

FOOD FERMENTATION TECHNOLOGY (LAB MANUAL)

Name _____

Roll No. _____



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1. Root Beer Production

Introduction:

In this laboratory you will be demonstrating the action of yeast on a mixture of sugar, water and flavorings through both aerobic and anaerobic respiration. Yeast are microbes that will break down sugar into alcohol and carbon dioxide--the latter causing the root beer to become carbonated. The oxygen present will eventually be depleted, causing the yeast to revert to anaerobic respiration. Be sure to follow aseptic technique and use only food-grade containers for this experiment. You will be responsible for making a control sample following the formula below and at least one other batch of root beer in which you have changed only one factor. You need to hypothesize what differences your experimental group will have as compared to your control. You must submit a proposal on what your experimental group(s) will entail before beginning this laboratory work.

Materials:

- Bottles--washed and sterilized
- Wine corks or caps and crowns
- Stirring spoon
- Large (20 liter or 5 gallon) enamel kettle or pot--DO NOT USE ALUMINUM!!!
- *59 mL Schillings root beer concentrate
- *2.27 kg sucrose (table sugar)
- *19 liters chlorine-free water
- *Containers to measure out needed volumes of materials--your choice--see procedures
- *9.5 g yeast dissolved in 236 mL warm water
- *see caution 2 below

Cautions:

- Wash hands thoroughly with antibacterial soap and water before and after completing each step of the laboratory.
- Alter the volumes and measures (by using proportions) to best suit your needs.
- Wear goggles and laboratory apron.

Control Group Procedure:

Day 1

- I. Make sure all materials, equipment, and your hands are as clean as possible. Wash hands before handling any materials.
- II. Place sucrose and root beer extract into kettle.
- III. Gradually add chlorine-free water
- IV. Add yeast/warm water mixture and stir well.
- V. Immediately place mixture in clean/sterile bottles, leaving approximately a 5 cm airspace at the top.
- VI. Tightly cork or seal the bottles and place them on their sides in a warm (25-30 degrees celsius) location.

Day 2

I. Record observations

Day 3

I. Record observations

Day 4

I. Once refrigerated, your root beer should be ready for consumption

Reference:

Nancy Heitel, et. al., "Production of Home Brewed Root Beer," Mankato State University, Mankato, MN, 1988

2. 2. Make Your Own Sauerkraut

Introduction:

Sauerkraut is naturally fermented cabbage. Natural fermentation is one of the oldest means of food preservation and reduces the risk of foodborne illness and food spoilage. The juice extracted from shredded cabbage by adding salt* contains fermentable sugars, and in the absence of air, the microorganisms feed on the cabbage leaves and will produce lactic acid. This lactic acid creates an acidic environment unsuitable for other organisms to survive. In this lab you will make your own sauerkraut.

*The salt used should be a non-iodized pickling or canning salt. Iodine, which is in table salt, prevents the bacterial fermentation necessary to change cabbage into sauerkraut.

Materials:

- Cabbage sliced into thin strips
- Non-iodized salt
- Container (Consider the fact that cabbage will require anaerobic conditions while fermenting in this container. If a fermenting crock is your container of choice, be careful that it is not chipped or cracked. Food-grade sturdy plastic pails are excellent containers. Do not use metal containers of any type.)

Procedure:

- I. Clean off cabbage head to remove residual insecticide spray or dust. There are important bacteria existing on the cabbage leaves which are necessary in the fermenting process. Therefore, do not overclean the cabbage.
- II. Cut, slice or shred the cabbage.
- III. Place cabbage in container and sprinkle with salt. Add 2.25 to 2.5 percent salt by weight.
- IV. Cover and weight down the cabbage to produce anaerobic conditions for the fermenting process to take place.
- V. Set the cabbage back and allow fermentation process to proceed. Remember the effects temperature can play on fermentation.
- VI. Check the container daily for film yeasts or molds which may appear on the surface. This can be removed by skimming the surface of the cabbage. Kraut should be ready in 3 to 4 weeks.

Reference:

Mennes, Mary E., "Make Your Own Sauerkraut," Food Science, University of Wisconsin - Madison, Food Management Specialist, UW-Extension

3. Fermenting Power of Bread Yeasts

Introduction:

Bread dough is usually leavened by baker's yeast (actively gas-producing strains of *Saccharomyces cerevesiae*). Yeasts ferment the sugar in the dough, producing ethanol and carbon dioxide. CO₂ is the leavening agent and the alcohol evaporates off during baking. Sometimes other gas-producing microorganisms are involved in bread leavening--these usually are heterofermenting lactic acid bacteria (sourdough bread or salt-rising bread).

Commercial yeast is prepared and sold in two forms: yeast cakes and active dry yeasts. Yeast cakes contain, in addition to yeast cells, small amounts of starch, vegetable oils, and some lactic acid bacteria. Active dry yeast is made by drying the yeast cells to less than 80% moisture. These yeast cells are dried carefully at low temperatures so the cells will survive. When these yeast cells are stored at room temperature, they will retain their "dough-raising" ability for many months.

You will be studying the difference between the active dry yeast and the yeast cakes.

Materials:

- 50 or 100 mL graduated cylinder (to measure 30 mL distilled water)
- 100 mL graduated cylinder, greased with vaseline
- Flour
- Two brands, A and B, of active dry yeast (either A or B should be a yeast cake)
- *Saccharomyces cerevesiae*, young streak culture (solid medium)
- Square sheets of brown wrapping paper
- Buffered methylene blue stain:
 - Mix 1 part of 1:5000 methylene blue and 1 part of a phosphate buffer solution (99.25 mL of 0.2 M KH₂PO₄ to 0.25 mL of 0.2 M Na₂HPO₄) to give pH or 4.6
- Microscope
- Microscope slides and coverslips

Procedure:

A. Examine yeast cells

- prepare wet mounts of yeasts
- stain slides using methylene blue stain
- microscopically examine yeast cells
- record the percent of living cells (will appear white)
- record the percent of dead cells (will appear blue)

B. Fermenting ("dough-raising") power of yeasts

- combine 50 g flour, 1 g dry yeast A, and 30 mL water on brown wrapping paper--knead vigorously for 5 minutes
- repeat step above using yeast B
- place into separate greased cylinders and record volume
- incubate at room temperature and record volume at various time intervals

References:

- Boyd, Robert F. 1988. General Microbiology 2nd ed. Times Mirror/Mosby College Publishing, St. Louis
- Frazier, W.C., and D.C. Westhoff. 1978. Food Microbiology, 3rd ed. McGraw-Hill, Inc., New York
- Gula, Dr. Mary M., Oklahoma State University, Dept. of Botany/Microbiology, Stillwater, OK 74.78-0289

4. Making Yogurt

Introduction

Yogurt production demonstrates fermentation by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Heated milk is inoculated and maintained at a given temperature causing bacteria to grow and ferment lactose, the sugar in milk. The bacteria produce lactic acid which causes the milk to coagulate and adds a sour flavor.

Be sure to follow aseptic technique and use only food-grade containers for this experiment. You must submit a proposal on what your experimental group(s) will entail before beginning this laboratory work.

Materials:

- Food-grade containers, washed
- Food-grade thermometer
- Hotplate
- Beaker tongs
- 2 cups milk
- 1/3 cup nonfat dry milk
- 2 tablespoons plain yogurt (Old Home Black Label works well)
- pH paper or meter
- Microscope slides
- Bunsen burner
- Inoculating loop (a toothpick will work)
- Crystal violet stain
- Microscope (oil immersion if available)

Procedure:

- I. Combine milk with nonfat dry milk and heat in a double boiler to 190 degrees F. Hold at that temperature for 10 to 20 minutes so that the protein in the milk mixture will take up more water and make a better gel. Cool to 115 degrees F (warm) and record the pH of the mixture.
- II. Place the plain yogurt in a jar and gradually blend in the warm milk.
- III. Cover. Place in a bowl of warm water (115 degrees F), a slightly warm oven or a styrofoam cooler. The temperature within the oven or cooler should be about 110 to 120 degrees F to provide optimum conditions for yogurt culture activity.
- IV. Allow to stand undisturbed until the mixture is firm when the jar is gently wiggled. This may take as long as 6 to 8 hours. Note the time so that less care will be needed for the next batch. Record the pH of the yogurt.
- V. Chill yogurt as soon as it is set. It can be stored in the refrigerator for up to 3 weeks.
- VI. Place a drop of water on the slide. Use the inoculating loop to mix a little yogurt with the water and spread it around the middle 1/3 of the slide.
- VII. Let the slide air dry.
- VIII. Quickly pass the slide through the flame of the Bunsen burner 3 to 4 times.
- IX. Let the slide cool.
- X. Cover the slide with 2 to 3 drops of crystal violet stain.

- XI. After 30 seconds, rinse the slide with water.
- XII. Examine the slide under the microscope and draw the bacteria.
- XIII. Add 2 tablespoons sugar and 1/4 cup fresh, crushed or frozen fruit to the yogurt.
- XIV. Taste your yogurt. *(Do not eat any yogurt that smells or looks bad! If in doubt, throw it out!)*

References:

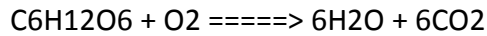
- "Bacteria in Yogurt" pages 44-47 of Laboratory Experiments in Biotechnology and Related Areas Volume III: Experiments with Microorganisms. Dept. of Biological Sciences, Mankato State University, Mankato, MN, 1988
- "Bacteria in Yogurt" pages 5-6 of The BioNet Booklet, Volume 3, Spring 1995, BioNet c/o Rod Johnson, Eau Claire North High School, 2700 Mercury Ave, Eau Claire, WI 54703
- "Homemade Yogurt, Sour Cream and Buttermilk" by M. Wagner, R. Bradleu, and M. Mennes, University of Wisconsin Extension publication B2768, available from Agricultural Bulletin, Rm 245, 30 N. Murray St., Madison, WI, 53715, 608-262-3346

5. Yeast Fermentation Lab

Introduction:

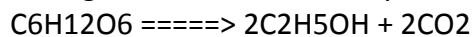
During anaerobic conditions high levels of NADH develop, leaving a shortage of NAD⁺. Low levels of NAD⁺ slow the rate of glycolysis. Fermentation restores NAD⁺ levels while producing alcohol and CO₂.

During aerobic respiration glucose is broken down into water and carbon dioxide.



Under ideal conditions most eukaryotic cells produce 36 ATP molecules from one molecule of glucose.

During fermentation baker's yeast breaks glucose into ethyl alcohol and carbon dioxide



This process only yields 2 ATP per glucose molecule.

Materials:

- 15 mL plastic centrifuge tubes with caps
- 7% yeast solution (4 packages yeast and 400 mL water)
- 5% glucose solution (20 grams glucose in 400 mL water)
- TES-TAPE (available at Wal-Mart and pharmacies)
- Large beakers (250 to 500 mL)
- Water bath at 40 degrees C
- Permanent fine point lab markers

Prelab preparation:

- I. Poke 3 to 4 small holes in the centrifuge tube caps using a pin or thumbtack.
- II. Prepare the yeast solution by mixing 4 packages active dry yeast in 400 mL tap water.
- III. Prepare the sugar solution by mixing 20 grams glucose in 400 mL tap water.
- IV. Preheat the water bath and the solutions to 40 degrees C.
- V. Fill beakers with water and place in the water bath to preheat.
- VI. Fill beakers with water and leave at room temperature.

Procedure:

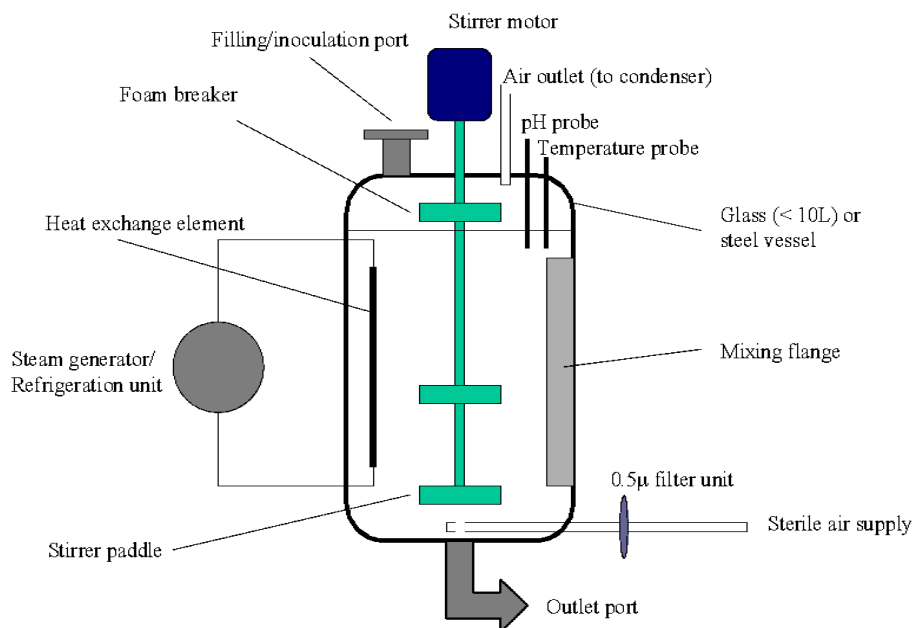
- 1 Fill each tube halfway with sugar solution
- 2 Fill the rest of each tube with yeast solution, extending the fluid level above the top of the tube
- 3 Take a small piece of TES-TAPE and measure the amount of glucose
- 4 Screw the cap on the tubes (a few drops will spurt out the holes)
- 5 Check to make sure there are no bubbles visible in the tube
- 6 Invert the tubes and mark two tubes L and the other two tubes C
- 7 Place one L tube and one C tube in each beaker
- 8 Return the 40 degree C beaker to the water bath
- 9 After 5, 10, 15, and 20 minutes...
 - a. take the L tubes out of the water
 - b. dry the tops with a paper towel

- c. mark the level of gas bubbles (include any foam as part of the bubble)
- d. return the L tubes to the beakers
- e. take the C tubes out of the water
- f. turn the tubes upright and remove the caps
- g. take two small pieces of TES-TAPE and measure the amount of glucose
- h. return the tubes are return to the beakers
- 10 After 20 minutes, empty the contents of all tubes into the waste beaker
- 11 Record the mL of CO₂ at each mark
- 12 Wash and rinse out your tubes
- 13 Graph the change in glucose and CO₂ over time in your journal
- 14 Answer the following questions in your journal
 - a. Why did you have two tubes at each each temperature?
 - b. What was the variable in this experiment?
 - c. What was the gas that formed in the tubes?
 - d. Describe an experiment that you could use to test if a different type of sugar would give the same results.

Reference:

"Fermentation, Respiration, and Enzyme Specificity: A Simple Device and Key Experiments with Yeast," by L. Reinking, J. Reinking, and K. Miller, The American Biology Teacher, Vol. 56, March 1994, pp. 164-168.

The essential components of a fermentor



6. Study of Fermentor

What is a fermentor?

An apparatus that maintains optimal conditions for the growth of microorganisms, used in large-scale fermentation and in the commercial production of antibiotics and hormones.

Biofermenters

40 to 120 liters
mobile pilot plant
probes for:
pH
dissolved O₂
temperature
agitation

new brunswick scientific
<http://www.nbsc.com/>



Biofermenters

75 to 500 liters

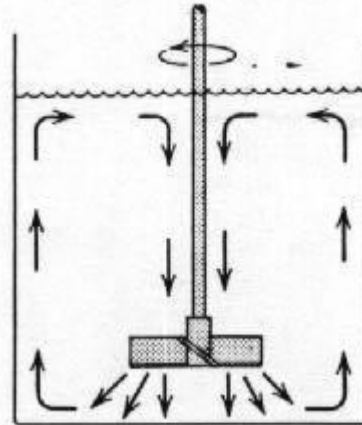


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What are different parts of a fermentor?

Agitators/ Impellers-

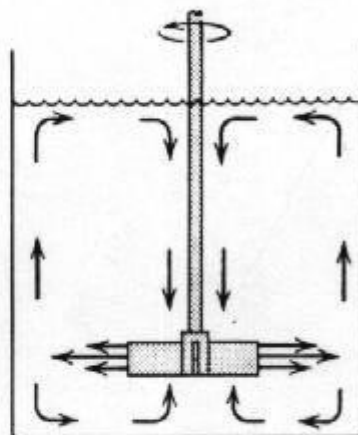
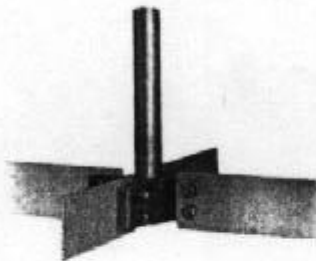
Two types of impellers are commonly used in industrial fermentation processes. Propeller agitators resemble marine propellers, with the main difference being that in industrial use, the vessel remains stationary while the fluid moves. Axial impellers are similar to radial ones, except that the blades are pitched, generally at a 45 degree angle. This causes flow to move downward parallel to the shaft, and then up along the wall of the tank. They are generally installed vertically in a tank, so as to allow for the fluid to circulate in one direction along the axis, while going in the opposite direction along the walls. Propellers are generally only employed in small-scale applications where their flexibility is desirable. They are often used to disperse gases or non-wetting solids in liquids due to the deep vortex they are able to create. They generally have diameters of not greater than 1.5 meters, and are characterized by their high rotational speed. They may range in power from those of laboratory size up to 50 kW. This type of impeller also serves as a means to achieve bulk mixing and produce a homologous sample. The accompanying figure shows both a picture and the circulation pattern associated with this type of impeller.



(A)

Propeller Agitators

The second type is a turbine impeller. Turbine impellers are mounted on shafts like propellers, but are usually much larger, and rotate at slower speeds. Turbines, because they are available in many impeller designs, are more flexible and efficient than propellers in several ways. Radial-flow impellers are similar to centrifugal pumps, in that they discharge liquids at high velocities in the radial direction. This acts like a jet mixer, causing entrainment of the surrounding fluid, while setting up two circulation systems. One of these is above the impeller, the other below. Liquid flowing outward separates at the wall, with some flowing up to the surface and returning to the eye of the impeller along the shaft. The remainder flows down along the wall, across the bottom of the tank, and returns back to the center of the impeller. Practical turbine impellers are limited to approximately 5 meters in diameter, translating to multiple tanks or multiple agitators necessary for larger plant capacities. In Fermentation plants, these impellers serve the purpose of creating mass transfer in order to break up oxygen bubbles. The high shear associated with the impeller necessitates a power demand 10-15 times that of the propeller-type impeller. The accompanying figure again shows a picture and the circulation pattern associated with this type of impeller.



(a)

Turbine Impellers

Commercially Available Impellers

Smoothline Impeller
P-4 or Pitched Blade Impeller
S-4 or Straight Blade Impeller
HE-3 Impeller
SC-3 Impeller
Maxflo W Impeller

JP-3 Impeller
Maxflo WSE
Maxflo Mark II & III Impellers
ChemShear Impeller
D-6 or Rushton Impeller

CD-6 Impeller
BT-6 Impeller
Double Helical Ribbon Impeller
Single Flight Helical Ribbon with Screw
Anchor Impeller

Screw (Auger) Impeller

Top vs. Bottom Installed Agitators

Why use a bottom-installed agitator?

- Easier to get into for cleaning and maintenance purpose.
- Easier to open lid

Why use a top-installed agitator?

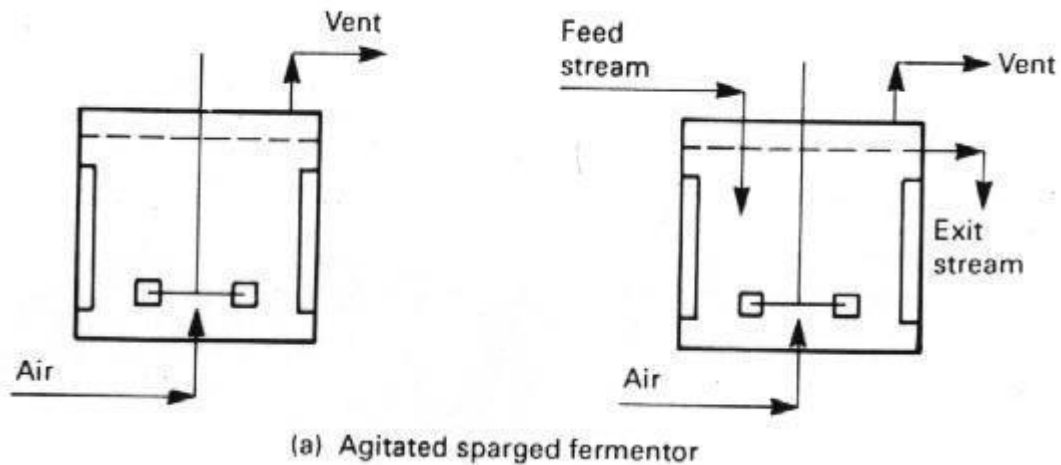
Less chance for contamination to occur due to a leaky seal.

What difference does it actually make?

If there is a problem with the seal on a bottom-installed agitator, the fermentation medium may leak out through the bottom of the tank, creating quite a mess, and most likely sacrificing the whole batch. If the same problem occurred in a top-installed agitator, the leak would not be serious enough to threaten the integrity of the batch. There would be no mess to clean, merely a seal to fix after completing the run.

Spargers/ Aerators

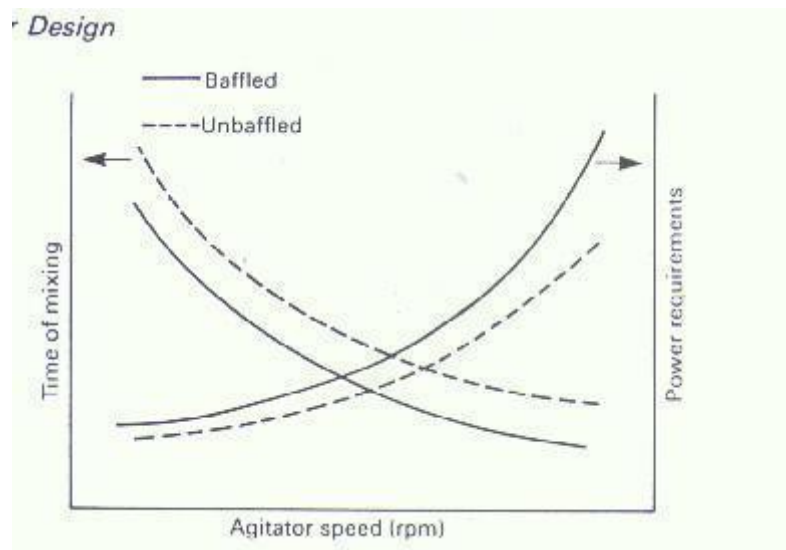
Spargers are a type of process equipment that introduces the smallest bubbles into a fermenter. The simplest type is known as a single-orifice sparger. As the size of the fermentation vessel increases, the gas requirements increase. To satisfy this requirement, the diameter of the orifice can be increased, but the goal is to keep bubbles as small as possible. Therefore, for larger aeration needs, the ring-sparger is incorporated. The ring-sparger has many holes, the sum of which equals the maximum anticipated air introduced into the fermenter. Each of these holes is therefore considered to be a critical orifice. Modern practice is to aim for a diameter that will deliver the air at about the speed of sound because that will tend to insure small bubbles. The Sparge line is located directly under the disk of the lower impeller so that the air stream is directed directly through the impellers.



Baffles

There is need for agitation in a fermentor for both aerobic and anaerobic operation. Liquids placed into cylindrical fermentation tanks containing centrally mounted agitators exhibit poor mixing characteristics if baffles are absent. Baffles reduce the time for reaching homogeneity. In other words, if a dye is poured into a region of the tank, it will be blended in less time when baffles are present. Their main drawback is the additional power required to operate the agitator. Baffles enable the impellers in the fermentation tank to deliver power to the fluid by preventing it from swirling.

The effects of baffles on agitation in a fermentor can best be seen in the accompanying figure below.



Agitation with and without baffles

Compressors

Compressors are necessary pieces of process equipment in industrial fermentation. They supply the fermentation tank with the air necessary to run the process. Most tanks require a compressor capable of delivering 300 to 400 hp. Plants may choose to use one, or several

compressors. For plants that choose a single compressor, the power level rises to 3000 to 4000 hp.

Single-compressor Plants

In single-compressor plants, the costs associated with the day-to-day operation are minimized. If costs were the only means by which process designs are accomplished, then the choice would be simple. There is risk involved with operation of only one, single compressor. If and when it breaks down, the processes that are already being process stand the chance of being compromised, as well as the inability to start new batches until the compressor is fixed.

Multiple-compressor Plants

The redundancy of having multiple compressors in the plant, or a compressor for each unit solves the problems associated with a single-compressor plant. The maintenance and equipment cost for such operation will be much greater than for the single-compressor.

Process control

Fermentor controls

- Temperature control
- Critical for optimum growth
- Aeration
- Related to flow rate and stirrer speed
- Dependent on temperature
- Mixing
- Depends on fermentor dimensions, paddle design and flanging
- Control aeration rate
- May cause cell damage (shear)
- pH control
- Important for culture stability/survival
- Foaming control

Temperature control

- Heat supplied by direct heating probes or by heat exchange
- Microbial growth is very sensitive to temperature changes - accurate temperature feed-back control is required
- Fermentations are exothermic – cooling may be required

Aeration

- Filtered air is supplied by forced airflow at the base of the fermentor
- The size of air bubbles is dictated by the air-supply tube hole diameter
- The time taken for air bubbles to rise to the surface (residence time) is dependent on bubble size and stirrer rate.
- The rate of oxygen dissolution is dependent on surface area (bubble size) and residence time
- The dO_2 (dissolved oxygen concentration) is dependent on the dissolution rate and the microbial uptake rate.
- To maintain constant dO_2 , an O_2 electrode is used to give feed-back information to the air-supply pump

Mixing

- Efficient mixing is critical for:
 - Homogeneous distribution of nutrients
 - Even temperature distribution
 - Rapid pH adjustment
 - Retention of air bubbles
- Different designs of stirrer blades give different circulation patterns
- Mixing is assisted by the presence of flanges on the fermentor walls
- Stirrer tip speed dictates the degree of shear stress
- Some cells types are very susceptible to shear stress
- Excessive stirring can promote foaming

pH control

- Most cultures have narrow pH growth ranges
- The buffering in culture media is generally low
- Most cultures cause the pH of the medium to rise during fermentation
- pH is controlled by using a pH probe, linked via computer to NaOH and HCl input pumps.

Foaming

- Foaming is caused when
 - Microbial cultures excrete high levels of proteins and/or emulsifiers
 - High aeration rates are used
- Excess foaming causes loss of culture volume and may result in culture contamination
- Foaming is controlled by use of a foam-breaker and/or the addition of anti-foaming agents (silicon-based reagents)

Causes of Foaming

I.Surface-active cell products

II. Nutrient Limitation

III. Cells running out of nutrient break open and lyse

Control of Foaming

To inhibit conditions which lead to foaming:

- Manipulate agitation and aeration -> lower power by dropping agitation speed or aeration
- Raise back pressure

To keep surface-active cell products from causing foaming:

- Add anti-foam e.g. (polymeric glycol, oil (lard oil), silicone-based antifoam)
- To prevent production of foaming gases, keep cells in good health by working on the nutrient feed strategy.

Sterilisation

- The fermentor and all additions (medium, air) must be completely sterile
- Sterilisation is performed by:
 - Small fermentors (<10L); autoclaving
 - Large fermentors;
 - Vessel: steam sterilisation; gas (ethylene oxide)
 - Medium: autoclaving or ultrafiltration
 - Air: ultrafiltration

Maintenance of Fermenter

IV. The fermenter vessel is sterilised as usual (e.g. 30 minutes at 120°C) and then cooled off.

V. Generally, acid, base and the other solutions are sterilised in Pyrex flasks, together with the degassing filters and tubes etc., simultaneously with the fermenter vessel. All these elements have then to be protected from contamination as customary.

VI. Due to the narrowness of the vessel, it is important that it be supported adequately during sterilization.

VII. Use the magnetic support for the supply bottles. The vibrations resulting from the mixer could otherwise start moving them.

VIII. The overheating of the regulation gate could damage the valve. An automatic protection will switch off the valve and change the selected air flow to zero. So don't take manual option for this.

IX. Fermenter does not need any special maintenance. Keep the fermenter clean. Clean it with a humid cloth. Common detergents or ethanol can be used as well.

X. If a liquid or saline solution gets into the back side of the fermenter (power supply), pull out the line cord **immediately** and contact expert mechanic.

Equipment Inspection:

Bottling bucket spigots use a rubber gasket to seal tight. Rubber will dry out and in this case can wear out. Remove the spigot from the bucket and inspect it.

Fit the spigot back on to the bucket and test for leaks.

Remove and inspect the air lock grommet on plastic fermenter lids (rubber again).
The lid should also be tested for wear.
Fill the fermenter half way with water. Attach the lid and fit the air lock in to place.
Plug the air lock with a cork or tightly wrapped piece of cloth. Turn the fermenter upside down and check for leaks.
If it leaks, try to determine if the problem is with the lid or the rim of the bucket.
Replace the faulty part.
Plastic fermenters and bottling buckets need to be inspected for scratches. A tiny scratch on the inside of the pail can hold millions of beer loving bugs, even through the sanitation process.

7. Alcohol Content (with hydrometer)



Homebrewer using a hydrometer (floating in the wort) and thermometer (clipped to the rim of the bucket)

Alcohol content is determined by measuring the specific gravity before and after fermentation by use of a hydrometer. Using a hydrometer, the original gravity (OG) of the wort is measured at 60°F before the admixture of yeast. After fermentation, a second reading is taken for final gravity (FG). These values are used in the following expressions to determine alcohol by weight (ABW) and alcohol by volume (ABV):

$$\%ABW = 76.08 * \frac{OG - FG}{1.775 - OG}$$

$$\%ABV = ABW * \frac{FG}{.794}$$

Specific gravity adjustments for different temperatures:

Temperature	50°F	55°F	60°F	65°F	70°F	75°F	80°F	85°F	90°F
Offset	0.0005	0.0003	0.00	0.002	0.012	0.018	0.026	0.033	0.04

$$\text{offset} = 1.313454 - (0.132674 * \text{Temperature}) + (.002057793 * \text{Temperature}^2) - (.000002627634 * \text{Temperature}^3)$$

Many homebrewing hydrometers offer a scale, in addition to specific gravity, called potential alcohol, which makes it much easier to estimate the alcoholic strength of the finished product. Potential alcohol represents what percentage would be in the brew if all the sugar were fully fermented. The homebrewer simply takes a before and an after reading, and notes the difference between them as the alcohol by volume.

Alcohol content without hydrometer

When using a typical malt extract home brew kit, plus sugar, the maximum possible ABV can be calculated, with the rider that if some sugar is left unfermented, the ABV will be lower.

$$MAX\%ABV = \frac{(S + .8M) * 11.39}{W} - .5$$

Where S = Weight of sugar in Kg

M = Weight of malt extract in Kg

W = Volume of wort in gallons

What is a Hydrometer?

A Hydrometer is a scientific instrument which measures the density of a liquid in relation to water (water being 1.000 on the Specific Gravity Scale). In the Brewing Industry, this instrument allows us to predict and finally measure the alcohol content of the beverage being made. Like many scientific processes, using a Hydrometer requires careful steps to insure accurate results. The steps involved are not difficult but do require concentration and consistency.

Using the Hydrometer

Place a sample of the liquid to be tested in the testing jar and gently lower the Hydrometer into the sample. Spin the Hydrometer until no air bubbles cling to the exterior of the instrument. Once the Hydrometer stops moving, take the "Original Gravity Reading" (before fermentation) on the Specific Gravity Scale. Be sure to take the reading according to the "True Reading" principle as shown in Figure 1. Use the "Original Gravity Reading" is accurate. Record the Results. Once the beer or wine has fermented, it is now time to take the "Final Gravity Reading". To do this, repeat all previous steps and Record the Results. Using the following equation, the alcohol content of the beer or wine can be calculated.



Figure 1

$$(\text{Original Gravity Reading}) - (\text{Final Gravity Reading}) \times (131) = \% \text{ Alcohol Content}$$

Temperature Correction Chart

This hydrometer has been calibrated to give a 100% accurate reading at 60 degrees F. This means that if the temperature of the liquid being tested is other than 60 degrees F, the Temperature Correction Chart below must be used to obtain an accurate reading.

		<p>Correction Example</p> <hr/> <hr/>
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Helpful Hints-

Cleanliness

It is extremely important to be sure that the Hydrometer is clean and free of any dirt or debris. Be sure to sanitize the Hydrometer and test the jar after every use. Once the reading has been taken, it is best to discard tested liquid rather than risk contamination of the beer or wine.

Temperature

Since liquids become less dense at higher temperatures and more dense at lower temperatures, it is important to be consistent with the temperature corrections. Please refer to the chart on this page for more details.

Just note your liquid temperature and use the chart below to add or subtract to the decimal portion of your Specific Gravity reading to correct.

°F	Adjust
40	- .002
50	- .001
60	0
70	+ .001
80	+ .002
90	+ .004
100	+ .005
110	+ .007
120	+ .008
130	+ .010
140	+ .013
150	+ .015

8. Fruit Beer Production








Tips on making Fruit Beers Using Oregon Real Fruit Puree

Always use the fruit puree in the primary or secondary fermenter. Never use it in the boil. Boiling will set the pectin, insure a chill haze and change the flavor. You don't have to worry about getting an infection because the puree has been commercially sterilized.

When substituting your favorite fruit for the one a recipe calls for, keep in mind that a fruit like raspberry has a stronger flavor than apricot and make the necessary adjustments. A yeast with low attenuation will help preserve the residual sweetness of the fruit. Never dump your beer out because the fruit flavor is too strong. It will mellow with age.

Fruit Beer Recipes for 5 gallons

Raspberry Relief

-  7 LB. Munton's plain light dry malt
-  1/2 LB. 20L crystal malt
-  1-1/2 oz. Hallertau hops (boil)
-  1/2 oz. Hallertau hops (finishing)
-  46 oz. Oregon Raspberry Puree
-  Wyeast #1338 European Ale
-  1 tsp. Irish Moss

Steep crushed crystal malt in cold water while heating to boil. Remove grain before boil. Add malt extract and 1 1/2 ounces Hallertau hops and boil for 45 minutes. Add Irish moss for the last 20 minutes of boil. (hydrate the Irish moss in 1/4 cup water overnight). At 45 minutes, turn off heat and add aroma hops. Cool down with wort chiller and rack into primary fermenter. Take and record the initial gravity reading. When the wort reaches 70 degrees pitch the yeast. (We recommend using a yeast starter). After two days, rack into secondary fermenter and add fruit puree. Ferment for 7 to 14 days. When your hydrometer reading shows no change for two to three days, bottle. Use 1/2 to 3/4 cups priming sugar. Beer is drinkable in one month if you like "zippy" fruit beers., Aging 6 to 8 months will let the raspberry mellow

Options: Add 2 to 4 ounces lactose before bottling to mellow raspberries.

True Framboise

- 6 LB. light malt extract
- 2 LB. plain dry wheat malt extract
- 1/3 oz. ea Saaz, Fuggles, Hallertau
- 2 cans Oregon Raspberry Puree
- 1 oz. vanilla extract
- Wyeast #3278 Lambic Blend
- 3/4 cup corn sugar for priming

Boil extract and hops for 2 hours. This is the standard length of boil for Belgian style beer which removes the cheesy characteristics of the aged hops. Belgians age their hops 3 years. Original gravity should be 1048 to 1052. Fill a primary fermenter with cooled wort and pitch yeast and ferment at 63 degrees. After one week add raspberry puree and vanilla to a sterilized carboy and siphon wort on top. This ferment would normally be done in oak but

you may add 1/2 to 1 pound oak chips for a few days to one week to achieve an oak flavor. Check the oak flavor periodically to make sure it's where you want it. Ferment at 69 degrees for 2 weeks then, 62 degrees for one week. Rack and ferment 3 weeks at 62 degrees. Bottle and age for at least 8 months.

Dark Cherry Lambic

- 5 LB. Munton's plain light DME
- 2 LB. wheat malt extract
- 3-1/2 oz. malto dextrin
- 1 oz. Hallertau hops
- 1 can Oregon Dark Sweet Cherry Puree
- Wyeast #3278 Lambic Blend

Dissolve the light malt extract, wheat malt extract and malto dextrin in warm water. Bring to a boil and add hops. Boil for 45 minutes. Strain out hops. Add enough water to the fermenter to make 4.5 gallons and cool to 70 degrees. Pitch yeast, add fruit puree and ferment for 3 weeks Rack into secondary fermenter and condition for a week at 60-65 degrees. Bottle condition using corn sugar for priming.

Deep Blue Stout

- 7 LB. Munton's plain dark extract
- 1/2 LB. roasted barley malt
- 1/3 LB. black patent malt
- 1 LB. crystal malt
- 1 1/2 oz. Fuggles hops
- Wyeast #1084 Irish Ale
- 1/2 cup corn sugar (priming)
- 1 can Oregon Blueberry Puree

Steep grains in cold tap water and bring to a boil. Remove grains just before boil and add hops. Boil for 45 minutes. Cool and add water to fermenter to make 4.5 gallons. When wort reaches 70 degrees, pitch yeast. After 2-3 days transfer to secondary fermenter and add fruit puree. Ferment 7 to 14 days. Use 1/2 cup corn sugar to prime for bottling.

Peach Wheat Ale

- 7 LB. Munton's plain wheat DME
- 1-1/2 oz. Tettnang hops
- 1/2 oz. East Kent Golding hops
- 1 can Oregon Peach Puree
- 1/2 teaspoon Irish Moss
- Wyeast #1338 European Ale yeast

Bring water and extract mixture to a boil. Add Irish Moss to boil. Add Tettnang hops for a minimum 45 minute boil. Five minutes from end of boil, add Golding pellets. After boil, strain hops and cool to 70 degrees. You can add the puree now or when you rack beer into secondary fermenter. Add your yeast when wort is 70 degrees. After three days of strong fermentation, rack your beer to a secondary fermenter and add the puree if you have not already. Ferment for 7 to 10 days and bottle.

Blueberry Weizen

- 6.6 LB. Munton's plain wheat extract
- 1/2 LB. crystal malt
- 1 oz. Hallertau or Saaz hops (boil)
- 1 oz. Saaz (finishing)
- 1 teaspoon Irish moss
- 1 can Oregon Blueberry Puree
- Wyeast #3056 Bavarian Wheat

9. Vinegar Production

Vinegar is nothing more or less than an alcoholic beverage which has gone sour. In fact, that is exactly what the roots of the word mean, coming from the French *vin*, meaning wine, and *aigre*, meaning sour. When alcoholic beverages sour it is the act of certain bacteria, known as *acetobacter*, on alcohol, turning it to acetic acid and water. It is the alcohol gone acid which gives us the taste which we associate with vinegar. It is the other elements, specific to the actual source of the original alcohol, which give the vinegar its individual character and body. The information in the next two sections on the history and making of vinegar is drawn almost exclusively from the pamphlet 'Making Vinegar at Home'.

History of Vinegar

Vinegar has been around and in use for considerably longer than would be suggested if one only goes as far back as the introduction of the methods of production put forth in the next section. The use and production of vinegar probably goes back to a time not much more recent than that of the making of mead and wine, possibly by no more than a few months. Vinegar is mentioned in the Bible -- in the Book of Ruth and in Proverbs. It is also specifically called for in the making of *haroseth* in Pesachim, a section of the Talmud. Vinegar was known to the Egyptians and it was drunk by Caesar's armies. Hippocrates prescribed the drinking of vinegar for his patients in ancient Greece. It would appear that in all the places that we have seen the production of wine or beer in the ancient world, we also find the production of vinegar.

How Vinegar is Made

As mentioned above, the making of vinegar, in theory, is very simple. Make beer or weak wine and leave it out for the vinegar bacteria to attack it. In practice, this is not the best of methodologies, although the realities of a vinegar generator are not much more complex than this.

Note that I have said in the foregoing to leave the wine, or beer out for the bacteria to take hold. This is the first necessity. The *acetobacter* reaction, unlike that of yeast on sugar to make alcohol, is an aerobic reaction. It requires the presence of oxygen. The more oxygen, the better. Most of the improvements in vinegar production over the millenia during which it has been made and used by man have been in the form of finding better ways to get greater amounts of oxygen to the bacteria in a shorter period of time. The next necessity is to keep insects away from the acetifying must while allowing for the air flow. Put these two items together in a workable fashion and you have a vinegar generator.

One of the oldest actual methods for the production of vinegar is what has come to be known as the Orleans method. The vinegar generator used in this method is a large, wooden barrel laid on its side with the bung hole up. In each end of the barrel a hole, or holes, is drilled so that when the liquid in the barrel is just below these holes, the barrel will be about three-quarters full. The barrel is then filled to this point with beer or dilute wine and a starter of vinegar which has been untreated and still contains active mother of vinegar (another name for the vinegar bacteria). The holes in the ends are covered with a fine screen- ing, or loose cloth, to prevent the entrance of insects, and the generator is allowed to sit for several months. The optimum temperature for this conversion is about 85°F, or 29°C. After this resting time the alcohol has been almost entirely converted to vinegar and it

is drawn off through a spigot placed near the bottom of the barrel on one end, leaving about 15% behind to charge the next batch. The next batch would be added through the bung hole using a long funnel which would reach below the surface level of the charging vinegar. The reason for this is that a scum will form on the surface of the mash as it is converted to vinegar. This is a very active layer of acetobacter and forms on the surface, where there is the most oxygen (from contact with the air). While succeeding batches of vinegar will proceed even if this layer is broken up, they will get off to a much better start if this layer is left undisturbed.

More modern methods of production, as stated earlier, are designed to allow more oxygen to reach the acetobacter. The first of these methods was to use a larger generator and loosely pack it with a porous material, such as pomace (grape pulp, after pressing), or beachwood shavings. The mash was allowed to slowly trickle down onto these materials, thus greatly increasing the amount of surface area for the volume of mash. This allowed for much more rapid production of vinegar with better controls. Further improvements came with the addition of more holes in the generator, allowing for freer passage of air through the generator and the oxygen which it brought.

Vinegar generators grew in size, thus increasing the distance which the mash would travel over the porous materials and thereby increase the oxygen reaching the mash as well. The last of the advances which has been made only in much more recent times (circa 1952) is the use of submerged fermentation which consists of aerating the entire mash with tiny bubbles, much as an aquarium aerator would produce when attached to a pump and placed at the bottom of the generator. This method introduces oxygen to the entire volume of the mash at all times and can reduce the time necessary for conversion from several months, to several days. This is, however, quite out of the period of our study and if you wish to maintain period techniques you will be advised to adhere to the Orleans method and its early variations.

Uses of Vinegar

Vinegar has been used, both as a food, and also as a preservative of food. It has been prescribed, mixed with sugar or honey, as a gargle to be used as a remedy for sore throats. It can also be used as a cleaning agent, or furniture polish. It is not, however, to be recommended for the use of cleaning polished marble, as some suggest, as its acidity will eat away at the surface and leave it lightly pocked, causing it to lose its luster.

9.1. Cider Without Apples

To each gallon of cold water put 1 lb. common sugar, 1/2 ounce of tartaric acid, one tablespoonful of yeast, shake well, make in an evening and it will be fit for use next day. I make in a keg a few gallons at a time, leaving a few quarts to make into next time, not using yeast again until the keg needs rinsing. If it gets a little sour, make a little more into it or put as much water with it as there is cider and put it with the vinegar. If it is desired to bottle this cider by manufacturers of small drinks, you will proceed as follows: put in a barrel 5 gallons of hot water, 30 lbs. of brown sugar, 3/4 lb. of tartaric acid, 25 gallons of cold water, 3 pints of hop or brewer's yeast, work into paste with 3/4 lb. of flour, and one pint water will be required in making this paste; put all together in a barrel which it will fill and let it work 24 hours, the yeast running out at the bung all the time by putting in a little occasionally to

keep it full; then bottle, putting in two or three broken raisins to each bottle, and it will nearly equal champagne.

9.2. Spruce or Aromatic Beer

Take 3 gallons of water, 2 1/2 pints molasses, 3 eggs well beaten, 1 gill yeast, put into two quarts of the water boiling hot, put in 50 drops of any oil you wish the flavour of, or mix one ounce each, oil sarsafra, spruce, and wintergreen; then use the 50 drops. For ginger flavour take 2 ounces ginger root bruised and a few hops, and boil for 30 minutes in one gallon of the water, strain and mix all; let it stand 2 hours and bottle, using yeast, of course, as before.

9.3. Lemon Beer

To make 20 gallons, boil 6 ounces of ginger root bruised, 1/4 lb. cream-tartar for 20 or 30 minutes in 2 or 3 gallons of water; this will be strained into 13 lbs. of coffer sugar on which you have put 1 oz. oil of lemon and six good lemons all squeezed up together, having warm water enough to make the whole 20 gallons, just so you can hold your hand in it without burning, or some 70 degrees of heat; put in 1 1/2 pint hops or brewer's yeast worked into paste as for cider, with 5 or 6 oz. of flower; let it work over night, then strain and bottle for use. This will keep a number of days.

9.4. Philadelphia Beer

Take 30 gallons of water, brown sugar 20 lbs., ginger root bruised 1/4 lb., cream tartar 1 1/4 lb., carbonate of soda 3 ounces, oil of lemon 1 teaspoonful, put in a little alcohol, the white of 10 eggs well beaten, hops 2 ounces, yeast one quart. The ginger root and hops should be boiled for 20 or 30 minutes in enough of the water to make all milk warm; then strain into the rest, and the yeast added and allowed to work itself clear as the cider and bottled.

9.5. A Superior Ginger Beer

Take of sugar 10 lbs., lemon juice 9 oz., honey 1/2 lb., bruised ginger root 11 oz., water 9 galls., yeast 3 pints, boil the ginger in the water until the strength is all extracted, which you may tell by tasting the root, then pour it into a tub, throwing the roots away, let it stand until nearly luke warm, then put in all the rest of the ingredients, stir well until all dissolved, cover it over with a cloth, and if it be in the evening, let it remain until next morning, then strain through cloth, and bottle it, and in a short time it will be fit for use. Some use less sugar, and some less lemon juice, to make it with less expense; but it is not so elegant a drink as this.

9.6. Ginger Pop No. 1

Take of water 5 1/2 galls., ginger root bruised 3/4 lb., tartaric acid 1/2 oz., white sugar 2 1/4 lbs., the whites of 3 eggs well beat, a small teaspoonful of oil of lemon, yeast 1 gill; boil the root for 30 minutes in 1 gallon of the water, strain off, and put the oil in while hot, mix all well, make over night, in the morning skim, and bottle, keeping out sediment.

9.7. Ginger Pop No. 2

Take best white Jamaica ginger root bruised 2 oz., water 6 quarts, boil 20 minutes and strain, then add cream tartar 1 oz., white sugar 1 lb.; put on the fire, then stir until all the sugar is dissolved; then put into an earthen jar, now put in tartaric acid 1/4 oz., and the rind of 1 lemon, let it stand until 70 degrees of Fahrenheit, or until you can bear your hand in it with comfort, then add two tablespoonsful of yeast, stir well, bottle for use, and tie the corks; make a few days before it is wanted for use.

9.8. Improved English Strong Beer

If you have malt use it, if not, take 1 peck of barley, and put it into a stove oven, and steam the moisture from them, grind coarsely, and pour into them 3 1/2 gallons of water, at 170 or 172 degrees. (If you use malt it does not need quite so much water, as it does not absorb so much as the other. The tub should have a false bottom with many gimblet holes to keep back the grain.) Stir them well and let stand 3 hours and draw off, put on 7 gallons more water at 180 or 182 degrees, stir well, let stand 2 hours and draw off, then put 1 gallon or 2 of cold water, stir well and draw off; you should have about 5 or 6 gallons; mix 6 lbs., coarse brown sugar in equal amount of water, add 4 oz. of good hops, boil for 1 1/2 hour; you should have from 8 to 10 gallons when boiled; when cooled to 80 degrees, put in a teacupful of good yeast and let it work 18 hours covered with a sack. Use sound iron-hooped kegs, or porter bottles, bung or cork tight, and in two weeks it will be good sound beer, nearly equal in strength to London porter, or good ale, and will keep a long time.

9.9. Hop Beer

Take of hops 6 oz., molasses 5 quarts, boil the hops in water till the strength is out, strain them into a 30 gallon barrel, add the molasses and a teacupful of yeast, and fill up with water, shake it well and leave the bung out until fermented, which will be in about 24 hours; bung up, and it will be fit for use in about 3 days. A most excellent summer drink, smaller quantities in proportion.

Ginger Ale Observations

		A	B
Vikram Dogra	Taste	8	6
	Smell	8	6
	Colour	8	7
	Liking	8	6
Gaje Singh	Taste	5	7
	Smell	5	8
	Colour	6	8
	Liking	6	8
K S Ahlawat	Taste	8	9
	Smell	9	9
	Colour	8	9
	Liking	8	9
	Total	87	92