



# Preparing Ethanol by Fermentation

*prepared by Joe Jeffers, Ouachita Baptist University*

**PURPOSE OF THE EXPERIMENT** Prepare ethanol by yeast fermentation using sucrose. Purify the product by fractional distillation. Use density measurement to determine the percent ethanol.

**EXPERIMENTAL OPTIONS**

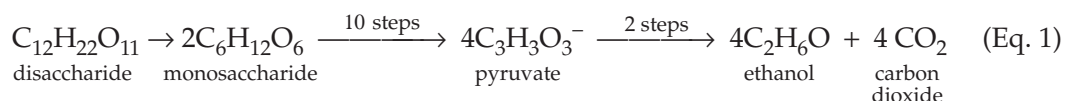
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**BACKGROUND REQUIRED** You should be familiar with vacuum filtration, distillation, and density measurement.

**BACKGROUND INFORMATION** People have used fermentation in brewing and baking for more than 5000 years. The action of yeast or other microorganisms converts disaccharides such as sucrose or maltose to ethanol and carbon dioxide (CO<sub>2</sub>). In brewing, the desired product is ethanol; in baking, the desired product is CO<sub>2</sub>.

In 1789, Lavoisier first showed that sugar undergoes fermentation to yield ethanol and CO<sub>2</sub>. Gay-Lussac quantified that process in 1815 to show that each mole of glucose gives two moles of ethanol and two moles of CO<sub>2</sub>. Pasteur demonstrated in 1857 that the process requires yeast. Then in 1897, Büchner found that a yeast extract not containing living organisms could cause the conversion. Twentieth-century chemists continued these studies to identify the enzymes and reaction intermediates that are part of the chemical pathways involved.

Sucrose is hydrolyzed to yield the monosaccharides glucose and fructose; maltose is hydrolyzed to yield two units of glucose. In either case, the monosaccharides are converted to their respective phosphate derivatives. Then, through a series of reactions, each six-carbon monosaccharide-phosphate yields two three-carbon pyruvate molecules. Each pyruvate is converted under the anaerobic conditions of fermentation into ethanol and CO<sub>2</sub>. The overall process is summarized in Equation 1.



Ethanol concentrations near 12 percent inhibit fermentation. As a result, distillation is required to generate ethanol solutions higher than 12 percent.

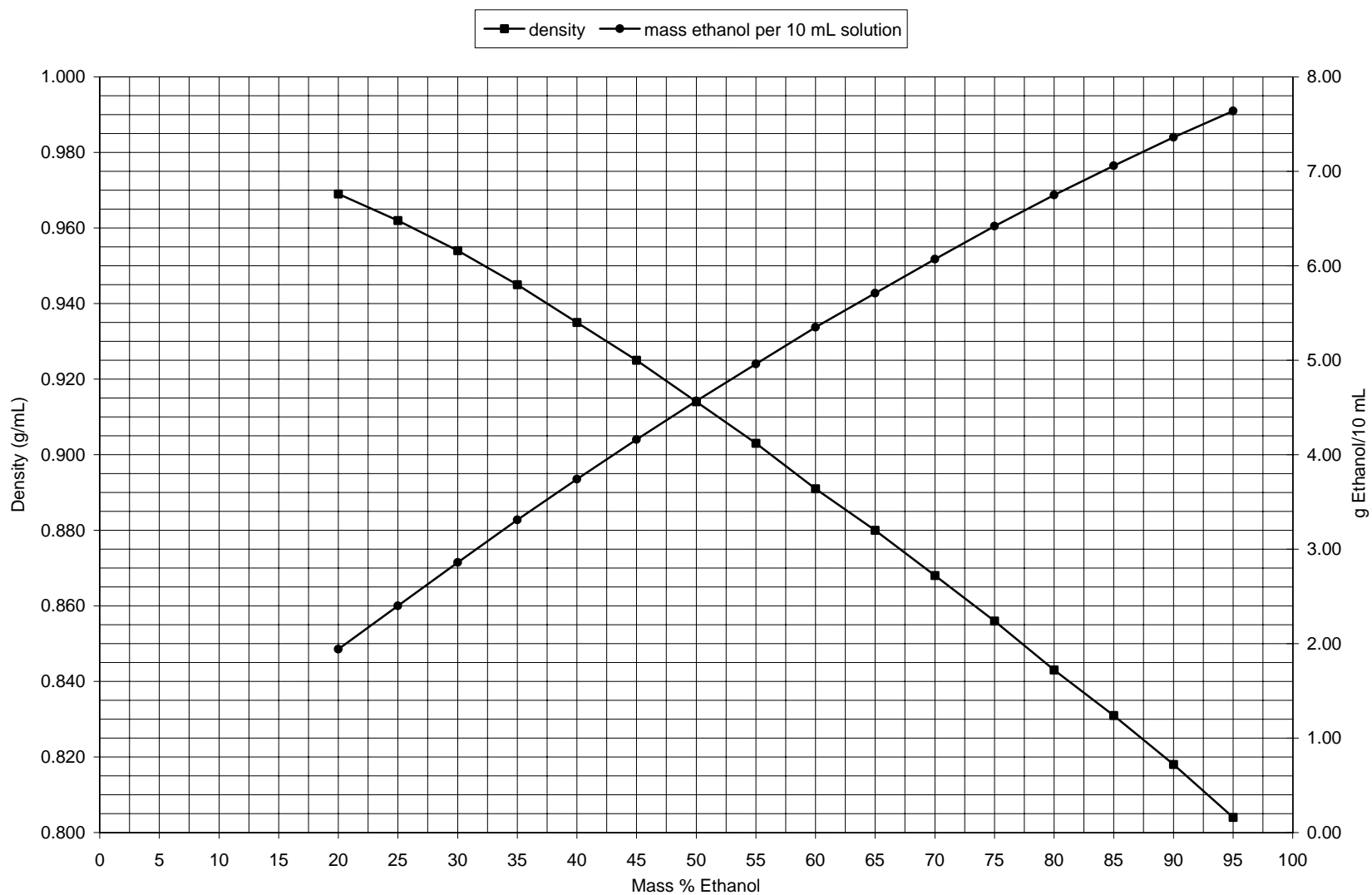
Ethanol and water form an **azeotrope**, a constant boiling mixture, at 95 percent ethanol, so pure ethanol cannot be obtained by distillation. Azeotropes have the same percentage composition of mixture components in the vapor as in the liquid, so they cannot be enriched in the vapor phase by continued distillation. Because azeotropes boil at temperatures lower than any component of the mixture, the azeotrope distills first from the mixture.

At 20 °C, the density of water is 0.998 g/mL and the density of ethanol is 0.789 g/mL. The densities of ethanol–water mixtures lie between these values. The mass percent of ethanol in an ethanol–water mixture can be determined from the mixture density. Figure 1 shows two standard curves for ethanol–water mixtures: density versus mass percent ethanol and grams ethanol per 10 mL solution versus mass percent ethanol.

You can use standard curves like those of Figure 1 to determine the mass percent of distillate samples if you know the sample's density. For example, an ethanol–water mixture with a density of 0.820 g/mL has a mass percent ethanol of 89. A mixture that is 89 mass percent ethanol contains 7.60 g of ethanol in each 10 mL of solution. If your distillate volume is 7.5 mL, you can calculate your yield using Equation 2.

$$\begin{aligned}\text{mass ethanol obtained, g} &= (\text{volume distillate obtained, mL}) \left( \frac{\text{mass ethanol in 10 mL, g}}{10 \text{ mL}} \right) \quad (\text{Eq. 2}) \\ &= (7.5 \text{ mL}) \left( \frac{7.60 \text{ g}}{10 \text{ mL}} \right) = 5.7 \text{ g ethanol}\end{aligned}$$

The procedure in this experiment requires time from two consecutive laboratory periods: one laboratory period plus a small portion of the immediately preceding laboratory period. You will start the fermentation reaction in Laboratory Period One by mixing sucrose, baker's yeast, and a phosphate salt. In Laboratory Period Two, you will distill the fermented mixture. Then you will measure the density of the product and calculate the yield of ethanol using your results and information from standard curves like those of Figure 1.



**Figure 1** Standard curves for density vs. mass % ethanol and mass ethanol per 10 mL solution, g vs. mass % ethanol

## Macroscale Fermentation

### Equipment

#### Laboratory Period One

250-mL beaker	500-mL round-bottom flask, with cork ring*
bent-glass tubing	spatula
100-mL graduated cylinder	18 × 150-mm test tube
one-hole rubber stopper	

#### Laboratory Period Two

aluminum foil	standard taper glassware
4 boiling chips	condenser, with adapter and rubber tubing
Büchner funnel, with adapter	distilling head
copper metal sponge	fractionating column
electric flask heater, with regulator	100-mL round-bottom flask
10-mL Erlenmeyer flask, with stopper <sup>†</sup>	–10 to 260 °C thermometer, with adapter
500-mL filter flask, with vacuum tubing	support ring
filter paper	2 support stands
10-mL graduated cylinder	10.0-mL transfer pipet <sup>†</sup>
100-mL graduated cylinder	2 utility clamps
spatula	

\*or 500-mL Florence flask      <sup>†</sup>or 10-mL density vial

### Reagents and Properties

<i>substance</i>	<i>quantity</i>	<i>molar mass</i> (g/mol)	<i>bp</i> (°C)	<i>d</i> (g/mL)
calcium hydroxide, sat. aq.	12 mL			
Celite <sup>®</sup> filter agent	10 g			
ethanol, 95%*		46.1	78.1	
disodium hydrogen phosphate	0.35 g	142		
sucrose	40 g	342.30		
yeast	3.5 g <sup>†</sup>			

\*product

<sup>†</sup>1/2 package of dry yeast

### Preview

#### Laboratory Period One

- Set up fermentation apparatus containing sucrose, disodium hydrogen phosphate, and yeast
- Set up trap containing saturated calcium hydroxide solution
- Allow the reaction to proceed for one week

#### Laboratory Period Two

- Add Celite<sup>®</sup> to the reaction mixture and conduct vacuum filtration
- Conduct simple distillation
- Measure volume and density
- Conduct fractional distillation
- Measure volume and density

**PROCEDURE** **Caution:** Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

Always use caution in the laboratory. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Avoid ingesting any of the reagents.

### Laboratory Period One

#### 1. Setting Up the Fermentation

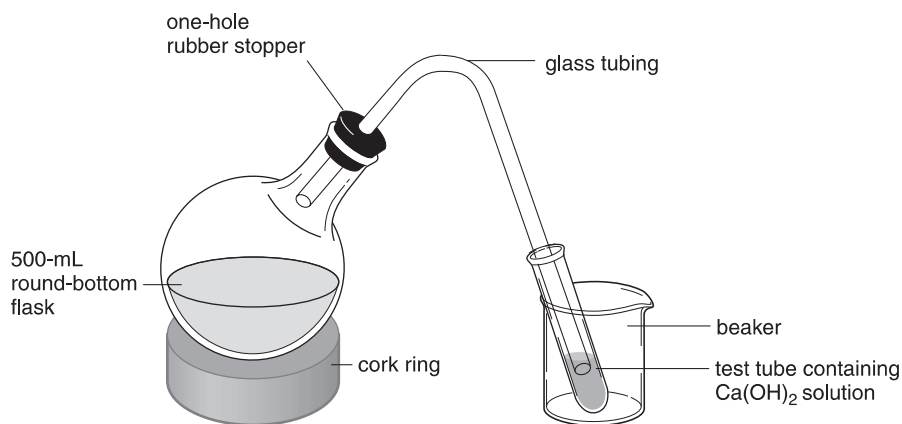
**Caution:** Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) is irritating and hygroscopic. Calcium hydroxide,  $\text{Ca}(\text{OH})_2$ , is corrosive.

Place 40 g of sucrose into a 500-mL round-bottom flask. Add 200 mL of distilled or deionized water. Swirl the flask to dissolve the sucrose. Set the flask onto a cork ring.

Add 3.5 g ( $1/2$  package) of dried baker's yeast. Swirl the flask to distribute the yeast throughout the solution.

Add 0.35 g of  $\text{Na}_2\text{HPO}_4$  to the mixture. Swirl the flask to dissolve the salt.

Using Figure 2 as a guide, insert bent-glass tubing into a one-hole rubber stopper. Close the fermentation flask with the stopper.



**Figure 2** Macroscale fermentation apparatus

**NOTE 1:** The  $\text{Ca}(\text{OH})_2$  trap will prevent oxygen from entering the fermentation flask and oxidizing ethanol to acetic acid. As fermentation proceeds,  $\text{CO}_2$  will bubble through the  $\text{Ca}(\text{OH})_2$  solution, forming a  $\text{CaCO}_3$  precipitate.

**NOTE 2:** The optimal temperature range for fermentation is 30–35 °C. Temperatures higher than 35 °C may kill the yeast.

Half fill a 18 × 150-mm test tube with saturated aqueous  $\text{Ca}(\text{OH})_2$  solution. Place the test tube into a 250-mL beaker. Insert the long end of the glass tubing into the test tube so that the tubing extends 3–5 cm into the solution. [NOTE 1]

Place the apparatus in a warm area of the laboratory for one week. [NOTE 2]

### Laboratory Period Two

#### 2. Filtering the Fermentation Mixture

**NOTE 3:** The Celite® filter agent will prevent the yeast cell debris from clogging the filter paper and thereby slowing the filtration process.

When the fermentation is complete, add 10 g of Celite® filter agent to the fermentation flask. Swirl the flask vigorously to wet the Celite® and distribute it throughout the solution. [NOTE 3]

Set up a vacuum filtration apparatus using a Büchner funnel and a 500-mL filter flask. Wet the filter paper with distilled or deionized water and turn on the aspirator.

Swirl the fermentation flask and pour the suspension into the Büchner funnel. Continue swirling and pouring until the entire suspension is filtered. Rinse the flask with 20 mL of water and add the rinse to the funnel.

**3. Conducting a Simple Distillation**

Clean the 500-mL round-bottom flask. Set up a simple distillation apparatus using the 500-mL round-bottom flask as the pot. Use a 100-mL graduated cylinder as the receiver.

Pour the filtrate from the filter flask into the pot. Add two boiling chips. Distill the solution at a rate of one drop per second.

Collect the fraction that boils *below* 100 °C. Measure and record the volume of distillate.

Tare a 10-mL Erlenmeyer flask with stopper on a 0.001-g balance.

**NOTE 4:** A 10.0-mL density vial may be used instead.

[NOTE 4] Use a 10.0-mL pipet to transfer *exactly* 10.0 mL of distillate into the Erlenmeyer flask. Replace the stopper. Weigh the flask to the nearest 0.001 g. Record the result in your laboratory notebook.

**4. Conducting a Fractional Distillation**

Assemble a fractional distillation apparatus using a 100-mL round-bottom flask for a pot and a 10-mL graduated cylinder for a receiver. Pack the fractionating column with copper metal sponge. Wrap the fractionating column with an aluminum foil jacket so that the foil can be opened on the front of the column.

Transfer the distillate from both the 100-mL graduated cylinder and the 10-mL Erlenmeyer flask into the pot. Add two boiling chips.

Heat the pot, *gradually increasing* the heat to *slowly* raise the vapor line up the fractionating column, allowing time for equilibration of vapor and condensate. As the vapor line rises, close the foil jacket below the vapor line.

Distill the ethanol at a rate of one drop per 2–3 s. Collect the fraction that distills from 77–80 °C. Measure and record the final volume.

Repeat the density measurement procedure described in Part 3. If your total distillate volume is less than 10.0 mL, use 5.0 mL for density measurement.

**5. Cleaning Up**

Use the labeled collection containers provided by your laboratory instructor.

**Caution:** Wash your hands thoroughly with soap or detergent before leaving the laboratory.

## Semi-Microscale Fermentation

### Equipment

#### Laboratory Period One

beaker, 100-mL	microspatula
2 beakers, 400-mL*	one-hole rubber stopper
bent-glass tubing	100-mL round-bottom flask
cotton	13 × 100-mm test tube
50-mL graduated cylinder	−10 to 260 °C thermometer
hot plate	

#### Laboratory Period Two

aluminum foil	25-mL round-bottom flask
2 boiling chips	50-mL round-bottom flask
5.0-mL conical vial	standard taper glassware:
copper metal sponge	condenser, with adapter and
electric flask heater, with regulator	rubber tubing
10-mL Erlenmeyer flask, with stopper <sup>†</sup>	distilling head
50-mL filter flask, with vacuum tubing	fractionating column
filter paper	−10 to 260 °C thermometer, with adapter
10-mL graduated cylinder	support ring
Hirsch funnel, with adapter	2 support stands
microspatula	1.0-mL transfer pipet <sup>†</sup>
	2 utility clamps

\*for warm-water bath and to hold fermentation flask

<sup>†</sup>or 1.00-mL density vial or 1000-μL micropipet

### Reagents and Properties

<i>substance</i>	<i>quantity</i>	<i>molar mass</i> (g/mol)	<i>bp</i> (°C)	<i>d</i> (g/mL)
calcium hydroxide, sat. aq.	5 mL			
Celite <sup>®</sup> filter agent	2 g			
ethanol, 95%*		46.1	78.1	
disodium hydrogen phosphate	0.070 g	142		
sucrose	8 g	342.30		
yeast	0.70 g			
*product				

### Preview

#### Laboratory Period One

- Set up fermentation apparatus containing sucrose, disodium hydrogen phosphate, and yeast
- Set up trap containing saturated calcium hydroxide solution
- Allow the reaction to proceed for one week

**Laboratory Period Two**

- Add Celite® to the reaction mixture and conduct vacuum filtration
- Conduct simple distillation
- Measure volume and density
- Conduct fractional distillation
- Measure volume and density

**PROCEDURE**

**Caution:** Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

Always use caution in the laboratory. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Avoid ingesting any of the reagents.

**Laboratory Period One****1. Setting Up the Fermentation**

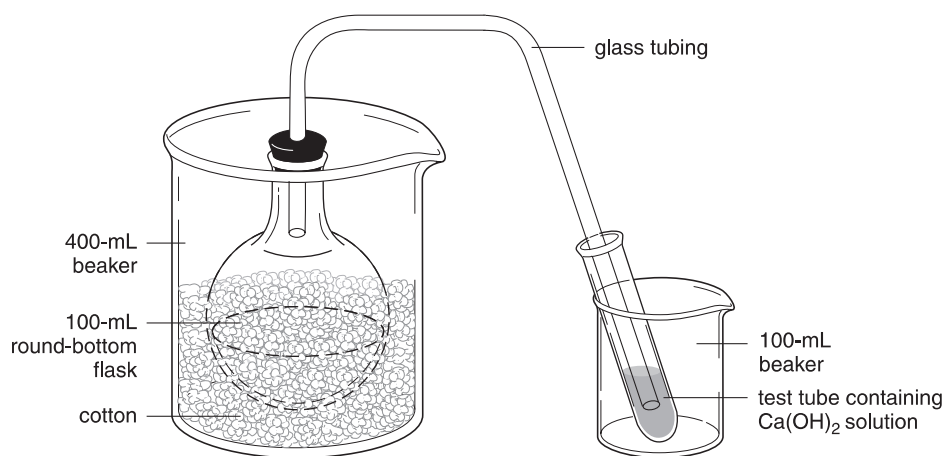
**Caution:** Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) is irritating and hygroscopic. Calcium hydroxide,  $\text{Ca}(\text{OH})_2$ , is corrosive.

Place 8 g of sucrose into a 100-mL round-bottom flask. Add 40 mL of distilled or deionized water. Swirl the flask to dissolve the sucrose. Set the flask into a 400-mL beaker.

Add 0.70 g of dried baker's yeast. Swirl the flask to distribute the yeast throughout the solution.

Add .070 g (70 mg) of  $\text{Na}_2\text{HPO}_4$  to the mixture. Swirl the flask to dissolve the salt.

Using Figure 3 as a guide, insert bent-glass tubing into a one-hole rubber stopper. Close the fermentation flask with the stopper.



**Figure 3** Semi-microscale fermentation apparatus

**NOTE 1:** The  $\text{Ca}(\text{OH})_2$  trap will prevent oxygen from entering the fermentation flask and oxidizing ethanol to acetic acid. As fermentation proceeds,  $\text{CO}_2$  will bubble through the  $\text{Ca}(\text{OH})_2$ , forming  $\text{CaCO}_3$ .

Half fill a  $13 \times 100$ -mm test tube with saturated aqueous  $\text{Ca}(\text{OH})_2$  solution. Place the tube into a 100-mL beaker. Insert the long end of the glass tubing into the test tube so that the tube extends 2–3 cm into the solution. [NOTE 1]

Prepare a 30 °C warm-water bath using a second 400-mL beaker.

Remove the fermentation flask from the first 400-mL beaker. Line this beaker with cotton to form an insulated nest for the flask.



**NOTE 2:** The optimal temperature range for fermentation is 30–35 °C. Temperatures higher than 35 °C may kill the yeast.

Warm the flask to 30 °C in the warm-water bath. Dry the outside of the flask and place it in the cotton nest. Place the apparatus in a warm area of the laboratory for one week. [NOTE 2]

## Laboratory Period Two

### 2. Filtering the Fermentation Mixture

**NOTE 3:** The Celite<sup>®</sup> filter agent will prevent the yeast cell debris from clogging the filter paper and thereby slowing the filtration process.

When the fermentation is complete, add 2 g of Celite<sup>®</sup> filter agent to the flask. Swirl the flask vigorously to wet the Celite<sup>®</sup> and distribute it throughout the solution. [NOTE 3]

Set up a vacuum filtration apparatus using a Hirsch funnel and a 50-mL filter flask. Wet the filter paper with distilled or deionized water and turn on the aspirator.

Swirl the fermentation flask and pour the suspension into the Hirsch funnel. Continue swirling and pouring until the entire suspension is filtered. Rinse the flask with 2 mL of distilled or deionized water and add the rinse to the funnel.

### 3. Conducting a Simple Distillation

Set up a simple distillation apparatus using a 50-mL round-bottom flask as the pot. Use a 10-mL graduated cylinder as the receiver.

Pour one-half of the filtrate from the filter flask into the pot. Add a boiling chip. Distill the solution at a rate of one drop per second. Collect the fraction that boils *below* 100 °C.

Allow the pot to cool. Remove the pot and discard the pot residue.

Pour the remaining half of the fermentation filtrate into the pot. Add a boiling chip. Distill the solution at a rate of one drop per second. Continue to use the 10-mL graduate as the receiver. Collect the fraction that boils *below* 100 °C.

Measure and record the total volume of distillates.

**NOTE 4:** A 1.0-mL density vial may be used instead.

Tare a 10-mL Erlenmeyer flask with stopper on a 0.001-g balance. [NOTE 4] Use a 1.00-mL pipet to transfer *exactly* 1.00 mL of distillate into the Erlenmeyer flask. Replace the stopper. Weigh the flask to the nearest 0.001 g. Record the result in your laboratory notebook.

### 4. Conducting a Fractional Distillation

Assemble a fractional distillation apparatus using a 25-mL round-bottom flask for a pot and a 5.0-mL conical vial for a receiver. Pack the fractionating column with copper metal sponge. Wrap the fractionating column with an aluminum foil jacket so that the foil can be opened on the front of the column.

Transfer the distillate from both the 10-mL graduated cylinder and the 10-mL Erlenmeyer flask into the pot. Add a boiling chip.

Heat the pot, *gradually increasing* the heat to *slowly* raise the vapor line up the fractionating column, allowing time for equilibration of vapor and condensate. As the vapor line rises, close the foil jacket below the vapor line.

Distill the ethanol at a rate of one drop per 2–3 s. Collect the fraction that distills from 77–80 °C. Measure and record the final volume.

Repeat the density measurement procedure described in Part 3.

### 5. Cleaning Up

Use the labeled collection containers provided by your laboratory instructor.

**Caution:** Wash your hands thoroughly with soap or detergent before leaving the laboratory.

## Microscale Fermentation

### Equipment

#### Laboratory Period One

2 beakers, 100-mL	hot plate
250-mL beaker*	microspatula
bent-glass tubing	one-hole rubber stopper
cotton	13 × 100-mm test tube
25-mL Erlenmeyer flask	−10 to 260 °C thermometer
10-mL graduated cylinder	

#### Laboratory Period Two

aluminum foil	5.0-mL conical vial <sup>†</sup>
2 boiling chips	copper metal sponge
distillation glassware, elastomeric connectors assembly <sup>‡</sup>	25-mL filter flask, with vacuum tubing
distilling column	filter paper
distilling head	10-mL graduated cylinder
with air condenser	Hirsch funnel, with adapter
elastomeric connectors	1000-μL micropipet
receiver vial	microspatula
5-mL round-bottom flask	Pasteur pipet, with latex bulb
−10 to 260 °C thermometer, with adapter	25-mL round-bottom flask
distillation glassware, Hickman still assembly <sup>‡</sup>	50-mL round-bottom flask
5.0-mL conical vial	sand bath, with −10 to 260 °C thermometer <sup>§</sup>
Hickman still head	support ring
10-mL round-bottom flask	support stand
−10 to 260 °C thermometer, with adapter	2 utility clamps

\*for warm-water bath

<sup>†</sup>or 10-mL Erlenmeyer flask

<sup>‡</sup>use glassware provided by your laboratory instructor

<sup>§</sup>sand in crystallizing dish on electric hot plate or sand in electric heating well with heat controller

### Reagents and Properties

substance	quantity	molar mass (g/mol)	bp (°C)	d (g/mL)
calcium hydroxide, sat. aq.	5 mL			
Celite <sup>®</sup> filter agent	0.5 g			
ethanol, 95%*		46.1	78.1	
disodium hydrogen phosphate	0.018 g	142		
sucrose	2 g	342.30		
yeast	0.18 g			
*product				

### Preview

#### Laboratory Period One

- Set up fermentation apparatus containing sucrose, sodium hydrogen phosphate, and yeast
- Set up trap containing saturated calcium hydroxide
- Allow the reaction to proceed for one week

#### Laboratory Period Two

- Add Celite<sup>®</sup> to the reaction mixture and conduct vacuum filtration
- Conduct simple distillation
- Measure volume and density
- Conduct fractional distillation
- Measure volume and density

**PROCEDURE** **Caution:** Wear departmentally approved safety goggles at all times while in the chemistry laboratory.  
Always use caution in the laboratory. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Avoid ingesting any of the reagents.

#### Laboratory Period One

##### 1. Setting Up the Fermentation

**Caution:** Sodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) is irritating and hygroscopic. Calcium hydroxide,  $\text{Ca}(\text{OH})_2$ , is corrosive.

Place 2 g of sucrose into a 25-mL Erlenmeyer flask. Add 10 mL of distilled or deionized water. Swirl the flask to dissolve the sucrose.

Add 0.18 g of dried baker's yeast. Swirl the flask to distribute the yeast throughout the solution.

Add 0.018 g (18 mg) of  $\text{Na}_2\text{HPO}_4$  to the mixture. Swirl the flask to dissolve the salt.

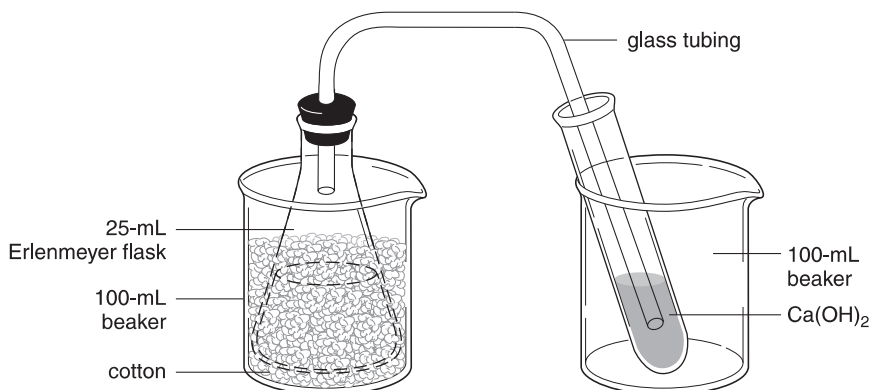
Using Figure 4 as a guide, insert bent-glass tubing into a one-hole rubber stopper. Close the fermentation flask with the stopper.

Half fill a 13 × 100-mm test tube with saturated aqueous  $\text{Ca}(\text{OH})_2$  solution. Place the tube into a 100-mL beaker. Insert the long end of the glass tubing into the test tube so that the tube extends 2–3 cm into the solution. [NOTE 1]

Prepare a 30 °C warm-water bath using a 250-mL beaker.

**NOTE 1:** The  $\text{Ca}(\text{OH})_2$  trap will prevent oxygen from entering the fermentation flask and oxidizing ethanol to acetic acid. As fermentation proceeds,  $\text{CO}_2$  will bubble through the  $\text{Ca}(\text{OH})_2$ , forming  $\text{CaCO}_3$ .

**Figure 4** Microscale fermentation apparatus



**NOTE 2:** The optimal temperature range is 30–35 °C. Temperatures higher than 35 °C may kill the yeast.

## Laboratory Period Two

### 2. Filtering the Fermentation Mixture

**NOTE 3:** The Celite® filter agent will prevent the yeast cell debris from clogging the filter paper and thereby slowing the filtration process.

### 3. Conducting a Simple Distillation

**NOTE 4:** If you use a 5-mL round-bottom flask, conduct the distillation in two batches. Then combine the two distillates.

Line a second 100-mL beaker with cotton to form an insulated nest for the flask.

Warm the flask to 30 °C in the warm-water bath. Dry the outside of the flask and place it in the cotton nest. Place the apparatus in a warm area of the lab for one week. [NOTE 2]

When the fermentation is complete, add 0.5 g of Celite® filter agent to the flask. Swirl the flask vigorously to wet the Celite® and distribute it throughout the solution. [NOTE 3]

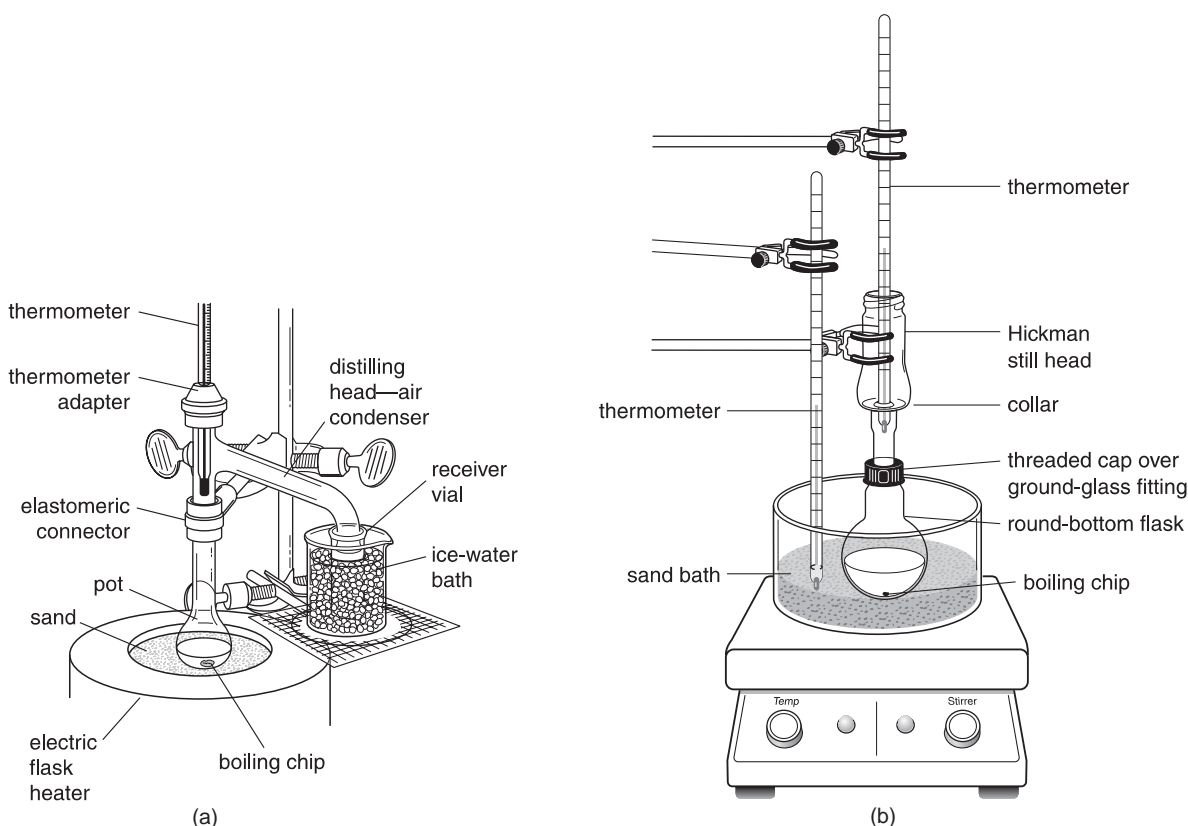
Set up a vacuum filtration apparatus using a Hirsch funnel and a 25-mL filter flask. Wet the filter paper with distilled or deionized water and turn on the aspirator.

Swirl the fermentation flask and pour the suspension into the Hirsch funnel. Continue swirling and pouring until the entire suspension is filtered. Rinse the flask with 1 mL of distilled or deionized water and add the rinse to the funnel.

Set up a simple distillation apparatus using a 5-mL round-bottom flask or a 10-mL round-bottom flask as the pot, as shown in Figure 5(a) or Figure 5(b).

Pour the filtrate from the filter flask into the pot. [NOTE 4] Add a boiling chip. Distill the solution at a rate of one drop per second.

Collect the fraction that boils *below* 100 °C. If you use a Hickman still, use a Pasteur pipet to transfer the distillate to a 10-mL graduated cylinder.



**Figure 5** Simple distillation apparatus using (a) glassware with elastomeric connectors or (b) Hickman still

When the temperature reaches 100 °C, remove the heat. Allow the apparatus to cool. Discard the pot residue.

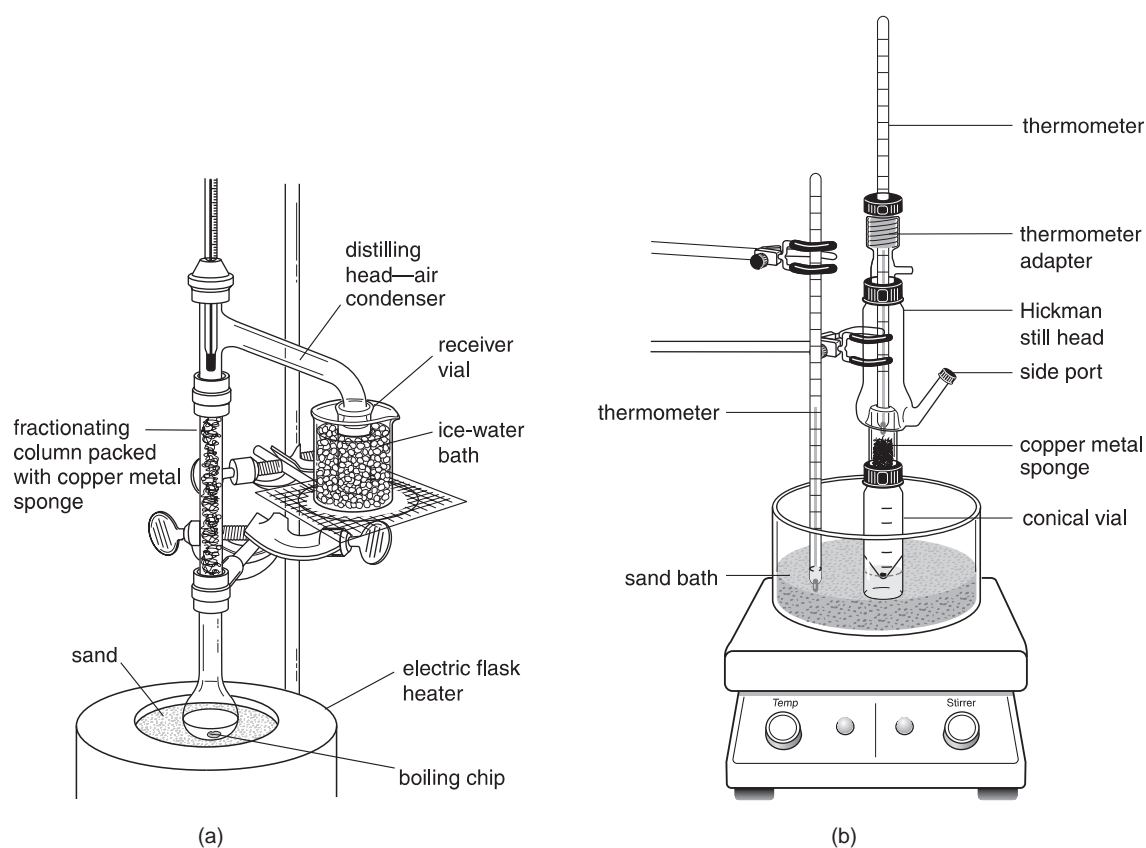
If you use a 5-mL round-bottom flask, add the other half of the filtrate into the pot. Again distill the solution, collecting the fraction that boils *below* 100 °C.

Use a 10-mL graduated cylinder to measure the total volume of distillate. Record the volume.

Tare a 5.0-mL conical vial or a 10-mL Erlenmeyer flask. Using a 1000- $\mu$ L micropipet, transfer 1.000 mL (1000- $\mu$ L) of distillate into the vial (flask). Weigh the vial (flask) to the nearest 0.001 g. Record the result in your laboratory notebook.

#### 4. Conducting a Fractional Distillation

Assemble a fractional distillation apparatus using a 5-mL round-bottom flask or a 5.0-mL conical vial as the pot, as shown in Figure 6(a) or 6(b).



**Figure 6** Fractional distillation apparatus using (a) glassware with elastomeric connectors or (b) Hickman still

Pack the fractionating column or Hickman still stem with copper metal sponge. Wrap the fractionating column with an aluminum foil jacket so that the foil can be opened on the front of the column.

Transfer the distillate from both the 10-mL graduated cylinder and the vial (flask) into the pot. Add a boiling chip.

Heat the pot, *gradually increasing* the heat to *slowly* raise the vapor line up the fractionating column, allowing time for equilibration of vapor and condensate. As the vapor line rises, close the foil jacket below the vapor line.

Distill the ethanol at a rate of one drop per 2–3 s. Collect the fraction that distills from 77–80 °C. Measure and record the final volume.

Repeat the density measurement described in Part 3. If the yield is less than 1.0 mL, perform the density measurement with 0.5 mL (500 µL).

- 5. Cleaning Up** Use the labeled collection containers provided by your laboratory instructor.

**Caution:** Wash your hands thoroughly with soap or detergent before leaving the laboratory.

### Post-Laboratory Questions

1. Calculate the densities of your simple and fractional distillates, using Equation 3. Make certain that you subtract the mass of the device, unless you tared the device on the balance. Show your calculations.

$$\text{density, g / mL} = \left( \frac{\text{distillate mass, g}}{\text{distillate volume, mL}} \right) \quad (\text{Eq. 3})$$

2. Use a graphing program to generate standard curves like those shown in Figure 1. Use the data table provided by your laboratory instructor. Plot density versus mass percent ethanol; plot mass ethanol per 10 mL versus mass percent ethanol.
3. Calculate your initial ethanol yield from fermentation, both as mass percent ethanol and total grams, using the simple distillation volume, the distillate density, the standard curves from Post-Laboratory Question 2, and Equation 2. Show your calculations. [NOTE 5]
4. Calculate your final ethanol yield from fermentation, both as mass percent ethanol and total grams, using the fractional distillation volume, the distillate density, the standard curves from Post-Laboratory Question 2, and Equation 2. Show your calculations. [NOTE 5]
5. Use the results of Post-Laboratory Questions 3 and 4 to calculate the percent recovery of ethanol, using Equation 4. Show your calculations.

$$\text{percent recovery, \%} = \left( \frac{\text{mass ethanol after fractional distillation, g}}{\text{mass ethanol after simple distillation, g}} \right) (100\%) \quad (\text{Eq. 4})$$

6. Calculate your percent yield for fermentation, using Equation 5. Recalculate your theoretical yield from Pre-Laboratory Assignment 5 using the actual mass of sucrose you used to start the fermentation. Show your calculations.

$$\text{percent yield, \%} = \left( \frac{\text{mass ethanol after simple distillation, g}}{\text{theoretical mass ethanol produced, g}} \right) (100\%) \quad (\text{Eq. 5})$$

**NOTE 5:** If you do not generate the standard curves in Post-Laboratory Question 2, use the standard curves in Figure 1.

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NAME

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SECTION

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DATE

SYNT 740/Preparing Ethanol by Fermentation

### **Pre-Laboratory Assignment**

1. What is the purpose of the  $\text{Ca}(\text{OH})_2$  trap?
2. What is the purpose of Celite<sup>®</sup>?
3. Why must the vapor line in the fractional distillation rise slowly?
4. What quantity of ethanol is in an 8-mL distillate with a density of 0.812 g/mL? Show your calculations.

5. What is the theoretical yield of ethanol produced by the fermentation of 40 g (or 8.0 g or 2.0 g) of sucrose? Using Equation 6, insert the mass corresponding to the scale assigned by your laboratory instructor. Convert the moles to grams using Equation 7. Show your calculations. Record your results here and in your laboratory notebook.

$$\text{theoretical yield of ethanol, mol} = \left( \frac{\text{mass sucrose used, g}}{\text{g sucrose per mole}} \right) \left( \frac{4 \text{ mol ethanol}}{1 \text{ mol sucrose}} \right) \quad (\text{Eq. 6})$$

$$\text{grams of ethanol, g} = (\text{mol ethanol}) \left( \frac{46.1 \text{ g}}{1 \text{ mol ethanol}} \right) = \text{g} \quad (\text{Eq. 7})$$