Yeast is arguably the most important ingredient in beer. Although the yeast itself is not often in evidence in the finished beer (with some notable exceptions) the flavors and aromas generated by the yeast are key sensory qualities. Many brewers go to great expense to accommodate difficult yeast strains to achieve their desired flavor profile.

Yeasts cover a huge range of functions in nature but there are only a few species we are concerned with in brewing. Brewer’s yeast is not found in nature: It is a domesticated microorganism. As a brewer you will keep it in the brewery and reuse it resulting in a controlled mutation. Unfortunately yeast, like all biological systems, follows the rules of chaos theory, and is inherently difficult to control. By applying some basic principles of yeast management you can make your yeast healthy and somewhat predictable.

**Yeast Structure**

Yeast cells have a structure that is in many ways similar to human cells. There are many of the same structures present, and the function of these structures (organs) is similar as well. A mature brewing yeast cell is 8-14μm in diameter, and weighs about 40 pg (pico grams, or 1 x 10^{-12} g).¹

**Cell Wall**

The cell wall forms the outermost layer of the yeast cell giving the cell shape. In addition to structure the cell wall has several other functions:

- Act as a barrier for yeast cell.
- Also acts as an osmotic barrier, increasing ethanol and sugar tolerance.
- Flocculation properties in some yeast strains, and also determines whether cell is top or bottom cropping.

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¹ Hough, Briggs, Stevens and Young

Diagram of an electron micrograph of a section through a resting cell of bakers' yeast (Saccharomyces cerevisiae). ER, endoplasmic reticulum; Mt, mitochondrion; N, nucleus; Nm, nuclear membrane; Nn, nucleolus; Pi, invagination; Pl, plasmalemma; V, vacuole; Vp, polymetaphosphate granule; W, cell wall; Ws, bud scar; L, lipid granule (sphaerosome).
The yeast cell wall is composed of:

- β-glucans 40%
- α-mannans 40%
- Proteins 8%
- Lipids 7%
- Mineral constituents 12%

The cell wall makes up about 30% of the total cell mass (dry weight). The outer surface of the cell wall has a high amount of phosphate and carboxyl groupings (side chains of the proteins) giving it an overall negative charge at the pH of beer.

The cell wall also contains several enzymes that are excreted from the cell. The most important of these is invertase, which can convert sucrose to glucose and fructose for yeast metabolism. Another enzyme found associated with the cell wall of some yeasts is melibiase (which can degrade the simple sugar melibiose), while not key in beer fermentation, it important for identification of yeast cells (called taxonomy, see Ale vs. Lager below for more information).

Cell Membrane

Inside the cell wall is a secondary protection for the cell, called the cell membrane, or plasmalemma. The cell membrane consists of a phospholipid bilayer, with proteins penetrating the membrane. These proteins add to the structural integrity of the membrane and many are used for transport of material across the membrane. The cell membrane not only surrounds the cytoplasm, but also connects with the other membranous organs inside the cell (such as the endoplasmic reticulum and the Golgi apparatus).

The cell membrane is key to uptake of nutrients for the yeast cell, and the proteins in the cell membrane facilitate this transport. The cell membrane must be fluid to allow transport of these nutrients, and as will be seen in Fermentation Biochemistry, aeration of the yeast at the beginning of fermentation is key in membrane fluidity.

There are four different types of transportation that can occur across the plasmalemma:

Passive (simple) Diffusion

Passive diffusion is the simplest form of transport into the cell, and requires no energy from the cell. Molecules simply pass from outside of the cell into the cytoplasm, directly through the membrane, or through a “pore” consisting of a protein bridging the lipid bilayer. The rate of diffusion depends on:

- Charge of the molecule (electrical charge prevents diffusion)
- Size (smaller molecules diffuse faster than larger ones)
- Lipid solubility (highly lipid-soluble molecules diffuse faster, since they can penetrate the hydrophobic portion of the lipid bilayer)
- Concentration gradient (the greater the concentration gradient, the faster the diffusion)
- Molecules always diffuse down the concentration gradient, meaning from areas with a high concentration of the molecule to a lower concentration.

Examples of molecules that can penetrate the cell by passive diffusion are:
Facilitated Diffusion

Facilitated diffusion or passive mediated transport is similar to passive diffusion, but requires a permease (carrier protein) to allow diffusion. This allows control of the diffusion, but the diffusion is still down the concentration gradient. In general facilitated diffusion is faster than simple diffusion, and no energy output by the cell is required. The permeases are specific to the structure of the transported molecules, and they are controlled depending on the needs of the cell. Examples of specific permeases will be given in fermentation biochemistry.

Molecules that enter the cell by facilitated diffusion include:
- Glucose
- Fructose
- Maltose
- Maltotriose
- Cl⁻, HCO₃⁻
Saturated
Stearate

Unsaturated
Oleate

Polyunsaturated
Linolenate

Desaturation
Active Transport

Active transport is the only mechanism for moving molecules up a concentration gradient. It is therefore critical to the health of the yeast cell, since there are many nutrients that must be “hoarded” inside of the cell, at a higher concentration than they exist outside the cell. Active transport also uses carrier proteins to transport molecules across the membrane, but they require energy to allow this transport. This energy may be in the form of ATP (adenosine triphosphate) or the energy of a Na⁺ gradient. The transport proteins are for specific molecules, or groups of molecules.

Molecules that enter the cell by active transport include:
- Amino acids (at differing rates)
- Ca²⁺ (if required in high concentrations)

Mitochondria

In most eukaryotic cells the mitochondria are the site of the electron transport system, Kreb’s cycle and ATP synthesis during respiration. During fermentation brewing yeast are not allowed to respire (due to the Crabtree effect) and therefore the mitochondria small and serve little function. If brewing yeast are exposed to oxygen in low glucose conditions, they will overcome the Crabtree effect and develop mitochondria within 6-8 hours. Mitochondria contain their own DNA, used in the synthesis of proteins for mitochondrial function. Mutations in the mitochondrial DNA may cause petite or respiratory deficient yeast.

Nucleus

In a normal yeast cell the nucleus is approximately 1.5 mm in diameter, and has a membrane similar to the plasmalemma (a lipid bilayer with some pores). The nucleus is the storage place of Deoxyribonucleic acid (DNA), the genetic material of the cell.

Endoplasmic Reticulum

The Endoplasmic Reticulum (ER) is a membranous structure found throughout the cytoplasm. The ER is important for channeling materials around the cell, and many metabolic sequences take place inside of the ER. The ER is also the site of most lipid synthesis inside of the cell including triglycerides, phospholipids and steroids. The Rough ER is covered with ribosomes, which are responsible for the production of proteins.
Golgi Apparatus

The Golgi apparatus is linked to the ER, and is responsible for continuing the synthesis of glycoproteins and lipo proteins. It also plays a role in secretions from the cell.

The Vacuole

Brewing yeast typically contain only one vacuole, a storage area for enzymes and nutrients. The vacuole is bounded by a single membrane, and typically contains several dense granules of phosphate material that will be used during fermentation. The vacuole contains hydrolytic enzymes that are used to recycle large molecules from the cell (RNA and protein for example). If the cell is subjected to conditions that destroy the vacuole (i.e. high temperature or pH), the vacuole will release its contents and the hydrolytic enzymes will destroy the cell (autolysis).

Yeast Life Cycle

Yeast reproduce by budding, and each yeast cell contains all the genetic material necessary to reproduce (called a diplophase, meaning they have two sets of chromosomes). The budding process leaves behind a bud scar on the mother cell, and once a yeast cell has too many of these scars, it will lose the ability to reproduce (a normal yeast cell can bud up to 50 times). During a normal fermentation the yeast will bud several times, resulting in a 3-5 fold increase in the total cell population (as each daughter cell will bud several times as well). It is important to keep this in mind when judging the how many “generations” the yeast can sustain and still avoid mutation.

Sources of Yeast

There are several options for finding the yeast you will use the brewery, and each varies in reliability and cost. One of the most common methods for smaller brewpubs is to get a pitching quantity of yeast from another brewery. You can probably get the yeast at a lower cost than from a laboratory, but there are no guarantees as to the quality of the yeast. One advantage to getting your yeast from another brewery is that you can easily get a pitching quantity, which will save you the hassle and risk of propagating in your
Yeast

Yeast

Brewery. In addition to problems with contamination and variation in yeast, your brewing cycle will also be tied to the other brewery.

The next option is to get your yeast from a qualified yeast laboratory, either a pitching quantity, or a smaller amount that you will propagate up in your brewery. Costs are much more substantial for a pitching quantity of yeast, and in many cases you may be forced to underpitch substantially. Propagating your own yeast from a starter culture is a reasonable option for most smaller brewpubs, and gives you some level of control without having a full laboratory setup.

Microbreweries often propagate their own yeast, either from a culture they have “found” or one purchased from a commercial yeast library. By propagating your own yeast, you will gain greater control over the yeast health, but you must have the knowledge and experience to handle the yeast. Propagating your own yeast does not have to be expensive, but it requires some knowledge and forethought before each new generation of yeast.

There are several laboratories that will aid you in finding the proper yeast, and growing up yeast for you if you require. A full listing of all they yeast types available is included in your handout material (courtesy of the Brewing Techniques Market Guide).

Yeast Quality

There are several qualities that brewers look for in their yeast:

- Flavor and aroma qualities
- Sedimentation/Flocculation
- Attenuation
- Head Size
- Mutation Rate
- Consistency of crop

Flavor and aroma are often regarded as the most important characteristic of a yeast, as far as the brewer is concerned. In breweries that use only one yeast strain, the flavor and aroma of all of the beers will exhibit the “house character” of that yeast. Many brewers use multiple strains to develop distinct characters in their specialty beers. Many brewers feel that the flavor and aroma qualities of a particular strain of yeast are so important that they will tailor their process to the idiosyncracies of that particular strain.

Flocculation is the aggregation (grouping) of cells into masses at the end of fermentation. Some yeast flocculate and settle to the top, while others settle to the bottom of the tank. Most modern yeast can be forced to settle to the bottom of a cylindroconical fermentor, but there are several tenacious top

Yeast Labs

- Weihenstephen (Freising, Bavaria)
- Brewing Research Foundation (England)
- Siebel
- White Labs
- Wyeast
- UC Davis
- Scott Labs/Lallemand (dried yeast)
fermentors (such as Bavarian Weiss strains), can only be harvested from the top of the fermentor (most often requiring open fermentations). Flocculation also describes how quickly and densely the yeast tend to drop out of the wort. A yeast that settles too quickly will tend to leave fermentable sugars in the beer (see attenuation, below), while a poorly flocculating yeast will cause problems in filtering or fining the beer (since it stays in suspension). Flocculation may also affect the flavor characteristics produced by the yeast, as will be seen in flavor biochemistry.

Attenuation describes the ability of the yeast to remove fermentable sugars. A highly attenuative yeast will produce a relatively dry beer, while a poorly attenuating yeast will leave sweetness in the beer.

The size of the head formed by the yeast while fermenting is important when deciding the size of tank required for fermentation. Yeast typically produced a head that rises from 15-25% above the level of the wort in the tank, and fermentors should be sized accordingly.

Mutation rate describes the genetic stability of the yeast strain. Although this would seem to be the determining factor in the amount of re-pitchings the yeast could withstand, the bacterial load is more often the determining factor. Mutations such as respiratory deficiency do cause problems in flavor and aroma characteristics and flocculation and will be discussed later in harvesting of yeast.

The consistency of the crop is related to mutation, and affected strongly by flocculation characteristics of the yeast. See Harvesting for more details.

**Dried or Liquid?**

**Liquid Yeast**

Liquid yeast gives a brewer more options, but is typically harder to deal with since it must be grown up from a small sample, or even a slant.

Pitching rate is more difficult to determine, and you must brew often to keep the yeast viable in the cone of the tank, or invest in a yeast propagation system.

Liquid yeast is cheaper than dried yeast (in the long run), since it can be reused for many generations.

**Dried Yeast**

Dried yeast is easier to use, since all it requires is hydrating and dumping the yeast into the fermentor. Typical dried yeasts are grown to give a high glycogen content, to allow recovery from dried form.

Although it is easier to control the pitching quantity (simply the weight of yeast added), there are less choices of variety of dried yeasts.
YEAST

LAGER VS. ALE

Strictly speaking, yeast that can metabolize melibiose (a simple sugar), are classified as Saccharomyces Uvarum, or lager yeast, and brewing yeasts that lack this ability are classified as Saccharomyces Cerevisiae, or ale yeast. In recent years the distinction between these yeast on a taxonomic level does not correspond well with the actual performance of the yeast in brewery fermentations. A yeast may have an ale yeast taxonomy (i.e. does not metabolize melibiose) but performs like a lager yeast in the brewery (i.e. low temperature fermentation, bottom fermenting).

Top fermenters vs. bottom yeasts can be determined by cell wall composition. Can be tested by placing in a clean test tube with H₂O and shaking. Those that make a “skin” at the top are probably top fermenters.

Saccharomyces Cerevisiae can ferment glucose, sucrose and maltose
S. Uvarum (Carlsbergensis) can ferment all including melibiose
S. Diastaticus can ferment all plus dextrins. Produces considerable quantities of diacetyl and is considered a wild yeast.

YEAST TRACKING

Keeping track of the history of your yeast is an important part of yeast management. A brewer should keep track of the following:

- Generation
- Age at pitching
- Pitching rate
- Cell count
- Viability
- Sensory qualities
- Time of trub removal
- Time of cooling

YEAST GENERATION

How many generations are too many? This varies from brewery to brewery and strain to strain, but typically yeast can stay vital and genetically stable for 10-30 generations, depending on many factors. If yeast has been stressed for any of the following reasons, it can reduce the viability/vitality of the yeast:

- Fermentation of strong beers or barley wine. The stress caused by the fermentation of high gravity worts will often affect the viability and vitality of yeast in subsequent pitches. This is especially true if yeast is stored in the high alcohol environment of the finished strong beer, which will lead to accelerated cell death.
Yeast

- Yeast washing. Although yeast washing (particularly acid washing) will destroy many brewery bacteria, it also can be detrimental to the health of yeast. Acid washing tends to destroy enzymes in the cell wall, particularly invertase, and can increase the lag phase of subsequent fermentations. See below for details on yeast washing.
- Poor selection/harvesting. See below for details on harvesting.
- Fermentation of fruit/spice beers. Although the yeast from a fruit or spice beer may be quite healthy, it is important to keep in mind that pitching yeast also contains a fair amount of beer, which will carry along flavor constituents. With strongly flavored fruit or spice beers, the characters of the beer can permeate the beer being pitched.
- Fermentation under CO₂ saturation conditions. Carbon dioxide can be toxic to the yeast cells, causing extended lag phases and other problems with yeast metabolism. More on CO₂ toxicity below.
- Poor aeration of wort. Yeast requires aeration at the beginning of fermentation to aid in the construction of robust membranes that will lead to healthy yeast cells generation after generation.
- Poor sanitation leading to contamination. The primary reason that brewers repropagate yeast is to avoid contamination.
- Low pitching rates
  Also note that you often will pitch several batches from the same generation, thus conserving generations. Typically you will go through one generation per week, regardless of how often you brew.
  Lager yeasts typically can handle less re-pitching, since the pH drop of a lager fermentation is smaller and slower, which encourages bacterial growth, along with a slower fermentation that favors contamination as well. Lager yeast are often re-propagated every 5-10 generations.

Yeast Harvesting

As a brewer you want to select the best possible yeast and keep genetic mutation to a minimum. You are also looking to harvest the highly viable yeast. Yeast harvesting is in essence a form of evolution, and by using cylindroconical fermentors you can have some level of control over the quality of the yeast to harvest for re-pitching.

“Natural” Selection

The cone of a cylindroconical fermentor is an excellent tool for harvesting the healthiest and most viable yeast possible. To harvest correctly you must realize that the yeast will form strata in the cone, and by draining off the right amount you can get to the prime yeast.
- Trub/yeast. Bitter and generally darker in color.
- Early settling yeast. Also mixed with more trub, hence more bitter, and exhibits poor attenuation (since it is not in contact with the beer long enough). May also have lost ability to metabolize maltose or maltotriose due to catabolite repression.
- Prime yeast. A moderation of both flocculation and attenuation. Generally brighter in color, with a more bread-like (or more beer-like), tangy flavor. These yeast tend to have higher viability than early settling yeast.
Late settling yeast. Generally poor flocculation qualities, and mixed with other undesirables that have dropped out of the wort.

It is important to note that early or late settling yeast may produce other off characters in the beer. For example, respiratory deficient yeast have poor flocculation abilities and in addition they tend to leave diacetyl in the finished beer. The sensory characteristics of the prime yeast are as follows:

- Fresh yeasty smell
- Tart taste
- Low bitterness
- Light tan color

Open fermentors also allow for good selection of yeast. You can either skim the yeast off of the top of the fermenting beer or harvest off of the bottom of the tank after raking.

**Yeast Settling Pattern**
“Unnatural” Selection

If using a flat or dished bottom fermenter, the pitching is much more difficult. If you simply open the bottom valve on the tank to harvest, you will pull out a plug of yeast from the center of the tank, which will not be a selection of the best yeast, and will probably be an insufficient quantity for repitching. You may also rack off the beer with a stand-pipe or racking arm and then scoop out the yeast through a manway. This will get you a greater quantity of yeast but either way, you are getting a mixture of trub, dead yeast and viable yeast. Brewers who harvest from dish bottoms typically get less generations out of the yeast, and produce beers with more yeast autolysis characters.

Yeast Nutrition

It is important to consider the composition of the wort that you will supply for the yeast to feed on. Although wort composition is often not the limiting factor in yeast performance (at least with all malt brews), it can have an effect on the flavor profile produced by the yeast during fermentation. Proper wort composition includes:

- Proper mix of fermentable carbohydrates
- Available nitrogen compounds
- Calcium, magnesium and zinc
- Temperature and pH within range
- Removal of excess proteins and trub
- Cooled wort aerated adequately
Fermentable Sugars

Keeping in mind that different sugars are taken up preferentially during the fermentation, it is important that wort contains a spectrum of fermentable sugars. Yeast will require glucose and fructose early in the fermentation, which can be easily transported across the cell membrane, and enter directly into the various biochemical pathways (helping to kick start and establish these pathways that were put on hold during the stationary phase). Maltose and maltotriose will be used later in the fermentation at a slower rate, allowing for less production of by-products from the main biochemical pathways. A wort that contains high glucose will encourage rapid fermentation, resulting in many by-products which may lead to undesirable flavor profiles. The spectrum of fermentable sugars is influenced during the mashing procedures with proper temperature, time and pH controls.

Amino Acids

If sugars (carbohydrates) are the fuels for the cell, the amino acids are the building blocks. At their most basic level they are a source of nitrogen for the yeast cell, but they also provide many other constituents critical for yeast growth. Proteins will be formed from the amino acids, and by-products of these transformations will lead to various flavor constituents. Yeast health will be influenced by the amino acid composition, and like the fermentable sugars they will be taken up in sequence according to the needs of the yeast cell. The total amount of amino acids (also known as Free Amino Nitrogen or FAN) will also be important in determining the health of the yeast during fermentation and into future generations. It is important to note that with the majority of all malt worts the amount of FAN present is in excess of the yeast requirements.

Trace Ions

Several ions present in wort are critical to the health of the yeast during and after fermentation. Calcium (improves flocculation of yeast), Magnesium (important cofactor in many pathways) and Zinc (required for proper yeast function) all play a role in enzymatic functions of the cell, and their presence (at least in small amounts) is required to produce a vigorous fermentation. Using distilled or reverse osmosis water for brewing liquor may result in worts that are deficient in certain trace elements. Although this may not present a problem for many brewing yeast, some will have higher requirements for trace elements than others.

Temperature and pH

Yeast performs best at a certain pH and temperature. The optimal pH and temperature depends on the strain of yeast being used (ale strains 60-72°F, lagers 45-55°F, pH varies for both) and the flavor compounds that the brewer wishes to maximize (this will be discussed further in the Flavor Biochemistry lecture). It is important to note that yeast does not react well to sudden changes in temperature, such as pitching cold yeast into warm wort. This so called “temperature shock” can affect the overall health and performance of the yeast during fermentation.
Suspended Material

There is much argument about the optimal level of suspended material required in the wort. It is clear that worts with extremely high suspended solids will produce beers that are difficult to filter, while if a wort is too bright (for example filtration before fermentation) it will affect the nutrients available to the yeast and therefore the health of the yeast.

Wort Aeration

Aeration of the wort prior to fermentation is critical to yeast metabolism. The yeast uses the oxygen to produce unsaturated fatty acids that are used to make membranes for the growing yeast cells. Without sufficient oxygen, the membranes produced will be rigid, and affect transport of nutrients into the cell, and metabolism inside of the cell. Although many brewing yeast ferment fairly well even if they have no oxygen at the beginning of the fermentation, they will have a long lag phase, and the yeast will not be in healthy enough at the end of the fermentation to be harvested and pitched again.

Inhibitory Effect of CO₂

CO₂ saturation has a negative effect on yeast health and performance. The yeast essentially suffocates in high CO₂ environments, and the uptake of nutrients is inhibited. It is important to note that it is not CO₂ pressure alone which cause inhibition of yeast, but rather saturation of the yeast cells with CO₂ (saturation can build up while yeast is in a tank under pressure). This is why yeast stored in a Unitank under carbonated beer may show signs of CO₂ inhibition when pitched into fresh wort, even though there is no CO₂ top pressure on the tank of freshly pitched wort.

Carbon dioxide toxicity causes many changes in the metabolism of the yeast during fermentation. Cell division is decreased, leading to prevention of cell division if CO₂ saturation conditions remain for too long during fermentation. This also leads to higher fatty acid contents in the cell membranes and high DNA and RNA contents in the nucleus.

Transport proteins are disabled causing changes in the overall metabolism during fermentation. In general fermentation and growth are inhibited, leading to longer lag phases and poor attenuation. Amino acids requiring active transport into cell will be reduced (this includes valine and leucine, which are
key components in the production of diacetyl, as will be seen in Flavor Biochemistry). The changes in the permeability of the cell membrane will cause substantial changes in the flavor of the finished beer: Acetaldehyde and vicinal diketones (diacetyl) levels will increase, while higher alcohol and ester concentration will decrease.

**Yeast Storage**

In many ways, yeast storage conditions are as important as yeast propagation. Storage of yeast leads to loss of growth factors, and the amount of this loss depends on the conditions of storage. Degeneration of the yeast is increased at higher temperatures, and in general the yeast must be kept cold and free from oxygen and bacteria. Alcohol in high concentrations is also toxic to yeast, so storage in beer may result in reduced performance as well. If the yeast is stored under CO₂ saturation conditions it may develop CO₂ toxicity (see section of CO₂ toxicity).

**Cone Storage**

- Can have high alcohol, CO₂ and pressure
- Low oxygen conditions
- May be high temperature due to insulated qualities of yeast. This can be reduced by cone cooling jackets on the fermentor.
- Fairly free from bacteria

Cone storage is very common in many microbreweries. It is important to get the yeast out as quickly as possible to avoid yeast autolysis characters in the beer.

**“Bucket” Storage**

This method can actually employ buckets, kegs or Corny Cans that can be placed in a walk-in cooler. This method can be a good way to store since the temperature is controlled and low, and the O₂ can be kept low as well. It also has advantages over cone storage because it allows for better pitching (see section on Propagation) and does not have the yeast autolysis problems found in cone storage. Use of a Corny Can or keg actually has many advantages of the Yeast Brink (see below).

**Yeast Brink**

Use of a dedicated yeast brink (or a propagation tank) has several major advantages over other yeast storage methods.
Aeration of the yeast prior to pitching

Aerating the yeast prior to pitching will aid in decreasing the lag phase by encouraging the yeast to start synthesizing unsaturated fatty acids and sterols, using the stored glycogen for fuel (more on this during Fermentation Biochemistry). The yeast should be aerated several times no more than 12 hours before pitching.

Studies have shown that aeration during the final stages of propagation will give good membrane production capabilities in the pitched yeast.

CO$_2$ Saturation

CO$_2$ saturation will affect many aspects of fermentation, and storage inside of a unitank will tend to lead to saturation conditions. By storing in a yeast brink it is possible to reduce the CO$_2$ pressure to a minimum to give optimal storage conditions.

Metered Pitch

With the proper valving it is possible to pitch the yeast directly in line with the wort as it fills the fermentor. By slowly adding the wort during the entire knockout the yeast will be evenly mixed throughout the wort, and will start fermentation earlier.

Temperature Control

It is important during the storage that the yeast metabolism be reduced to a minimum, and low temperatures are the best means to ensure this. Because a yeast brink is often relatively small (perhaps 1/10th the size of the fermentors), with high surface to volume ratios, it can have efficient heat transfer. Yeast is a relatively good insulator, and since yeast metabolism generates heat, storage inside of large vessels can result in “hot spots”, which cannot be effectively cooled due to the small surface to volume ratio.

Homogenous Slurry

Yeast slurries will tend to settle and stratify quickly if they are not stirred. It is useful to have a homogenous mixture when pitching, particularly when a sample taken from the slurry will be used to determine the overall pitching rate.

Yeast Enumeration

Pitching Rates

Underpitching

Underpitching will cause increased yeast growth, resulting in more production of the by-products of amino acid synthesis.

Production of more higher alcohols (fusel oils) and organic acids. Increased yeast growth, lower alcohol production. Also, many sugars are not metabolized and lower attenuation.

Greater likelihood of infection by opportunistic bacteria or wild yeast.

The production of more esters and diacetyl, less acetaldehyde.
**Overpitching**

Overpitching generally means that the yeast does not have to vigorously increase its population, and this means that more of the sugars will be converted into alcohol, rather than yeast mass. It also means that less of the fermentation characters will develop.

Excessive yeast autolysis resulting in astringency and yeasty “bite”. Also not as healthy for the yeast in general, since it hampers their growth cycle. Excessive yeast growth produces beers that lack in many fermentation qualities but increases “green beer” character called acetaldehyde.

**Enumeration Methods**

**Haemocytometer**

The simplest and cheapest means of accurately determining the pitching rate is the use of a haemocytometer and a microscope. A haemocytometer is an specialized slide which has a known volume and a counting chamber. By placing a slurry of diluted pitching yeast on the slide, the amount of cells in the chamber can be counted, giving a concentration of cells/ml in the pitching yeast.

**Packed Cell Volume**

Another simple method of determining the cell concentration is to spin down the slurry in a centrifuge and measure the % solids. The only problem with this method is that other solids in the slurry (such as trub) will affect the measurement. Yeast cell mass also changes depending on the growth stage of the yeast, and its health. Because of these factors this method is not terribly accurate, but it will give you a general idea of the thickness of the slurry.

**Coulter Counter**

A Coulter Counter can electronically measure the exact amount of cells contained in a slurry of yeast, but it cannot determine the viability of those cells. These counting devices are outside of the budget of most microbreweries, although there are several craft regional breweries that use them.

**Viability**

**Methylene Blue**

The most common method of measuring viability is to add methylene blue stain to the yeast before counting. Those cells that are not viable will stain dark blue. Viable cells will take up the stain as well, but can metabolize it inside the cell and will therefore appear unstained. Unfortunately this method is only accurate at viabilities above 85%, and many recent studies have shown that it may only be accurate above 95%.

**Slide Culture**

The most accurate method of determining yeast viability is to actually see what proportion grow. Slide culturing allows the yeast to grow for 8-16 hours and then the amount of cells that actually reproduce is counted. Slides are prepared by mixing molten growth media with a measured amount of yeast on a microscope slide. The cover slip is placed on the slide and it is incubated for 8-16 hours at
HAEMOCYTOMETER
room temperature. The slide is then inspected for the amount of individual cells that have formed microcolonies. By comparing the number of microcolonies to total cells the viability of the yeast can be determined. This method is fairly time consuming and tedious, but is the only other realistic option for microbrewers. This method is the standard by which new methods for viability are judged for accuracy.

**Protease Activity**

There is another method of determining viability which uses the activity of a protease as an indicator of yeast health. Although this method gives good results, it requires a spectrophotometer for determination of protease activity, putting it out of the reach of many microbreweries. In addition, this method has yet to be accepted by the ASBC.

**Vitality**

Vitality is the vigor with which a yeast will ferment and is much more difficult to measure than viability. One method used in Great Britain uses iodine into a slurry of lysed yeast cells to determine the glycogen concentration. As you will see during the Fermentation Biochemistry lecture, glycogen is a storage sugar laid down by healthy yeast during the stationary phase, and is one indicator of yeast vitality. This method requires careful technique and a spectrophotometer to measure the color of the iodine after the reaction. In most microbreweries you must look to careful yeast tracking to determine the vitality at pitching.

**Acid Washing**

Yeast is fairly resistant to acidic conditions, and in many cases has much greater resistance to acid than ordinary brewery bacteria. By washing the yeast slurry in cold water or acid the bacterial population can be significantly reduced. These methods are typically used either in an emergency or as a routine to keep the yeast free from bacteria. Many different acids can be used, but tartaric and phosphoric are most common. The pH of the slurry is adjusted to 2.0-2.4 for 45 minutes (an alternative method adjusts to 2.8-3.0 for 2 hours or more).

The yeast may also be washed using cold sterile hard water and perhaps washing the yeast several times. In either method the excess liquid is decanted off and the yeast is pitched in the normal fashion.

Note that all you need for this method is a clean receptacle and a pH meter. The problem with this method is that some strains can be unduly stressed by acid washing, reducing viability and vitality. Some yeasts react very well to this method though, and it can be an effective preventative measure.

Some breweries have noticed that their bacteria have gained resistance to acid conditions, and instead they employ chlorine dioxide as a washing agent (there will be more information on this disinfecting agent in the Sanitation lecture).
This is basically the ideal system for propagation of yeast under sterile conditions. Since this system costs as much as many microbreweries, it is not common to see such a system in a craft brewery. Note that the culture comes from a 10 L slurry, and that the wort is sterilized (with steam injection) in the tank and then cooled. Sterile air can be injected to aerate the wort and encourage yeast growth.

For most microbreweries, the options are much more modest. Small brewpubs may not even have any means of yeast propagation other than the use of the cone of a cylindroconical fermentors. In reality there are several viable options for even the smallest micro to allow more control over yeast propagation without spending a fortune.
The main options for yeast propagation employed in microbreweries are:
1) No propagation
2) Propagation from purchased or borrowed slurry
3) Propagation from slant preserved colony

No Propagation

If you choose to avoid “propagation” in the brewery you can get your yeast from another brewery or from a yeast laboratory. Getting the yeast from a laboratory can be expensive if you want to get a full pitching quantity, but it may be the safest option. It is important to keep in mind that laboratories are not fool proof, and that you should check the quality control record of the laboratory that you choose to get your yeast from.

Getting a full pitching quantity of yeast from another brewery can be inexpensive, but also has risks. You will inherit any microbial spoilage problems the other brewery may have, and your brewing schedule will be partially tied to the schedule of the other brewery. Be sure that you trust the propagation and brewing practices of the brewery that you are dealing with and have other options available in an emergency.

Propagation From Purchased or Borrowed Culture

The simplest method of propagation is to purchase or borrow a small culture, perhaps a one liter starter slurry and grow this culture up into a pitch-able quantity of yeast. If you are planning on purchasing from a laboratory, this is a much more economical method than purchasing a full pitching quantity.

To grow up the yeast from this starter slurry you will need a propagation system, although it may be as rudimentary as a homebrew fermentor. You will also need clean hopped wort for growing up the culture.

By assuming some of the risks of propagation, you will decrease your costs and increase your control over the propagation. If there are any problems with the yeast, you may notice them before you actually have to pitch a full batch, but you may also cause problems during your propagation as well.

Generally speaking, a one liter pitch of yeast will cost around $40 from a yeast laboratory, and the capital expense depends on the type of propagator you choose to purchase. It may take from 2-3 days to propagate up a pitching quantity of yeast from this starter culture, but you may want to allow 4-5 days for better yeast health (it is better for the yeast to complete fermentation and go into the stationary phase before re-pitching).
Slant Loop

Plate with Growth media
2-3 days

10 ml Test Tube
2-3 days 37 C Nutrient broth

5 gallon bucket
(2.5 gallons wort)
(6.25 L of hopped wort)
2 days at Fermentation Temp

1 Litre
2-3 days 37 C Nutrient broth

2 kegs w/0.5 bbl each
(31 gallons of hopped wort)
(117 L of hopped wort)
2-3 days at Fermentation Temp

100 ml
2-3 days 37 C Nutrient broth

Sufficient Yeast to pitch into 10 bbl wort
Propagation from slant preserved colony

Full propagation from a slant preserved colony requires more time and knowledge than simpler methods, but it also grants you full control over the propagation. You will need some basic laboratory equipment and a propagator as well.

The cost of full propagation is mainly in the capital costs of the lab equipment and propagation system, since the initial culture will be relatively cheap. Full propagation requires much more planning than other methods, since it may take from 10-15 days before the yeast will be ready for pitching from the start of the propagation.

PROPAGATION VESSELS

- Cylindroconical fermentor cone
- Bucket
- Soda Keg (Cornelius Cannister)
- Keg (with adaptations)
- Dedicated propagator

Bucket

Although a bucket is not the most elegant solution to the propagation question, it is a popular one that does work. Using plastic food grade buckets has its risks, since scars on the interior of the bucket can harbor bacteria. You can use disposable food grade liners for better sanitation, but you still must pitch the yeast through the door of the fermentor rather than in-line with the wort. There is also little or no temperature control options with bucket propagation, although storing the yeast in a walk-in cooler will allow you to preserve the yeast after it is harvest or propagated.
Corny Can

By adapting a Cornelius soda cannister with more sanitary fittings a brewer can create a relatively cheap yeast storage, pitching and even propagation system. Because the quick connects are prone to contamination and may be too small to allow flow of a thick yeast slurry, it is important to install a larger line that penetrates the lid of the “Corny Can”. Inside the can you can install a hose barb and attach a flexible 1/2” hose to the barb for dispensing the yeast. The inlet quick connect can still be employed for forcing pressure into the can to push out the yeast. You can also force in air into the downspout to help with the growth of the yeast during propagation, but this may be difficult due to foaming. Even if you have properly adapted the Corny Can it may still be difficult to sanitize because of the small fittings. The best option is to fill the Corny Can with a no rinse sanitizer after cleaning and leave it stored in this fashion for the next filling. You should also consider buying your Corny Can new (around $90) to avoid risk of off flavors caused by soda flavorings. At a minimum you should replace all of the gaskets on a used Corny Can.

One of the biggest problems with a Cornelius Can is its small volume which may not allow for enough yeast growth to pitch a 15 bbl. batch. In many ways an adapted Corny Can is better for yeast storage and pitching than it is for actual propagation.

Another advantage of a Corny Can is its use as a pitching device. By placing the yeast filled Corny Can on a scale and hooking it up in-line with the cooled wort flow into the fermentor, you can accurately weigh the amount of yeast being pitched.

Keg

Adapting a regular straight walled Sankey keg with a large sanitary fitting on the top, and inflow and outflow lines (see diagram) is relatively inexpensive and will give you greater capacity than a Corny Can. The keg size is better for propagation and is generally easier to clean than a Corny Can. You can even sterilize the wort for propagation use by placing the keg on a stove and boiling the wort.
An important adaptation for all of these propagation systems is a sterile air filter to allow you to push air into the propagator during the propagation. This would also be important after sterilizing the wort, since as the wort cools it will pull in air to compensate for compression.

As with the Corny Can or bucket, temperature control is difficult in a keg propagator, although it is possible to fit a glycol jacket around the keg, or to put it into an area attached to a walk-in cooler with a separate thermostat (cooled air can be blown in like in a dispense line cooling system).

Although a keg is larger than a Corny Can, it is just barely sufficient to grow up yeast for pitching a 15 bbl batch. Aeration of the yeast to encourage growth is important to get high enough concentration of the yeast for pitching.

To measure the pitch like a Cornelius Can you will need a scale with a capacity of 150 lbs, and 0.1 lb precision, which is an expensive item. Transferring to a Cornelius Can for pitching may be the best option.

Cylindroconical fermentor cone

This is the most common propagation system in most brewpubs, and it has some distinct advantages, the main one being that it is inexpensive (as long as you already have cylindroconical fermentors). There are several ways of making this system work, and they all depend on the number of free fermentors you have available, and whether you have some method of making up small quantities of wort. If you have a pilot system of some sort, you can make up one barrel of wort, and put it into the bottom of a 15 bbl fermentor along with your yeast and ample aeration. After the fermentation has finished (2-3 days), you can add an additional 14 bbl to the tank, again with aeration. Although this method results in underpitching, it is fairly sanitary, and there are no yeast transfers involved.

The other method requires two empty fermentors. You will do a full brew, and put 14 bbl into one fermentor, which you will grow up with the old yeast that you are phasing out. The remaining 1 bbl of wort will be put into the cone of the other fermentor, and the new yeast will be added along with aeration. The following day you can top up both tanks with the same style of wort. This method is simple, but the requirement for two empty fermentors, along with the need for two brews of the same style of beer make it less attractive. Using this method will also mean that you are adding additional sugars to the yeast when it is in the middle of the growth phase which will change the fermentation performance of the yeast.

Overall this method gives you very little control of the pitch rate, and in most cases you will be underpitching the wort. In addition you need to have cone cooling to use this method.
Dedicated propagator

The most expensive option is a dedicated propagation tank. This system gives you the greatest control and flexibility over the propagation process, and can have any or all of the following features (listed in order of importance):

- with cooling
- with aeration
- with stirring
- with heating
- with load cells/flow meter

Many dedicated propagators are actually just small tanks that are being used to propagate up yeast. Three barrel Grundy tanks can be used for this purpose, but they are less than ideal due to the sanitation risks. If you have the opportunity to design your own propagation tank, make sure you keep these factors in mind:

Sanitary welds and good polishing inside the tank to avoid areas that will harbor bacteria.

Although stirring is not required, it is an excellent feature that will increase the homogeneity of the pitch, and increase the growth rate during propagation. Sterile air can be used to stir the slurry by bubbling up from ports on the bottom, but as the slurry gets thicker this may not be sufficient.

A properly installed jacket can be used for both heating and cooling simply by installing quick connects to the jacket that allow a transfer from glycol to a steam source. This feature is definitely above what most brewpubs will require.

You may also consider transferring the settled yeast slurring into another container for pitching (i.e. a Cornelius Can) to allow for accurate measurement of the pitch.

<table>
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<td><strong>Disadvantages</strong></td>
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<td>Difficult to measure pitch</td>
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Mixer
Removable Lid
CIP/Vent Line
Jacket Coolant Out
Temperature Probe
Jacket Coolant In
Sterile Air Injection
Tank Drain
Yeast Removal
Exterior
Interior
CHAOS THEORY

Yeast is a living organism, and you should treat it as such. You must always be aware of the vitality/viability of your yeast, and often you must schedule your brews according not to demand, but yeast health. When you leave the brewery for the weekend, you will probably be more concerned about the yeast than about whether you will run out of beer!

As a living organism, yeast metabolism is even more complex than the enzymatic changes in the mash. Do not expect to be able to completely control the activity of the yeast, since it is subject to the forces of Chaos theory. Watch for patterns that develop in yeast performance and analysis, and pay attention to the many factors that might affect them.

If you think water chemistry and thermodynamics are complex, they are nothing in comparison to biological systems. The variables controlling yeast activity and flavor development are innumerable, and the best you can hope to do is shepherd it in the right direction.
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