



---

# SUPPLEMENTAL MATERIAL

## 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 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 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.



### 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 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 CO<sub>2</sub> saturation conditions. Carbon dioxide can be toxic to the yeast cells, causing extended lag phases and other problems with yeast metabolism. More on CO<sub>2</sub> 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.



## YEAST SUPPLEMENTAL MATERIAL

- 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.

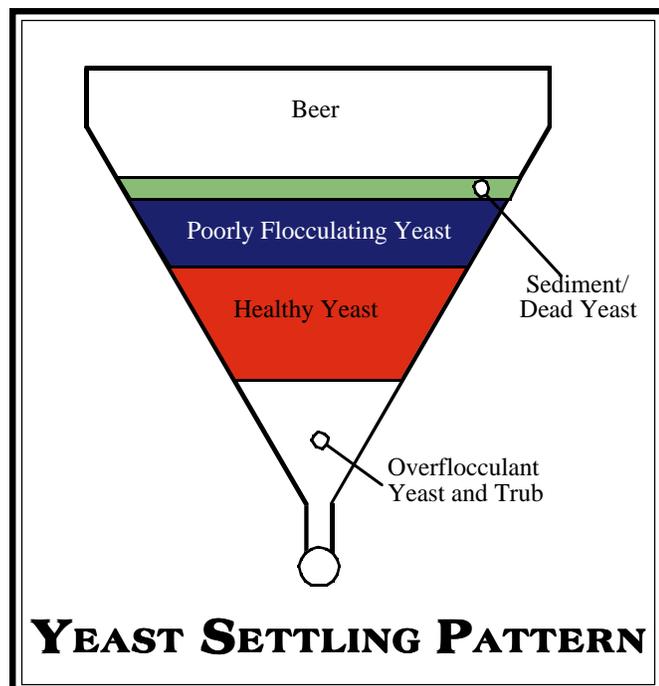
### Yeast Harvesting

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).
- 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.





## YEAST SUPPLEMENTAL MATERIAL

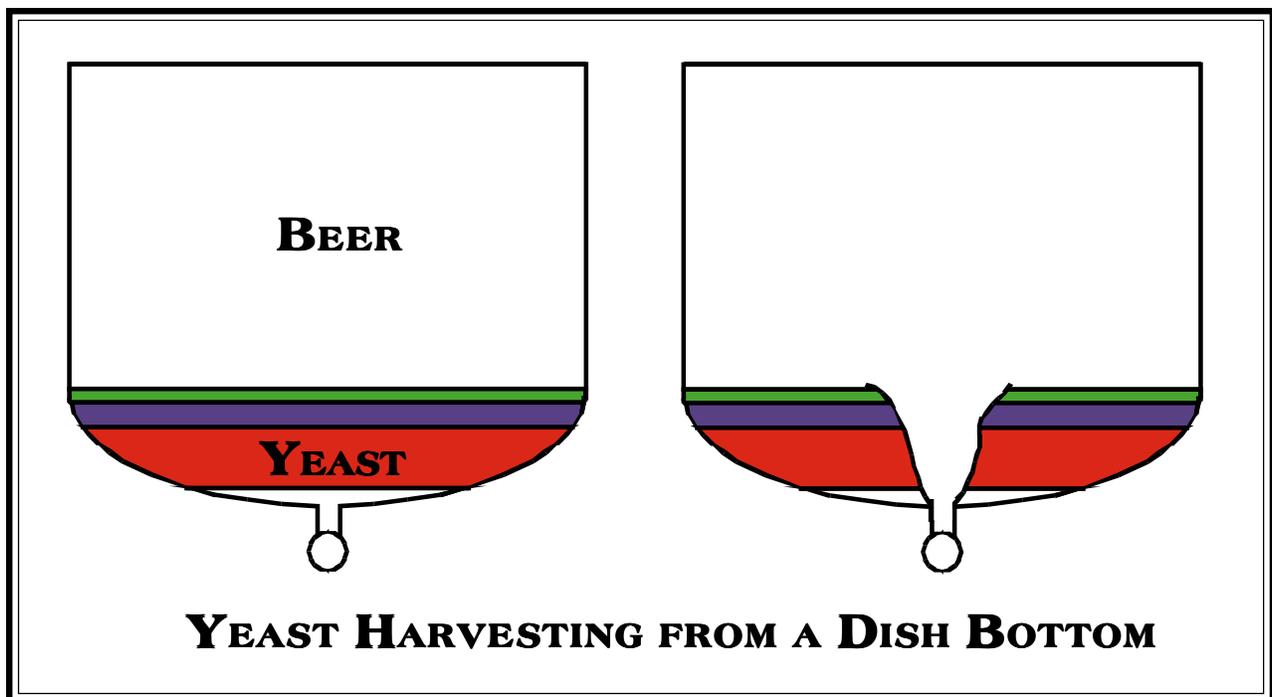
It is important to note that early or late settling yeast may produce other off characters in the beer. For example, respiratory deficient yeast have poor flocculation abilities and in addition they tend to leave diacetyl in the finished beer. The sensory characteristics of the prime yeast are as follows:

- 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 racking.

### “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 re-pitching. 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.





### INHIBITORY EFFECT OF CO<sub>2</sub>

CO<sub>2</sub> saturation has a negative effect on yeast health and performance. The yeast essentially suffocates in high CO<sub>2</sub> environments, and the uptake of nutrients is inhibited. It is important to note that it is not CO<sub>2</sub> pressure alone which cause inhibition of yeast, but rather saturation of the yeast cells with CO<sub>2</sub> (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 CO<sub>2</sub> inhibition when pitched into fresh wort, even though there is no CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> saturation conditions it may develop CO<sub>2</sub> toxicity (see section of CO<sub>2</sub> toxicity)

#### Cone Storage

- Can have high alcohol, CO<sub>2</sub> 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 O<sub>2</sub> 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).

### 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 a 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%.



---

## YEAST SUPPLEMENTAL MATERIAL

### 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 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.



---

## YEAST SUPPLEMENTAL MATERIAL

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 stress 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.