Biosynthesis of Ethanol from Molasses

Techniques

Jointware (Chapter 4)
Clamps & Clamping (Chapter 19)
Distillation (Chapter 20)
Theory of Distillation (Chapter 36)

Green Principles

Catalysts
Design for Degradation
Renewable Feedstocks

Introduction

Alcohols have been selected as green organic solvents in this course because they have few human health and environmental risks, particularly in comparison to methylene chloride or benzene. While these are two important concerns, there are others a green chemist must ask. One is the question of feedstocks. Ethanol may be a benign solvent, but what is its source? If the source is petroleum all we have done is move the hazard to another part of the process.

In fact, this has been the case until recently. Ethanol has been synthesized primarily from oil. Over the last few decades other feedstocks have become important sources of ethanol. One significant and growing source of ethanol is blackstrap molasses, which is a waste product of sugar production.

Sugar comes from two major feedstocks: sugar cane in tropical climates and sugar beets in temperate climates, giving most countries a source of sugar. Both sources of sugar are processed in a similar way. Sugar is extracted from the beet or cane pulp with hot water in diffusion towers forming the raw juice or mother liquor. Lime ($Ca(OH)_2$) and carbon dioxide are then added to the mother liquor to precipitate most of the water-soluble non-sugars. The mother liquor is then concentrated by evaporation, bringing the initial 10% – 15% sugar concentration to 60% – 65%. This leaves the solution super-saturated when cooled back to room temperature. Seed crystals are added to the cooled solution to promote sugar crystal growth. The crystals are grown until they reach the desired sugar crystal size and then are separated from the mother liquor. The initial sugar crystals collected are known as the A-product or white sugar. The evaporation crystallization cycle is repeated forming the light brown B-product or high raw sugar. This cycle is repeated a third time producing the dark brown C-product or low raw sugar. At this point, it is no longer cost effective to produce further sugar product and the mother liquor is now known as blackstrap molasses.

The need to effectively use this waste molasses has grown in the last 30 years as the demand for sugar has tripled. Global blackstrap molasses production is estimated to be about 60 tons per year. The majority of this waste molasses is combined with the dried pulp from the sugar beet or cane and used for animal feed. Ethanol production is one growing application of this plentiful material. Other applications include food and drink manufacture, fuels, rubber, printing, and binders.

In this experiment we will use commercially available molasses. It can be found in three forms: fancy, cooking, and blackstrap. Fancy molasses, also known as Gold Star molasses, is a direct product of sugar cane. Cooking molasses is a blend of fancy and blackstrap molasses. Blackstrap molasses is the by-product of the sugar making process. These products may or may not have sulfur added. Sulfur inhibits the breakdown of molasses sugars and will reduce the ethanol yield for our synthesis.

Greening the Chemistry

This laboratory experiment demonstrates three key green principles: the use of renewable feedstocks, catalysis, and design for degradation.

Cheap petroleum led to its use for the synthesis of most organic compounds. This feedstock is renewable only on a geological timescale and is dwindling rapidly. This timescale is unreasonable for most applications. In comparison, blackstrap molasses is an excellent source as it comes from a quickly renewable natural product. Sugar beets are harvested annually and sugar cane several times a year. In evaluating feedstocks we can further analyze the agricultural practices themselves. While the crop is renewable the practices used in growing the crop can vary from environmentally responsible to environmentally destructive. Even when well intentioned our use of chemical products can still result in serious environmental harm. We need to do more than just a surface analysis of the feedstock to know that we are making the best choice. Sometimes the petroleum product may actually do the least amount of harm when considering the big picture. Analyzing feedstocks can be a challenging task!

Catalysis is important for reducing the energy consumption and the waste production in a chemical reaction. This reaction takes advantage of the enzymes sucrase or invertase and zymase found in yeast. Enzymes are remarkable natural catalysts that are highly chemically selective and significantly reduce the activation energy of a reaction. Molasses contains disaccharides that are hydrolyzed into monosaccharides by sucrase. The glucose and fructose monosaccharides are then converted into ethanol by zymase.

This reaction is an excellent example of design for degradation. Only a small amount of yeast is necessary to initiate the reaction and the yeast reproduces while the reaction is occurring. Once the reaction has reached completion, the yeast dies and the ethanol and all by-products are easily, rapidly, and harmlessly degraded in the environment.

Theory/Discussion

Fermentation is one of the oldest chemical arts. The fermentation of molasses is the process used to make rum (although the rum produced in this reaction will not have a desirable flavor and is not fit for human consumption). In our case, the interest is the synthesis and purification ethanol by a green reaction pathway.

Molasses is a mixture of monosaccharides and disaccharides and other miscellaneous flavoring agents naturally produced in the sugar cane or sugar beet. Approximately 50% of the molasses mass is sugars. Yeast has two enzymes that convert the saccharides to ethanol. Invertase converts dissacharides (such as sucrose) to monosaccharides by a catalytic hydrolysis (addition of water) reaction. Glucose and fructose are then converted to ethanol and carbon dioxide by the enzyme zymase. One mole of sucrose will produce four moles of ethanol and four moles of carbon dioxide. When the alcohol level becomes high enough the yeast will die of alcohol poisoning. The reaction is vapor locked with limewater so that the reaction environment remains oxygen free keeping the ethanol from further oxidizing to acetic acid.

Boiling occurs when the vapor pressure of a liquid is equal to the atmospheric pressure. This is determined using Raoult's law for an ideal gas. Distillations take advantage of this behavior in a mix of solutions to separate two liquids. Some liquids do not behave in this ideal way and instead form azeotropes. Ethanol and water form a temperature minimum azeotrope, making pure ethanol very difficult to obtain. This azeotrope boils at 78.2°C with a 95.6% ethanol, 4.4% water blend. To better understand this process read the distillation theory section of the laboratory techniques manual.

Experimental

Mix 70 mL of molasses with 70 mL of water in a 250 mL Erlenmeyer flask. Add about 0.5 g of yeast to the flask and stir gently until everything is well mixed. Stopper the flask with a one-hole rubber stopper containing a bent glass tube. Attach a short rubber hose to the bent glass tube and insert a short straight section of glass tube into the other end of the rubber tube. Dip the straight glass tube into a test tube two-thirds full of limewater ($Ca(OH)_2$ solution). The test tube of limewater serves as a one-way vapor lock, keeping air from entering the flask while allowing the carbon dioxide to escape. If air were to enter the flask during the reaction, the ethanol produced would be further oxidized to acetic acid. Store this reaction in your drawer for one week while the fermentation reaction occurs.

Prepare a simple distillation, decanting the ethanol solution into the 250 mL round bottom flask (RBF). The simple distillation can be done fairly rapidly (one drop per second) and the alcohol fraction should be collected until just below the boiling point of water. Prepare a fractional distillation, placing the mixed alcohol product from the simple distillation in the RBF. Distill the ethanol slowly; record the temperature range for each fraction collected, stopping collection at 97°C. Fractions are identified by a rapid change in temperature and a concurrent increase/decrease in distillate production. Determine the density of each fraction by massing a 10 mL sample collected with a volumetric pipette. If the collected fraction is less than 10 mL, use the available pipette that is closest in volume to the fraction. Use the table below to determine the alcohol content in each fraction. Record your volume of "pure" ethanol collected and add your ethanol to the collection container. We will use this ethanol in the next experiment requiring ethanol as a solvent.

Aqueous Alcohol (EtOH) Content							
Density g/mL	% EtOH by wt.	% EtOH by vol.	g EtOH per 100 mL	Density g/mL	% EtOH by wt.	% EtOH by vol.	g EtOH per 100 mL
0.989	5	6.27	4.95	0.856	75	81.30	64.17
0.982	10	12.44	9.82	0.843	80	85.49	67.48
0.975	15	18.54	14.63	0.831	85	89.48	70.63
0.969	20	24.54	19.37	0.828	86	90.25	71.23
0.962	25	30.46	24.04	0.826	87	91.02	71.84
0.954	30	36.25	28.61	0.823	88	91.77	72.43
0.945	35	41.90	33.07	0.821	89	92.53	73.03
0.935	40	47.40	37.41	0.818	90	93.27	73.62
0.925	45	52.72	41.61	0.815	91	93.99	74.19
0.914	50	57.89	45.69	0.813	92	94.72	74.76
0.903	55	62.89	49.64	0.810	93	95.44	75.32
0.891	60	67.74	53.47	0.807	94	96.11	75.86
0.880	65	72.43	57.17	0.804	95	96.79	76.40
0.868	70	76.95	60.74	0.789	100	100.00	78.9

Biosynthesis of Ethanol from Molasses

Pre-Laboratory Questions

1. During the fermentation reaction it is important to prevent air from entering the reaction chamber. Why?

2. Explain the advantage of a fractional distillation over a simple distillation.

3. What is an azeotrope and why does it limit our ethanol purity even when we are doing a fractional distillation.

4. Complete the following tables of chemical data:

Liquids	b.p. °C	Density
ethanol		
water		

5. Consider the chemicals used for this experiment. What realistic hazards are present? What safety procedures are necessary beyond wearing goggles and gloves?

Biosynthesis of Ethanol from Molasses

Experiment Report

For this experiment I would like you to create a summary report. The report should be a typed one-page narrative and should include:

- A brief summary of the experiment (one paragraph)
- A discussion of your results that should include
  - Volumes and percent ethanol for each fraction collected
  - Percent yield of ethanol from molasses
  - Percent yield of ethanol from the reactive sugars in the molasses assuming the molasses is approximately half reactive sugars.
- An analysis of error and the quality of your experiment. Did the experiment go well or poorly and can you explain what went wrong? Do you find your results to be reliable?
- An evaluation of this experiment in terms of its greenness.