Biosynthesis and Purification by Distillation of Ethanol

Part I: Ethanol Biosynthesis

Introduction

Man has been brewing alcoholic beverages for thousands of years (longer than man has been making soap, believe it or not!). Well known liquors, e.g., wine from grapes, vodka from potatoes, whiskey from corn, come from foods with a source of carbohydrate which, while enjoying anaerobic conditions (what are these conditions?), will be metabolized to ethyl alcohol and carbon dioxide.

The source of carbohydrate from pants is, as you recall, starch. As grains begin to sprout (potatoes, as well), enzymes are activated which catabolize (what does this mean?) the starch to glucose. If the process is stopped, e.g., by heating until the "sprouts" are dried, the glucose wil not be further catabolized. If the dried residue is rehydrated, has its pH and temperature adjusted to optimal conditions, has yeast added and then the resulting mix isolated from an aerobic atmosphere, then anaerobic fermentation will proceed.

Anaerobic fermentation proceeds because the enzymes required to catabolize the glucose are in the yeast. The catabolism of glucose is enzymatically alike between yeast and animals down to pyruvate formation. Once at pyruvate, however, the metabolic similarities under anaerobic conditions cease. In animals, pyruvate is further catabolized to lactic acid (accumulates in muscles and is a cause for cramping). In yeast, the pyruvate is metabolized to ethanol via acetaldehyde and CO_2 synthesis.

In this part of the experiment, the student will initiate the fermentation of table sugar with baker's yeast.

Materials and Method

Materials

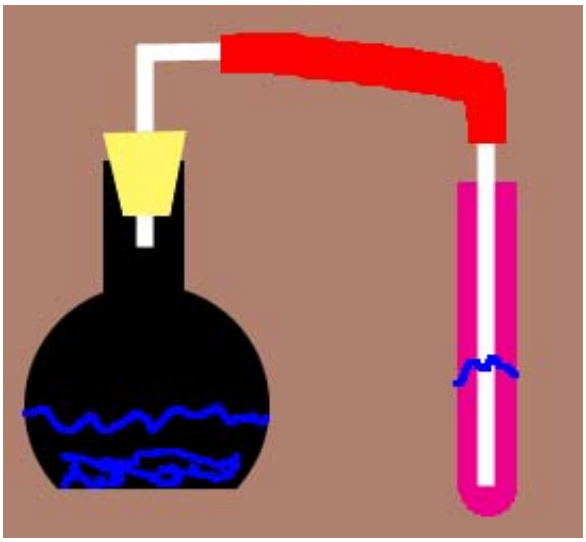
The table, below, lists the chemicals and supplies necessary for this part of the experiment:

Baker's yeast	Table sugar	Florence flask	One holed rubber stopper for flask
Na_2HPO_4	Sat'd $Ca(OH)_2$	Bent glass tubing	

Method

Place 50 mL of distilled water in a 100 mL beaker. Add half an envelope of Flesichmann's yeast and make a fine suspenstion (do NOT use the rapid start yeasts like Red Star as they don't work for this experiment). To the suspension, add 0.35 grams of Na_2HPO_4 and then pour the suspension into your Florence flask. Add to the Florence flask a solution of 51.5 grams of table sugar in 150 mL distilled water. Mix by gentle swirling – do NOT shake as this will denature the enzymes in the yeast.

Put a one-holed rubber stopper with a bent glass tube in it (attached to a piece of latex tubing with a piece of glass tubing at the other end) in the neck of the Florence flask. Insert the end opposite the Florence flask into a test tube containing saturated calcium hydroxide. Once you have done this, layer on about 0.5 cm of mineral oil over the limewater solution so that the limewater solution won't evaporate. Place your apparatus (and it ought to look sort of like the graphic, below) in the incubator until the next lab period.



NOTE: Once you have your samples ready for the incubator, leave the door closed, i.e., put all of your samples in at once – if the samples warm up, and you keep opening the door to the incubator, then your fermenting mixture will siphon the calcium hydroxide back into itself as it cools – remember Boyle's Law?

Part II: Ethanol Purification by Distillation

Introduction

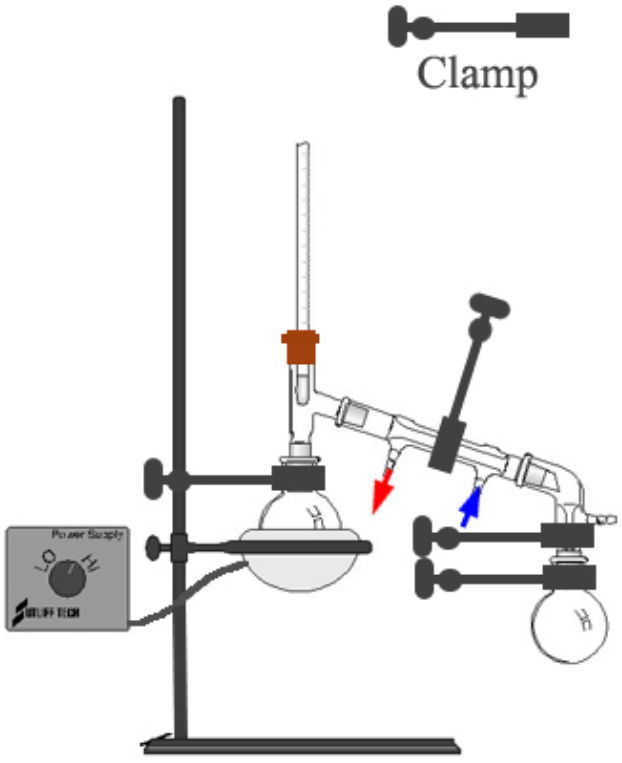
A liquid is said to boil when the vapor pressure of that liquid is equivalent to the pressure exerted on it surface. This exerting pressure is generally the atmospheric pressure. Some liquids boil at room temperature, e.g., liquid nitrogen, and evaporate very rapidly if not kept tightly covered, i.e., are very volatile.

Compound	Boiling Point
Diethyl ether	34.6 °C
Acetone	56.5 °C
Ethanol	78.5 °C
Toluene	110.6 °C

When heat is added to a liquid in an open vessel at room temperature, the heat excites the molecules. As the molecules become more excited, they gain more and more energy. Once enough energy has been gained, some of the molecules leave the liquid and become vapor. When that vapor pressure equals atmospheric pressure, the liquid is boiling. When a liquid is heated in an enclosed container, the vapor pressure will reach a constant equilibrium pressure. At this point, molecules leaving the liquid phase and vaporizing are equal to the molecules leaving the vapor phase and condensing.

If the vapor in an enclosed container comes into contact with a cold surface, it condenses. When this happens and the condensate is collected, it is called distillation. If two liquids with different boiling points are treated in this manner, the liquid that one desires to purify, isolate or separate may be so collected – while the other may be discarded.

During distillation, the liquid is being vaporized across the bulb of a thermometer (just beneath the red adapter in the graphic, below). This point, at the intersection of the downward arm off the adaptor where the bulb is always placed, is the hottest part of the still. As long as the same liquid is being vaporized, the temperature remains constant – this is key to obtaining the correct fraction from a mixture. The temperature changes only when there are different compounds being distilled. Hence, when collecting distillate fractions, it is very important to watch the temperature very closely and collect the fraction boiling in a specific range. The graphic, below, illustrates a very generic and simple still:



In the graphic, above, the red arrow shows where water comes out of the jacket and the blue arrow where water enters the jacket (why is this important?). There are ring stands left out for simplicity's sake – wherever a clamp is in the graphic, you need to make sure you have a ring

stand for it. Your lab instructor will describe each part of the still for you – be sure to label as s/he goes along.

Materials and Methods

Materials

The table, below, summarizes the supplies you'll need for this experiment:

Your fermentate	Distillation apparatus	Heating mantle	"Goose" grease
10 mL graduated cylinder	Pan balance	Variac	Rubber tubing
Three-pronged clamps	Ring stand	Thermometer	Buchner funnel
Filter paper	Diatomaceous earth	Spatula	Filter flask

Method

Remove your suspension from the incubator. Immediately remove the tubing from the $Ca(OH)_2$ tube so you don't siphon anything into your fermentate. Examine the test tube. Do you see a fine white precipitate? What is it?

Add about 10 grams of diatomaceous earth to the suspension and then filter the mix through the Buchner funnel. COLLECT the FILTRATE (the part that goes through the paper into the flask).

Assemble the distillation apparatus with the water hose going in on the bottom of the condenser jacket and the top outlet running into the sink through tubing.

NOTE: for a large class with limited sink nozzles, "piggy-back" stills to each other, no more than 4 in series, though, as it gets rather cumbersome. Be sure that the students remember that they are "piggy-backed" so that one doesn't turn off the water before the others are done.

When assembling the still, remember to grease the glass joints as shown by your instructor. Clamp every where that you can to protect the glassware, as well. Keep joints snug when distilling so you don't lose any vapor.

Pour enough of your filtrate into the distillation flask (the one that rests in the heating mantle). Reset all seals and turn on the Variac (plug it in, too). Heat the solution slowly, watching your thermometer closely. Record the temperature when the first drop of condensate leaves the condensing jacket. Collect the fraction that distills across in the temperature range of 77-79 °C ± 2 °C. Once you have collected the fraction boiling between this range, remove your collection flask and turn off the Variac. Catch any other condensate which may come across the jacket in a beaker as your still cools down.

Determine the volume of distillate you collected. Determine its mass just as you did in CHEM 101 (soon to be CHEM 121 effective Fall 2003). Using this information, determine the density of the ethanol you've distilled. Using the following equation (which is REAL crude), determine the percent alcohol you have collected.

$$\frac{Alcohol\ Density - 1.062}{-0.00267} = \%\ EtOH$$

NOTE: the denominator has a NEGATIVE sign!!!!!

Once you've done that, record it here:

Now, double the percent to determine the proof of the alcohol you've obtained by distillation:

Questions

1) Using Stryer or another Biochemistry book (or waiting until we've covered it in lecture), draw the complete biochemical pathway for the fermentation of glucose to ethanol on a separate piece of paper and attach it to this lab for turn in.

2) What are enzymes?

3) If you were to have added more sucrose to the suspension, would you have been able to make more EtOH in your flask in the incubator? HINT: review non-competitive inhibition of enzymes in lecture for this course, BIOL 223 or BIOL 251. Why or why not?

4) What would you have predicted would be the effect on the alcohol production in your flask had you added EtOH to the mixture before it began fermenting?

5) What is meant by the term “boiling point”?

6) What would you expect to happen to the boiling point of any liquid if the pressure exerted on it were reduced? What if it was increased? How does this explain how a pressure cooker (or autoclave, for that matter) works?

7) What is Raoult's Law? What is an azeotropic mixture? Attach separate paper for this one, as well, when you've answered it.

8) If impurities lower the melting point of solids, what do you think would happen to the boiling point of a liquid with an impurity in it? (HINT: think colligative properties)