Fig. 13-1

Eduard Buchner (1860 - 1917) 1907 Nobel prize winner for helping to elucidate mechanism of fermentation. http://en.wikipedia.org/wiki/Eduard_Buchner

Experiment 13

Fermentation of Sucrose

Text Topics

Enzymatic fermentation.

Comment

As the fermentation process in this experiment takes several days, you should set up the reaction in a hood and let it run for about a week. A more extensive investigation of fermentation of fruit juice, corn, potato starch and grass is available: Epstein, J. L.; Vieira, M.; Aryal, B.; Vera, N.; Solis, M. *J. Chem. Ed.*, **2010**, *87*, 708-710.

Discussion

Some might say that the discovery about 5000 years ago that the fermentation of sucrose (table sugar) produces ethanol was the beginning of the discipline of chemistry. However, chemistry like all sciences, encompasses more than observation. Chemistry involves questioning, proposing explanations and testing. It was not until Boyle, Lavoisier and others in the 17th, 18th and 19th centuries began to gain insight and understanding from experimentation that the science of chemistry originated. And it was not until about the turn to the 20th century that Eduard Buchner (should be distinguished from Ernst Büchner who developed the funnel named after him) and others began to elucidate the mechanism of the fermentation of sucrose.

Because fermentation is used to produce liquors, wines and beers, this process remains today, for better or for worse, one of the most commonly used chemical reactions. While prudent consumption of alcoholic beverages is enjoyed by some and according to some research has health benefits for the heart, abuse leads to disruption of many lives and thousands of injuries and deaths every year in motor vehicle "accidents". Because of the recognized dangers of alcohol consumption, the United States in 1920 added the only amendment to the Constitution at that time to deal with a chemical. The amendment called the "noble experiment" banned alcoholic beverages and resulted in many more problems than it solved. As a result, a second "chemical" amendment in 1933 was added to the Constitution repealing Prohibition.
[For example: see http://www.cato.org/pubs/pas/pa-157.html]

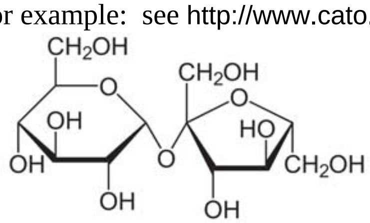


Fig. 13-2

sucrose

H_2O/invertase →

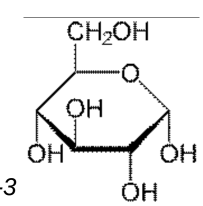


Fig. 13-3

α-D-glucopyranose

+

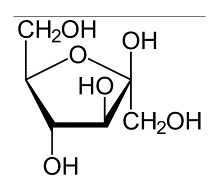


Fig. 13-4

β-D-fructofuranose

↓ zymase

4 CH_3CH_2OH + CO_2 + energy

The world supply of sucrose or table sugar is extracted primarily from sugar cane (15 - 20% sucrose) and sugar beets (10 - 17% sucrose). In this experiment, sucrose will be subjected to fermentation using the enzymes in baker's yeast. As the product, ethanol, inhibits fermentation, the reaction stops after the alcohol production reaches about 10 - 15% ethanol. The ethanol will be concentrated and partially purified using fractional distillation. Since water and ethanol form an azeotrope (constant boiling mixture) consisting of 95.57% by weight ethanol (94.9% by volume) that boils at 78.15 $^{\circ}$C, pure ethanol cannot be obtained from a binary distillation with water. The percentage of ethanol early and late in the distillate will be determined using nmr and/or other methods of your choice.

Procedure

Fig. 13-5

Add 20.0 g of sucrose and 175 mL of water to a 250 mL Erlenmeyer flask. Warm the solution to about 30°C and swirl until all of the sucrose has dissolved. Now add 20 mL of Pasteur's salts (2.0 g of potassium phosphate, 0.20 g calcium phosphate, 0.20 g of magnesium sulfate and 10.0 g of ammonium tartrate dissolved in 860 mL of water) and 2.0 g of dried baker's yeast to the flask. Shake vigorously to mix the contents and insert a one hole rubber stopper with a piece of glass tubing with a 90° bend. The glass tubing should be connected to another piece of glass tubing with a 90° bend that extends down into a gas bottle containing limewater with a 1 cm layer of mineral oil on top of it. The mineral oil protects the limewater from the air. The carbon dioxide produced by the reaction helps to protect the products from air oxidation. Ethanol air oxidizes to acetic acid and eventually to carbon dioxide. If acetic acid is produced the solution will acquire an undesirable vinegar taste.

The mixture should be allowed to stand (preferably at 30°C or slightly higher if possible) until carbon dioxide is no longer evolved by the reaction. For you, this means you will probably allow it to stand for a week and considerably longer if it is at room temperature. After a suitable time has elapsed for the fermentation, carefully decant the liquid into another flask trying as best as possible to leave the sediment in the reaction flask. Unless the liquid is not cloudy, it should be filtered through a Büchner funnel that has had a thin layer of Celite deposited on the filter paper. To do this, add about 1 tablespoon of Celite to a beaker containing 100 mL of water. Stir vigorously and add it to the Büchner funnel while a vacuum is applied. Discard the filtrate and filter the decanted fermentation liquid with the aspirator set on a slow water flow. Set aside about 0.5 mL of the Celite filtered liquid in a vial for an nmr.

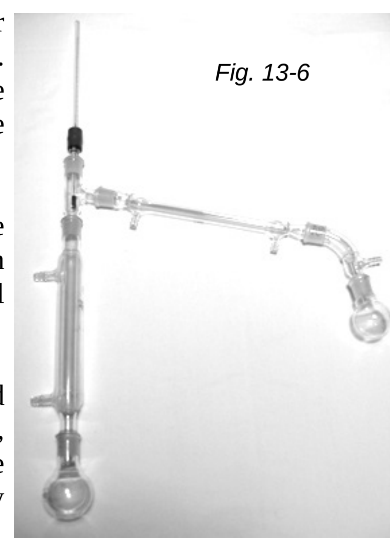


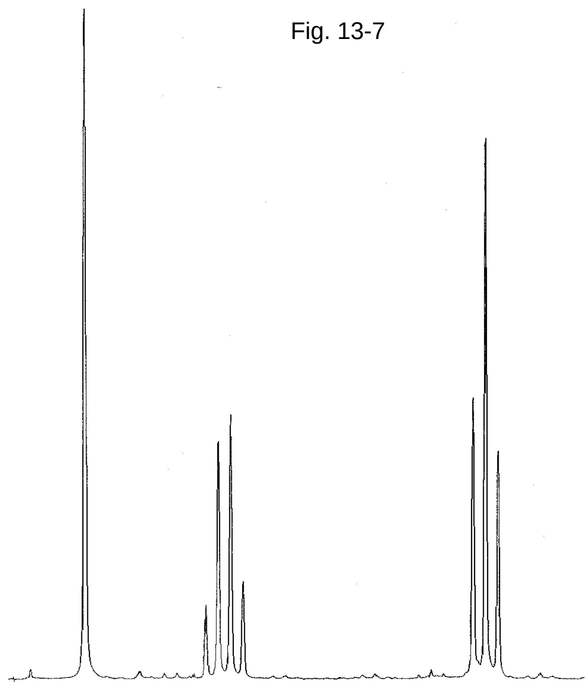
Fig. 13-6

Select a round bottom flask that is about twice the volume of the filtrate, add a couple of boiling chips and set up a fractional distillation apparatus. Distill the Celite filtered liquid and collect the fractions that boil between about 77 and 82°C, 82 and 90°C and 90 and 100°C.

Run an ^{1}H-nmr of the Celite filtered liquid and each fraction and determine the % by mass ethanol using the procedure below. Also, determine the density of each fraction and use the graph below to determine the % by mass of ethanol. Compare the results and attempt to explain any differences outside the range of experimental error.

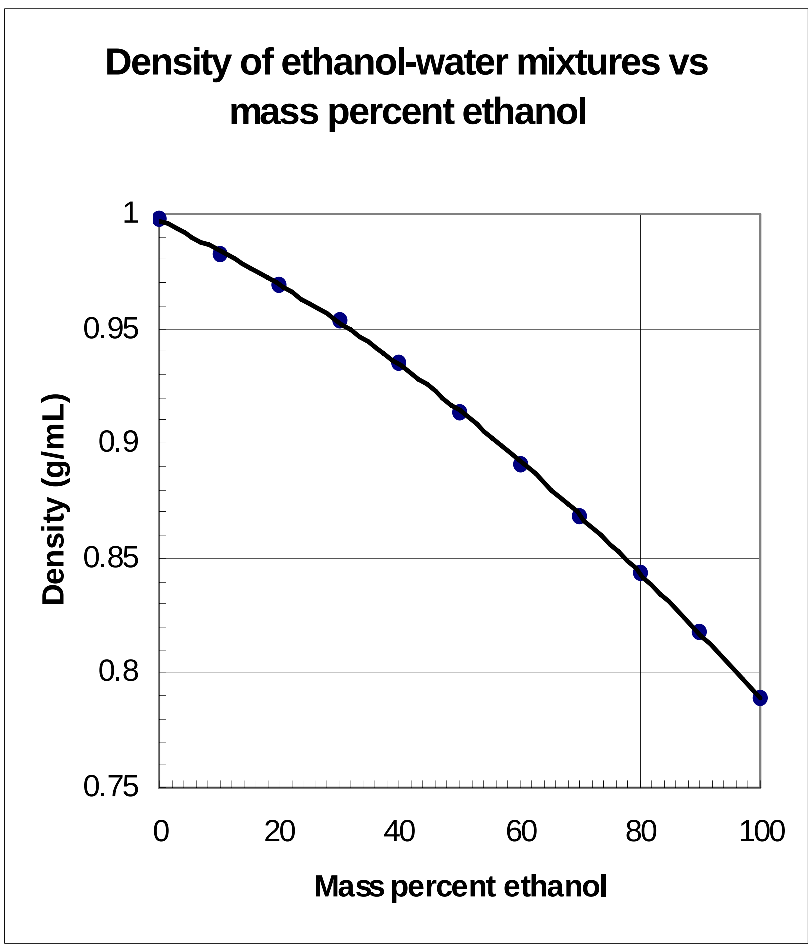
Although nmr integration is inherently subject to error, its use to determine the mass percent of ethanol in your distillate is one of the better methods available for the size sample that you will have. The nmr to the right is an example of a fermentation result. The integrations left to right of the singlet, quartet and triplet were 1247, 1249 and 1851 respectively. As a test of the accuracy of the integration, the triplet should integrate to 1.5 times the integration of the quartet. The experimental results for this spectrum are 1851/1249 = 1.48. To determine the mass percent of ethanol, the integration of the methylene quartet is divided in half (1249/2 = 624) because it represents the integration of the two hydrogens. The singlet represents the integration of the two water hydrogens and the single ethanol hydrogen (on oxygen). The subtraction of 624 from 1247 and division by two gives the relative number of water molecules (312). The mol fraction of ethanol is 624/(624 + 312) = 0.67. The mass percent of ethanol is determined as follows:

Fig. 13-7



$$(624)(46.1)/[(624)(46.1) + (312)(18.0)] = 84\%$$

An alternative method to nmr for the determination of the % by mass is to measure the density of the ethanol-water mixture and use the accompanying graph to determine the % by mass of ethanol. The data used to prepare the graph came from the 70th edition of the *Handbook of Chemistry and Physics* (D230)



Reference

Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Engel, R. G., *Organic Laboratory Techniques*, Saunders, **1998**, pp. 85-92.

Prelaboratory Preparation - *Experiment 13*

First, be sure to list all the goals of the experiment. The hydrolysis of sucrose illustrated on the first page of this experiment is not complete as both monosaccharide products undergo partial isomerization to an equilibrium mixture of glucoses and fructoses. Why is this process called an inversion of sucrose? Based on the stoichiometry of the reaction, calculate the theoretical yield of ethanol. Since the reaction stops at about the 15% mark, calculate the yield that could result. On a previous page, a calculation of the percent by mass of ethanol for an ethanol-water mixture was performed from data obtained from an nmr spectrum. Confirm that the calculation of the % mass of ethanol was performed correctly.

Observations

Report all relevant observations including boiling points and spectra.

Conclusions

This section should include the following:

1. Were the goals of the experiment achieved? Explain your answer.
2. Did the nmr confirm that ethanol was produced by the fermentation? Was there any other evidence that ethanol was the product of the reaction?
3. Compare the % by mass determined by nmr and density for each fraction. Explain any differences that are outside the range of experimental error. Which method do you have more confidence in? Explain your answer.
4. Did the percent by mass of ethanol change as the distillation proceeded? If so, is the trend consistent with expectations? Explain your answer.
5. If you had been trying to prepare an alcoholic beverage by distillation, at what temperature would you have stopped the distillation? Explain your answer.
6. Did the nmr have any peaks that you could not attribute to ethanol or water? What tests would you perform to determine if the distilled product is consumable?
7. How did your yield compare to the expected yield?
8. How could the percent yield and recoveries have been improved?
9. Did the nmr of the Celite filtered liquid indicate approximately 15% ethanol? Could the Pasteur salts have interfered with the nmr? What is the function of the Pasteur salts and are they necessary?