The Fermentation of Sugar and the Isolation of Ethanol by Distillation

Under anaerobic conditions, enzymes in yeast can convert glucose and sucrose to ethanol through a process known as the Emden-Meyerhof-Parnas scheme. This same series of reactions, up to the formation of pyruvic acid, occurs in the human body. In the body, the pyruvic acid is oxidized to lactic acid that is partly responsible for the feeling of fatigue during exercise.

In the absence of oxygen, the yeast converts the sugar to ethanol through the following reaction

$$H_2O + \text{sucrose} \rightarrow 4\ CH_3CH_2OH + 4\ CO_2$$

A large scale fermentation

1. In a 250-500 ml Erlenmeyer flask, mix 51.5 g of sucrose in 150 ml of water.
2. In a separate beaker, mix a half a cake of yeast with 50 ml of water and 0.35 g of disodium hydrogen phosphate and then transfer this slurry to the Erlenmeyer flask and mix well.
3. Outfit the flask with a bubbler system. The bubbler keeps oxygen from the air from the reaction while allowing CO_2 to escape.

Distilling the Ethanol

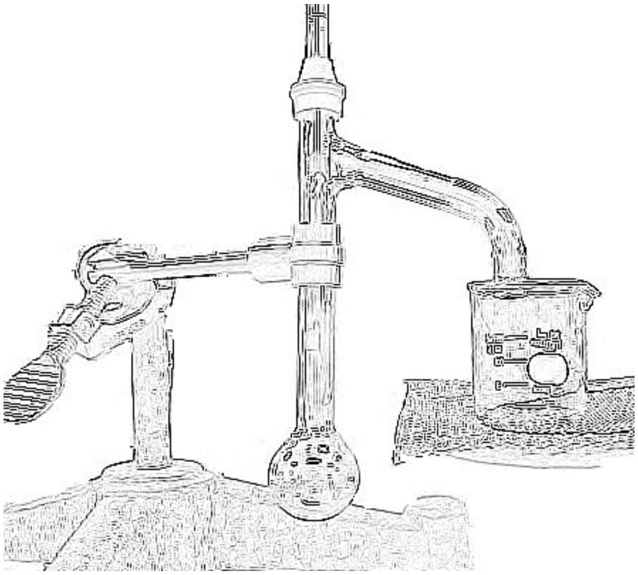
Please read the discussion on the separation of Cyclohexane and Toluene.
We did a large-scale fermentation. We will distill on a small scale by taking an aliquot[1] from the large pot. You will later have to back calculate how much ethanol we could have distilled from the large fermentation.

Water and ethanol form an azeotrope; a 95% ethanol/5% water combination boils at 78.15° C. Pure ethanol boils at 78.3°C and water boils at 100°C. It is therefore impossible for us to distill pure ethanol from our fermentation mixture.

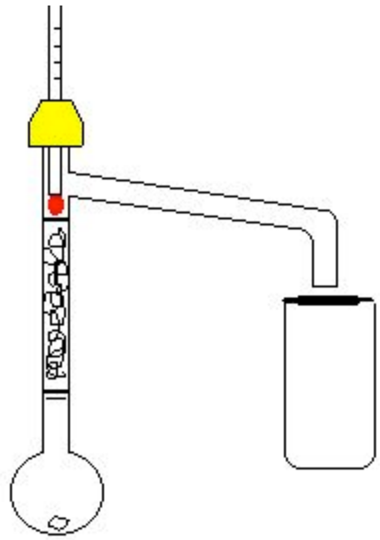
1. Carefully decant a measured amount of the liquid from the fermented mixture without disturbing the sediment into a round bottom flask. A small amount of K_2CO_3 is often added before distillation.

2. Set up a simple distillation apparatus (see picture below). Do not forget to use a boiling chip.

[1] Definition of aliquot: *Chemistry, Pharmacology* . comprising a known fraction of a whole and constituting a sample. (Dictionary.com)



or



Two distillation setups: The first shows a simpler setup. To improve the separation, you can add glass beads or copper ribbon to increase the surface area. This increase in surface area allows more condensations/re-vaporizations and therefore a purer product.

3. Record the temperature when the first drop of distillate comes over. Collect 1-3 drops before switching collection vessels. What one hopes to see is a distinct change from the 95:5 ethanol mixture to pure water. Collect until the ethanol is finished distilling. When this occurs, you should see the temperature go from a steady temperature (78-80°C) rapidly to 100°C. You might even the temperature drop for a minute as the apparatus has to re-warm to the higher 100° temperature. In reality, in this experiment, the cutoffs can be hard to see and so I leave it to your best judgment.
4. Determine a yield and find the refractive index for your sample.

Calculations

1. Calculate the theoretical yield of ethanol using sugar(51.5g) as the limiting reagent.
2. Steps to calculate the percentage ethanol
    - Graph refractive index vs. ethanol and find a best fit line. The following web site has RI vs % information. http://www.thewhiskystore.de/experts/alcohol.htm . The data was not linear. I fit the data to a quadratic function.
    - In our RI calculation we saw $y = -0.0575x^2 + 0.0873x + 1.3323$. To use the quadratic equation, we need to make the left side equal to zero. Plug your RI into y and then subtract from both sides to make the left side equal zero.
    - RI of sample = 1.3650
    - $1.3650 = -0.0575x^2 + 0.0873x + 1.3323$.
    - $0 = -0.0575x^2 + 0.0873x -.0327$
    - Plug into the quadratic equation at http://www.math.com/students/calculators/source/quadratic.htm

- You might get two answers. Choose the one you think is most reasonable and describe why you chose it.

3. Calculate the grams of ethanol isolated in your distillation (based on your % purity of ethanol. If your sample is 31.2 % ethanol, then 1.00g of our sample = 0.312g of EtOH)
4. Back calculate the amount of ethanol based on the aliquot ratio. (The aliquat ratio is 200 mL total/4 mL we used.)
5. Calculate the % yield. (% yield is actual /theoretical x 100%.)

Post Lab Questions:

1. Calculate how many ml of CO_2 are theoretically possible from the conversion of our 51.5 g of sucrose. (Assume 25.0°C and 1.00 atm)
2. Why is the air trap necessary in the yeast fermentation process?
3. Explain why a packed fractionating column is more efficient than an unpacked one.
4. Why is it unwise to attempt to distill with a completely closed apparatus (not open to the atmosphere)? Use complete sentences.
5. Boiling occurs when the vapor pressure of the liquid equals the pressure above the liquid. Will the boiling point (distillation temperature) increase, decrease or stay the same under reduced atmospheric pressure?