentation was unsuccessful. Add about 1 g of Celite™ filter aid (diatomaceous earth; to prevent fine particles of organic matter from clogging the filter paper) to the flask in small portions. Assemble a vacuum filtration apparatus as shown on the next page. After placing the filter paper on the Buchner funnel, add a few drops of water to stick the paper

to the funnel. Swirl the stoppered flask gently, turn on the vacuum, and pour the suspension slowly in portions onto the filter paper. Swirl the flask in between pours. Wash out the flask with 1-2 mL of distilled water. You will discard the matter on the filter paper and keep the filtrate. **Later you will obtain a GC of the filtrate.**



Pour about 50 mL of the filtrate into a 125 mL **ground glass Erlenmeyer flask** containing a stir bar. Assemble the fractional distillation apparatus on page 28, making sure to lightly grease the ground glass joints. **Do not turn on the heat until your TA has inspected your setup.** When it has been inspected, turn the water on **just a trickle**, and turn the stirrer to 6, then turn the heat to 350. Collect the first 3 mL of distillate in a 10 mL graduated cylinder and transfer it to a clean, dry 25 mL flask. Label it "Fraction 1." Record the temperature as it drips into the graduated cylinder. Collect the next 2-3 mL of distillate in the graduated cylinder, transfer it to a clean, dry 25 mL flask and label it "Fraction 2." Record the temperature as it distills. Check the composition of your samples on the GC, making copies for each partner. **Include the GC trace with your report. For your results section,** use the GC traces of Fraction 1 and Fraction 2 to calculate the grams of ethanol in your samples. Calculate your percent yield of ethanol and compare it to the theoretical yield.

Prelab

1. Define: a. distilland; b. distillate; c. carrier gas; d. retention time
2. What is the difference between "g" and "N" on the balance readout?
3. What should be circulated through your West condenser to cool vapors to liquid?
4. Is this distillation an open or closed system? What is the difference between the two?
5. In the experiment, what is the approximate temperature range at which the ethanol azeotrope will distill: 25-40 °C; 70-76 °C; 90-100° C? When should you stop collecting distillate?

Postlab

1. During the fermentation reaction it was important to prevent air from entering the reaction flask by tying the balloon on firmly with a rubber band. Why?
2. Under aerobic conditions certain bacteria can oxidize ethanol. Oxidation, which is the gain of oxygen or loss of hydrogen, is shown below with benzyl alcohol as an example. Draw the structures of the two **analogous** compounds that would form if **ethanol** were oxidized by bacteria in the same way.

