CHEM 105: BIOCHEMISTRY AND SOCIETY

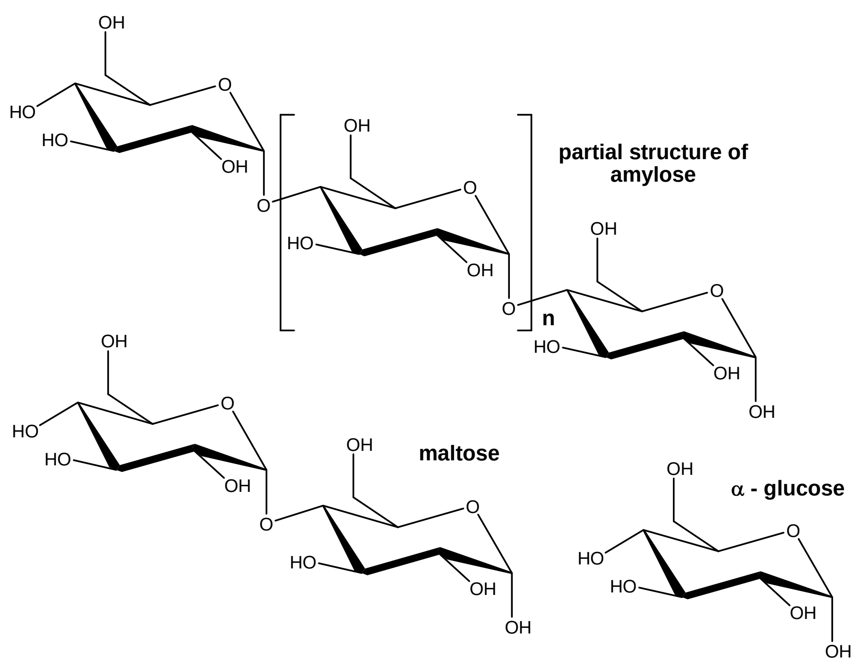
Dr. Jakubowski

Lab 5: Let's make Alcohol

Introduction

Ethanol, or ethyl alcohol, can be prepared by the fermentation by yeast of the sugars in fruit or grain. This process has been known for thousands of years, and is still the basis today for the preparation of alcoholic beverages. Ethanol is also used extensively for the production of gasohol, which contains about 10% ethanol prepared from corn. In this experiment, you will isolate ethanol from the corn mash you prepared last cycle. In addition, you will test your purified ethanol is see how pure it is.

The sugar for the fermentation came from starch that is present in corn (as well as in plants such as potatoes, wheat, and rice. Starch, present in the seeds of those plants and used by them for reserve energy, is actually composed of two substances with very similar structures, amylose (shown below) and amylopectin. Amylose is a covalent polymer of the monomer, α - D glucose, a simple sugar molecule. Detailed studies indicate that each amylose molecule contains about 1000 α - D glucose monomers.

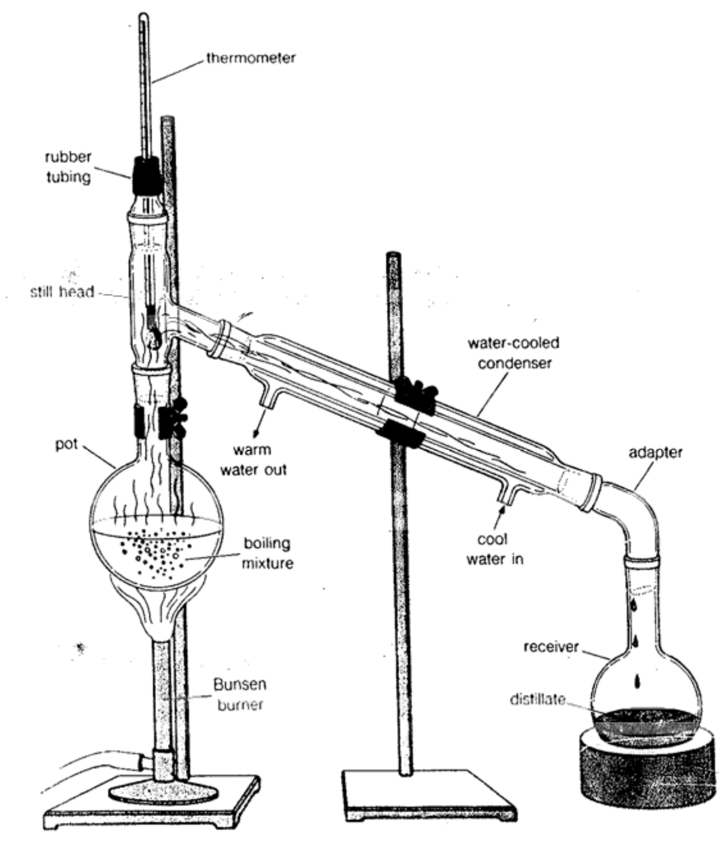


Upon treatment with acid or in the presence of certain digestive enzymes, amylose is broken down by water in a hydrolysis reaction into D-glucose and maltose, another sugar that is actually two α - D - glucose molecules linked together. Without these enzymes, these reactions would occur at very slow rates.

In the first part of this experiment, you will boil corn kernels to extract the starch. Then you will add the enzymes **amylase** and **amyloglucosidase** to break the starch (amylose) into maltose and D-glucose, respectively. Under acidic conditions, maltose decomposes to D-glucose. A buffer solution, which helps to maintain the pH at optimal levels for the enzyme, will also be added. The added yeast will act on the D-glucose to produce ethanol and carbon dioxide, as shown in the balanced reaction below.

$$C_6H_{12}O_6 (s) \rightarrow 2\ CH_3CH_2OH (l) + 2\ CO_2 (g)$$

Fermentation as described above can only produce solutions that contain about 10 to 15% alcohol, because at this concentration, yeast growth is inhibited. To achieve higher alcohol content, the ethanol must be distilled from the water. Distillation is a process by which two liquids with different boiling points may be separated from each other. In distillation, the mixture to be separated is placed in a distilling flask. When heat is applied, the component with the lower boiling point (in this case, ethanol) tends to vaporize first. The vapor then travels through the distillation apparatus until it contacts a cool surface, at which point it condenses (the copper coils in Hawkeye's still in M*A*S*H) Thus, the liquid that drips out the end of the distillation apparatus should be enriched in the lower boiling component. The boiling point of ethanol (78°C) is close enough to that of water that the separation is not complete. Even with a very efficient distillation apparatus, the **distillate** will be a 95% ethanol/5% water mixture. Since ethanol must be 99.6% dry before it can be used as a fuel blend in gasoline, further drying must be done. This is usually accomplished by passing the distillate through molecular sieves, which absorb the remaining water. He will distill the ethanol you produce in the second part of this experiment. A picture of a distillation device is shown below.



A simple distillation apparatus.

PROCEDURE

A. PREPARATION OF CORN SLURRY (1st day of lab)

1. Tare (set the weight to zero) a 250 mL beaker and weigh out 100 g of frozen corn. Record the exact weight in your notebook. Keep track of which balance you use.

2. Transfer the corn to a 600 mL beaker. Add 100 mL of deionized water. Place the beaker on a ringstand, cover it with a watchglass, and boil gently for 15 minutes. (If the mixture becomes too dry, more water may be added.)

3. Alter boiling is completed, remove the beaker trom me ringstand and allow it to cool somewhat. Add 100 mL of deionized water and 10 mL of the amylase solution, using a small graduated cylinder. Be sure to shake the amylase solution before using. Stir the resulting mixture occasionally with a glass rod during the next ten minutes.

4. At the end of the 10 minute period, add 35 mL of the buffer solution (to maintain a slightly acidic pH), 10 mL of the amyloglucosidase solution, and 10 mL of the yeast solution. Be sure to shake the buffer and amyloglucosidase solutions before mixing and stir the entire mixture after mixing.

5. Place a piece of Saran wrap over the mouth of the beaker and secure it with a rubber band.

6. Weigh the beaker using the triple beam balance — it is too heavy for the electronic balances—and record this weight in your notebook. You will be reweighing this beaker again next period. Weigh carefully and be consistent —with Saran wrap and rubber band each time.

7. Place your beaker in your drawer and allow it to sit until the next lab period so that the enzymes have time to work

B. Distillation of Ethanol from Corn Slurry (Mash) (2nd day of lab, next cycle)

1. Take the mass of the beaker (with the Saran wrap and rubber band) containing the corn and solution which you prepared during the last lab using a triple-beam balance. Record this mass in the data section.

2. Set up the distillation apparatus as demonstrated by the laboratory instructor. In order to do this properly:

    - Make sure to grease the ground glass joints before connecting them with the blue plastic “quick connects”. This helps to prevent any vapor from escaping and the joints from freezing together.

    - Put about 25—30 mL of your solution in the distilling flask so that it is a little more than half full. Avoid getting chunks of corn in the flask.

    - Place a boiling chip in the flask to promote even boiling.

- You will **use a heating mantle** to provide the heat necessary to do the distillation. **DO NOT USE A BUNSEN BURNER**! The inside of the mantle will eventually become extremely hot. In order to control the heat, you can raise or lower the mantle. The outside is relatively cool.

- The best separation of alcohol will occur if the distillation is done SLOWLY (Before you use your still, have your lab instructor inspect it.)

3. Collect the first 20 drops of **distillate** in a clean glass vial. Record the temperature range over which they are collected. The range is the temperature when it begins to boil until 20 drops are collected. Cap the vial and set it aside to take a refractive index measurement later.

4. After collecting the first 20 drops, place a second vial at the end of the distilling apparatus and collect an additional 20 drops. Record the temperature range.

5. When the final temperature has been reached, lower the heating mantle away from the distillation flask and allow it to cool.

C. Characterization of the Distillate.

How do you know that your distillate is ethanol and not just water? Figure out a way to do so.

Let's Make Ethanol

Chemical Information Sheet for Experiment 5

Disposal Procedures

chromium/zinc waste container — collect solution from step 4.

waste corn container — collect all left-over corn. DO NOT pour the corn slurry down the drain or put it in the waste basket.

ethanol/corn slurry	flammable
ethanol distillate	flammable
acetone	flammable, irritant
$Cr0_3$-H_2S0_4	toxic, strong acid

LABORATORY REPORT

Experiment 5: Let's Make Ethanol

Names: ______________________ ___________________________

Date: ______________________

DATA

Starting mass of corn plus solution (from end of Day 1) ___________

Final mass (start Day 2) _______________

Mass loss ______________

DISTILLATION DATA

temperature range over which first 20 drops were collected ___________ ^oC

refractive index of first 20 drops ___________

temperature range over which last 20 drops were collected ___________ ^oC

refractive index of last 20 drops ___________

PROPERTIES OF DISTILLED ETHANOL

1. Explain how you determined that the distillate was not water.

2. Compare the alcohol concentrations in the two distillates. Which one is higher? Can you explain why?

3. The "proof' value of alcohol-water mixtures is determined by doubling the % concentration, (for example, a glass of wine that is 10% alcohol is 20 proof.) What are the "proofs" of your two batches of distillate?

4. Explain any mass change observed from the masses of the beaker before and after the fermentation process.