Ethanol: corn mash and distillation
How is ethanol produced? What are the steps in ethanol production?

Materials
- Hot plate
- 110V heating mantle
- 100 or 1000 mL distillation apparatus
- Condenser tube
- Dial thermometer
- Graduated cylinders (10, 100 mL)
- Large watch glass covers
- Beakers (100, 250, 600 mL)
- Deionized water
- Cracked corn (ground up)
- Scale or triple beam balance
- Glass vials with caps (or a small beaker)
- Buffer solution (pH 5)
- Yeast solution (7g yeast/300 mL water)
- Amylase solution (1 tsp./500 mL water)
- Gluco-Amylase solution (1 tsp./500 mL water)
- Funnel
- Thermal gloves
- Glass stir rod
- Cheesecloth
- Optional: Pipette pump
- Optional: 10 mL pipettes

Background
Commercial production of fuel ethanol in the United States involves breaking down the starch present in corn into simple sugars (glucose), feeding these sugars to yeast (fermentation), and then recovering the main product (ethanol) and byproducts (animal feed, corn oil, and carbon dioxide). Ethanol is an alcohol produced by yeast from sugars. Fuel ethanol is ethanol that has been highly concentrated and blended with other compounds (gasoline) to render the alcohol undrinkable. For each pound of simple sugars, yeast can produce approximately .5 pounds (0.15 gallons) of ethanol and an equivalent amount of carbon dioxide.

The value of corn as a feedstock for ethanol production is due to its large volume of carbohydrates, specifically starch. Starch can be easily processed to break down into simple sugars, which can then be fed to yeast to produce ethanol. Modern ethanol production can produce approximately 2.8 - 3 gallons of fuel ethanol per bushel of corn. Dry-milled ethanol production uses only the starch portion of the corn, which is about 70% of the kernel.

All the remaining nutrients: protein, fat, minerals, and vitamins, are concentrated into distiller's dried grain (DDGs), a valuable feed for livestock. Some ethanol plants also remove the corn oil from DDGs to create renewable diesel. About 40% of the United States’ corn crop is used to produce ethanol.
**Directions**

**Day 1: Preparation of corn mash**

1. Weigh out 100 g of ground cracked corn and add to a 600 mL beaker.
2. Add 300 mL of warm distilled water. Record observations in Data section. Stir well. Place the beaker on a hotplate and set the temperature to 100° C (or more to get it to boil), cover it with a watch glass, boil gently and stir often for 15 minutes, *be careful not to let it burn*. (If the mixture becomes too dry, more water may be added).
3. After boiling is completed, remove the beaker from the hotplate and allow it to cool to touch. Record observations on consistency, color and smell in Data section. Measure 100 mL of distilled water and pour into a 250 mL beaker. Measure 10 mL of the amylase solution into a small graduated cylinder and add to the 250 mL beaker of water. *Be sure to shake the amylase solution before using.* Stir the resulting mixture and add it to the corn mash. Stir the mixture occasionally with a glass rod during the next 10 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 gluco-amylase solution, and 10 mL of the yeast solution together in a 100 mL beaker. Be sure to shake the buffer and gluco-amylase solutions before mixing and pour the mixture into the corn mash. Stir the entire mixture well.
5. Place a piece of plastic wrap over the mouth of beaker and secure it with a rubber band or use parafilm (fermentation will occur so do not secure it too tightly).
6. Place your beaker on the counter and allow it to sit overnight (next lab period) so that the enzymes have time to work.

**Day 2: Distillation of ethanol from corn mash**

1. Set up the distillation apparatus as demonstrated by the lab instructor and the figures below: (Before you use your distillation apparatus, have your lab instructor inspect it).
   a. Make sure to either grease or wet the ground glass joints before connecting them. This helps to prevent any vapor from escaping the joints and to keep the joints from freezing together.
   b. Put about 50–75 mL of your solution in the distilling flask so that it is a little more than half full. If you are using a large distillation flask (1000 mL), put the entire classes’ solution into the flask. *You will have to filter out the distiller's grains by using cheesecloth or coffee filters.* *Avoid getting chunks of corn in the flask.* If necessary, add enough distilled water to the flask to reach the half-way point of the flask to ensure even heating of distillate.
   c. 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 temperature of the heating mantle. Make sure the distillation flask and condenser tube are properly supported! The outside of the mantle is relatively cool to touch.
2. The best separation of alcohol will occur if the distillation is done slowly. Ethanol’s boiling point is 173.1°F/78.37°C and water’s is 212°F/100°C, please keep the temperature between these two boiling points. Collect the ethanol distillate samples into a small beaker to be used for the alcohol flame test (we recommend that you collect them in 4 dram vials). Pour the distillate samples into a capped vial for storage until ready to do the flame test.
3. When you are no longer able to distill your product, turn the heating mantle off and allow the distillation apparatus to cool before disconnecting.

**Alcohol flame test**

4. *This test should only be done in a fume hood after the ethanol distillation is complete and there is no vapor in the air.* You will be testing the ethanol distillate for alcohol concentration by lighting it on fire. The longer the flame burns, the greater the alcohol concentration. If the distillate does not burn, the water concentration is too high.

5. Place a watch glass in the fume hood and pour 2 mL of your ethanol distillate on it.

6. Turn the fume hood on and lower the window, light the distillate.

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**Physical changes of corn**

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<thead>
<tr>
<th>Day 1</th>
<th>Consistency</th>
<th>Color</th>
<th>Smell</th>
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<tbody>
<tr>
<td>Before boiling (step 2)</td>
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<td>After boiling (step 3)</td>
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<td>After enzyme addition (step 4)</td>
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<table>
<thead>
<tr>
<th>Day 2</th>
<th>Consistency</th>
<th>Color</th>
<th>Smell</th>
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<tbody>
<tr>
<td>Before distillation (step 1)</td>
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<tr>
<td>First distillate sample (step 3)</td>
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<td>Second distillate sample (step 4)</td>
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**Flame test**

<table>
<thead>
<tr>
<th>Time</th>
<th>Flame properties</th>
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<tbody>
<tr>
<td>Sample 1</td>
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<td>Sample 2</td>
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**Reflection**

1. What effect does the physical heating have on the corn mash?
2. Explain how each enzyme (amylase and gluco-amylase) change the corn mash mixture in preparation for fermentation.
3. What is the function of the yeast during the fermentation process?
4. Describe the physical changes that your corn went through during its transformation into ethanol.
5. Compare the alcohol concentrations in the two distillate samples. Which one is higher? Can you explain why? (If you were not able to light your ethanol distillate, explain why here).
6. What byproducts result from ethanol production?