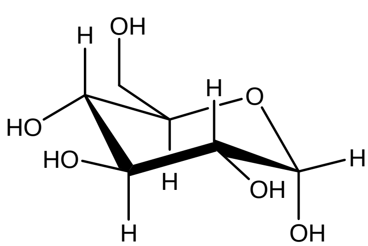
**Chem 251**

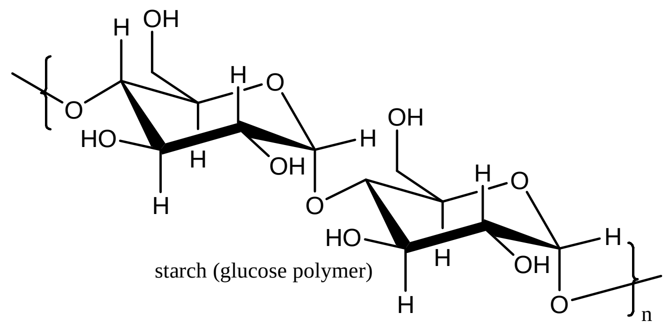
**Ethanol from Corn**

Most of the ethanol that is used as a biofuel in this country is produced from corn. In this experiment you will make ethanol from frozen corn kernels using a process similar to the method used in industry.  The first step is to break down the corn starch into glucose molecules.  This accomplished with two enzymes, amylase and amyloglucosidase.  Starch does not dissolve in water at lower temperatures, and it cannot be hydrolyzed by these enzymes unless it is dissolved.  To dissolve the starch, it must be heated in water to 100°C.  This causes the internal hydrogen bonds in starch to be broken and then water is absorbed by the starch.  When the mixture is cooled, starch remains in solution.

Starch is a polymer of D-glucose composed of two different components, amylose and amylopectin  (see sections 21,12 and 21,17 of Bruice). Amylose is a linear polymer of D-glucose connected by alpha (1—>4) linkages.  Amylopectin is a branched polymer of D-glucose with alpha (1—>4) linkages, as in amylose, and alpha (1—>6) linkages at the branches.




Glucose




starch (glucose polymer)

Amylase randomly hydrolyzes the alpha (1—>4) bonds to produce smaller fragments of starch.  Amyloglucosidase can attack both 1—>4 and 1—>6 linkages and it breaks off single glucose units on the end of the polymer.  Over time the combination of the two enzymes will break down starch completely into glucose.

Yeast, which is also added to the mixture, provides the enzymes that catalyze the fermentation of glucose into ethanol and carbon dioxide.  When the fermentation is complete, the mixture is filtered to remove the solid residue.  Using fractional distillation, ethanol is isolated from the mixture.  It is necessary to add anti-foam agent to prevent excessive frothing during the distillation.  Ethanol and water form an azeotropic mixture consisting of 95% ethanol and 5% water by weight, which is the most concentrated ethanol that can be obtained from fractional distillation of dilute ethanol-water mixtures.

**Required Reading**

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|---|---|
| Technique 14 | Simple distillation sec 14.1. 14.2, 14.4 |
| Technique 15 | Fractional Distillation,Azeotropes 15.1, 15.4, 15.6 |
| Essays | Ethanol and Fermentation Chemistry pg 142 |

**Special Instructions**

Start the fermentation at least 1 week before the period in which the ethanol will be isolated.

**Suggested Waste Disposal**

Discard all aqueous solutions in the waste container marked for the disposal of aqueous waste. Filter Aid may be discarded in the trash containers.

**PROCEDURE**

Grind 100 g of corn (frozen corn that has been thawed) for several minutes in a mortar and pestle. Transfer the corn to a 500-mL Erlenmeyer flask and add 100 mL of water. The water can be also be used to rinse the mortar so that all of the corn is transferred. Boil the mixture gently for 15 minutes, adding more water if the mixture becomes too dry. After letting the mixture cool until the temperature is about 55°C, add 50 mL of water, 10 mL of amylase solution,[1] and 10 mL of calcium acetate solution.[2] Mix thoroughly and let stand for 10 minutes. Add 35 mL of buffer solution, [3] 10 mL of amyloglucosidase solution, [4] and 0.5 g of dried baker's yeast. Mix thoroughly and weigh the flask. Cover the flask opening with saran wrap or other plastic wrap, using a rubber band to hold the plastic wrap firmly in place. Allow the mixture to stand until enxt week.

[1]Amylase solution: Mix 3 mL of stock solution (Bacterial amylase from Carolina Biological) with 97 mL water.

[2]Dissolve 0.5 g of calcium acetate in 100 mL water.

[3]Buffer solution: 3.75 g glacial acetic acid and 3.125 g sodium acetate in 250 mL water.

[4]Amyloglucosidase solution: Mix 3 mL of stock solution (amyloglucosidase from Carolina Biological) with 97 mL water.

**Procedure for the following week:**

When fermentation is complete, weigh the flask and compare this to the weight before fermentation. The difference in weight corresponds to the amount of carbon dioxide produced during the fermentation. Pour this mixture through an 8-inch square of 4-5 layers of cheese cloth into a 250-mL beaker. Most of the corn residue should be caught by the cheese cloth. After most of the liquid has drained out of the cheese cloth, carefully squeeze the cheese cloth with your hands so that the remaining liquid is recovered. To filter the mixture more completely, prepare a Buchner funnel with filter aid (celite). Place 4 grams of celite in a beaker with about 100 mL of water. Stir the mixture vigorously and then pour the contents into a Buchner funnel (with filter paper) while applying a vacuum, as in a vacuum filtration (Technique 8, Section 8.5, p. 622). This procedure will cause a thin layer of filter aid to be deposited on the filter paper. Discard the water that passes through this filter. Being careful not to disturb the bed of celite, pour the fermentation mixture into the Buchner funnel, using gentle suction. It may be helpful to hold a glass stirring rod over the opening of the flask while pouring the mixture into the Buchner funnel. The liquid will follow the length of the stirring rod and hit the bed of celite more gently. The liquid that goes through the filter should be clear.

**Fractional Distillation**

Add 2 mL of antifoam emulsion[6] to the filtered liquid to prevent frothing during the distillation (Make a 1/10 dilution of Antifoam B silicon emulsion in water. Assemble the fractional distillation apparatus shown in Technique 15, Figure 15.11, page 724 or the simple distillation on pg 714 (your instructor will let you know which to use). You will use a three-neck flask so that the temperature of the liquid in the distilling flask can be monitored with a thermometer that is held in place with a thermometer adapter. Place the bulb of the thermometer below the surface of the liquid in the flask. Plug the third neck with a glass stopper. Insulate the distilling head by covering it with a layer of cotton held in place with aluminum foil. Use a pre-weighed 25-mL round-bottom flask as the receiving flask and a heating mantle for the heat source.

It is important to distill the liquid **slowly** through the fractionating column to get the best possible separation. This can be done by carefully following these instructions: Distillation will begin when the temperature of the liquid in the distilling flask is about 85-90°C. When the liquid begins boiling, it is best to turn the heat down immediately and then gradually raise it so that the heat setting required to maintain boiling is at the lowest possible setting. As ethanol moves up the distillation column, it will not wet the stainless steel sponge and you will not be able to see the ethanol. After all of the ethanol has begun moving up the column, water will begin to enter the column. Since water will wet the stainless steel sponge, you will be able to see the water gradually moving up the column. (Note: for those doing a simple distillation will not observe this). To get a good separation, you should control the temperature in the distilling flask so that it takes about 10-15 minutes for the water to move up the column. Once ethanol reaches the top of the column, the temperature in the distillation head will increase to about 78°C and then rise gradually until the ethanol fraction is distilled. Collect the fraction boiling between 78°C and 84° and discard the residue in the distillation flask. You should collect about 1-5 mL of distillate. The distillation should then be interrupted by removing the apparatus from the heat source.

**Analysis of Distillate**

Determine the total weight of the distillate. Determine the approximate density of the distillate using the method given in Experiment 17 on pg 149. Using the table on page 149, determine the percentage composition by weight of ethanol in your distillate from the density of your sample. The extent of purification of the ethanol is limited because ethanol and water form a constant-boiling mixture, an azeotrope, with a composition of 95% ethanol and 5% water. Submit the ethanol to the instructor in a labeled vial.

**Calorimetry (optional)**

Determine the heat of combustion (in kjoules/gram) of your ethanol. Your instructor will provide instructions on how to use the bomb calorimeter and how to perform the calculations.

## REFERENCES

Maslowsky, E. "Ethanol form Corn: One Route to Gasohol." *Journal of Chemical Education, 60* (1983): 752.

## QUESTION

1. Using the weight of carbon dioxide that was produced during the fermentation, calculate the weight of ethanol that should have been produced. (See Essay Biofuels, p. 144, for the balanced equation.) Based on this weight, calculate the percent recovery of ethanol that you obtained from the distillation. To do this calculation, you will also need the weight of the distillate and the percentage composition of ethanol by weight that you determined from the density determination.