MODULE 2: Yeast and Beer

UNIT 2.4: Yeast in Brewing

SECTION 2.4.1: Yeast Cultures

ABSTRACT: The systematic use of clean, pure and highly viable yeast cells ensures that bacteria, wild yeasts or yeast mutations do not lead to inconsistent fermentations and off-flavour development. It is normal procedure in many breweries to propagate fresh yeast every 8-10 generations (yeast cycles) and the practice of using pure yeast culture to propagate for brewing has been employed for over 100 years.

LEARNING OUTCOMES: On completion and comprehension of this unit you will be able to:

1. Describe the methods used for preserving stock yeast cultures.
2. Explain the principles and procedures involved in yeast propagation from pure cultures.
3. Understand how and why yeast cultures must be kept free from contamination by bacteria and wild yeasts.

PREREQUISITE UNDERSTANDING: To have studied Section 2.1
2.4 Yeast in Brewing

2.4.1 Yeast Cultures

2.4.1.1 Introduction – Yeast Management

2.4.1.2 Preservation of Stock Yeast Cultures

2.4.1.3 Pure Yeast Culture Propagation

2.4.1.4 Contamination of Cultures with Bacteria

2.4.1.5 Contamination of Cultures with Wild Yeast
2.4 Yeast in Brewing

2.4.1 Yeast Cultures

2.4.1.1 Introduction - Yeast Management

It has been previously discussed in this document (but is such an important fact that it is worthy of repetition), that in brewing the cropped yeast is re-pitched into subsequent brews. The quality of the cropped yeast will significantly affect the overall performance of a subsequent fermentation into which this yeast is pitched, which in turn will influence the resulting beer quality and stability.

It is normal procedure in many breweries to propagate fresh yeast (particularly lager yeast) every 8-10 generations (fermentation cycles), or earlier if contaminated (the yeast could also be acid washed), or if a fermentation problem is identified. Fermentation problems include sluggish fermentations, usually slower rates of wort maltose and maltotriose uptake, higher levels of sulphur dioxide and hydrogen sulphide, prolonged diacetyl reduction times and increased flocculation and sedimentation rates.

2.4.1.2 Preservation of Stock Yeast Culture

The long term preservation of a brewing yeast culture requires that not only is optimal survival important, but it is imperative that no change in the character of the yeast occurs. Many yeast strains are difficult to maintain in a stable state and long term preservation by lyophilization, (freeze drying), which has proven useful for mycelial fungi, has been found to give poor results with many brewing yeast strains. Storage studies have been conducted with a number of ale and lager brewing strains. The most common preservation methods currently in use are:

- Subculture;
- Drying or desiccation;
- Lyophilization (freeze drying);
- Low temperature (-70°C refrigeration or liquid nitrogen), freezing or cryopreservation.
(a) **Subculture**  
This traditional and popular method involves the use of two vials – one for transfer and one for laboratory use, for inoculation to scale up the culture for plant use. The cultures are maintained on a yeast growth medium (such as MYGP, PYN), incubated between 20 and 30 °C to stationary phase (about 72 hours), and then stored for up to 6 months at 1 to 4 °C. At 6 months, the culture is transferred to two fresh slopes from the vial reserved exclusively for transfer. Few cultures are lost using this method, but the cultures might change over time. Studies have shown that of 300 brewing yeast strains, 25% lost their ability to utilize maltotriose and 10% showed a change in flocculation ability. To summarise, although this method is inexpensive and versatile, and the slopes are convenient for distribution, there is a danger of unacceptable levels of strain degeneration and should, therefore, not be recommended for long term storage. In addition, there is always a risk of cross-contamination, comprising strain purity.

(b) **Drying or Desiccation**  
Here, there are several methods available. For example, silica gel can be used as a desiccant, but not usually employed for industrial strains (usually research). Another method for drying uses squares of filter paper and tinned milk as the suspending medium. The damaging effects of drying appear to be strain-specific.

(c) **Lyophilization (freeze drying)** can also be used, being very popular for distillers, but less so for brewers. The process differs from desiccation in that water is removed by sublimation from the frozen material using a centrifugal dryer. The dried yeast is sealed under vacuum in a glass ampoule. Survival levels tend to be low (possibly as low as 5%), plus there is also concern as to whether the surviving cells truly represent the original population. Some studies have shown that there can be little change in morphological, physiological or industrial fermentation characteristics, although some strains of *Saccharomyces* showed an increase in the level of Respiratory Deficient (RD) mutants. Long term survival is often satisfactory, with a loss of viability at approximately 1% per year. The advantages of freeze drying include longevity of the culture, easy storage and distribution of ampoules, but the major disadvantages are the initial high level of diminished activity of the culture, with the technique being labour intensive, requiring special equipment.

(d) **Cryopreservation** is often the method of choice, as little molecular activity occurs at the low temperatures used and for long term storage, with maximum genetic stability, the favoured procedure involves liquid nitrogen at – 196 °C. Storage at – 20 °C to – 90 °C is acceptable, but for shorter storage periods. At such low temperatures, there have been very few reports of genetic instability.
or changes in fermentation characteristics and RD mutations are rare. Of 75 brewing yeast strains studied, the mean survival rate was 66%.

This method clearly yields the highest viability and superior stability, but the disadvantages include the use of liquid nitrogen (costs, safe handling, etc). However, freezers that operate at –130 °C are available and this can eliminate the disadvantages associated with the use of liquid nitrogen.

It is thought a wise precaution and safeguard to also keep a duplicate set of the most critical cultures on solid medium at 4 °C, in case of freezer failure!

In summary, the results of wort fermentation tests (including wort fermentation rate and wort sugar uptake efficiency, flocculation tests, sporulation ability, formation of respiratory deficient colonies and ease of revival), compared to the characteristics of the unstored control culture, showed that:

- Low temperature storage appears to be the storage method of choice if cost and availability of the appropriate equipment is not a significant factor. Cultures stored at -70°C or lower, had the lowest death rate and were the easiest to revitalise. Also, the degree of flocculation, wort fermentation ability, sporulation ability and proportion of respiratory deficient mutants present were all unaffected by this storage method.

- Storage at 4°C on nutrient agar slopes, sub-cultured every 6 months, was the next method of preference to low temperature storage.

- Lyophilization and other storage methods revealed yeast instability which varied from strain to strain.

Today many breweries store their strains (or have them stored under contract by a number of world-wide yeast culture storage specialists) at -70°C or less. Routine sub-culturing of cultures on solid media every six months is less desirable but is still a cost effective storage method. Very few brewers use lyophilisation of their yeast cultures, although some have adopted this as their standard procedure, with satisfactory results.
2.4.1.3 Pure Yeast Culture Propagation

The systematic use of clean, pure and highly viable cells ensures that bacteria, wild yeasts or yeast mutations (such as respiratory deficiency) do not lead to inconsistent fermentations and off-flavour development.

The practice of using a pure yeast culture for brewing was started by Emil C. Hansen in the Carlsberg laboratory over 100 years ago. Employing dilution techniques, he was able to isolate single cells of brewing yeast, test them individually and select the specific yeast strains that gave the desired brewing properties. The first pure yeast culture was introduced into a Carlsberg brewery on a production scale in 1883, and the benefits of using a pure culture quickly became clear. Soon, 23 countries had installed Hansen’s pure culture plant, for example, in North America, Pabst, Schlitz, Anheuser Busch and 50 smaller breweries were using pure lager cultures by 1892.

Hansen’s first propagation plant consisted of a steam-sterilised wort receiver and propagation vessel equipped with a supply of sterile air and an impeller. The basic principles of propagation devised in 1890 have changed little. Propagation can be batch or semi-continuous and usually consists of three stainless steel vessels of increasing size, equipped with attemperation control, sight glasses and non-contaminating venting systems (Figure 1).
Figure 1. Typical propagation vessel.

Each vessel is equipped with a CIP system and often has in place heat sterilising and cooling systems for both the equipment and the wort. Ideally the yeast propagation system should be located in a separate room from the fermenting area with positive air pressure, humidity control, an air sterilising system, disinfectant mats in doorways and limited access by brewing staff.

During yeast propagation, the aim is to obtain maximum yield of yeast but also to keep the flavour of the beer similar to a normal fermentation so that it can be blended into the production stream. As a result, the propagation is often carried out at only slightly higher temperatures and with intermittent aeration to stimulate yeast growth. The propagation of the master culture to the plant fermentation scale is a progression of fermentations of increasing size (typically 5-20 X) until sufficient yeast is grown to pitch a half or full size commercial brew.
Wort sterility is normally ensured by boiling for 30 minutes or it can be pasteurised using a plate heat exchanger, passed into a sterile vessel and then cooled. Wort gravities typically range from 10°P (1040 OG) to 16°P (1064 OG) but typically should be at the lower end of the range. Depending on the yeast strain, zinc or a commercial yeast food can be added. Aeration (oxygenation) is important for yeast growth, and ale wort is aerated using oxygen or sterile air, and anti-foam may be added depending on the yeast. Agitation is not normally necessary as the aeration process and CO₂ evolved during active fermentation are sufficient to maintain the yeast in suspension.

A typical brewery yeast propagation schedule would be as follows, but details will vary greatly with the size of the brewery and the particular propagation equipment available:

- Loop of culture from slope or petri dish;
- Transfer to 250 mL wort (1040, 10°P) or yeast extract - peptone broth in 500 mL flask, place on shaker for 2 days at 20°C (lager) or 27°C (ale);
- Transfer to 50 L vessel containing 25-35 L wort (1040-1048, 10-12°P), 3 days, slow shaking;
- Transfer to 20-30 hL yeast vessel, 15-20 hL, wort (1040-1049, 10-12°P), aerate/oxygenate (25 L/min.), 20-22°C, 2-3 days;
- Transfer to larger culture vessel 100-150 hL, 75-100 hL wort, 20-22°C, 2-3 days;
- Transfer to fermenting vessel, 300 hL. Ferment using normal procedures;
- Crop yeast and blend “green” beer at low rate (20-30%);
- Hygiene during the whole procedure is critical!

2.4.1.4 Contamination of Cultures with Bacteria

Detailed consideration of microbial contaminants of brewing yeast cultures are beyond the scope of this publication, but a brief review of the most important aspects is probably appropriate. Bacteria are common spoilage agents of beer. The most troublesome Gram-positive bacteria are the lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus*. At least ten species of lactobacillus can cause beer spoilage. When viewed under a light microscope,
lactobacilli are very pleomorphic in appearance and can range in shape from long slender rods to short coccobacilli. Brewing lactobacilli are heterofermentative (producing lactic acid as well as other acids and/or alcohols and some strains produce diacetyl) and homofermentative (producing only lactic acid). They are acid tolerant and have complex nutritional requirements. Some species such as *Lactobacillus brevis* and *Lactobacillus plantarum* can grow quickly during fermenting, ageing or storage, whereas others such as *Lactobacillus lindneri* grow relatively slowly. Lactobacillus spoilage is most problematic during conditioning of beer and after packaging where spoilage gives rise to a “silky turbidity” and off-flavours.

Pediococci are homofermentative cocci that occur in pairs and tetrads. Six species of Pediococci have been identified, but the species predominantly found in beer is *Pediococcus damnosus*. Pediococcus infection in the beer is characterised by lactic acid and diacetyl formation. Infection may also cause ropiness in beer due to the production of polysaccharide capsules.

Many Gram-positive bacteria are inhibited by hop bittering compounds, particularly the iso-α-acids, but Gram-negative bacteria are usually, but not always, unaffected. Some members of the *Micrococccaeae* can survive in beer, grow and cause spoilage as can some aerobic spore forming bacteria belonging to the genus *Bacillus*. Generally, these two genera are inhibited by hop components and prefer an aerobic environment and, therefore, are not a serious threat.

Important Gram-negative beer spoilage bacteria include acetic acid bacteria (*Acetobacter, Gluconobacter*) certain members of the family *Enterobacteriaceae* (*Escherichia, Aerobacter, Klebsiella, Citrobacter, Obesumbacterium*) as well as *Zymomonas, Pectinatus* and *Megasphaera*. Acetic acid bacteria can convert ethanol to acetic acid, producing a vinegar flavour in the beer and tend to produce a ropy slime. This type of spoilage is most often observed in draught beer. The bacteria are airborne and prefer an aerobic environment but can survive under microaerophilic conditions and infect the kegs as a result of air entering or beer standing too long on tap in a partly filled keg. The *Enterobacteriaceae* are aerobes and facultative aerobes and do not tolerate high ethanol levels. They are usually found early in the fermentation and can produce celery-like, cooked cabbage, cooked vegetable and rotten-egg aromas, especially if pitching of the wort is delayed. Figure 2 illustrates some of the bacterial contaminants encountered in brewing fermentation.
2.4.1.5 Contamination of Cultures with Wild Yeast

A wild yeast is any yeast, other than the culture yeast, that was unintentionally pitched. With breweries producing different types of beer, each with its own yeast or mixture of yeasts, it is important that cross contamination does not occur. Wild yeast can originate from a

Figure 2. Photomicrographs of typical bacteria found as brewing contaminants. Top – Pediococci and Bottom – Lactobacilli.
wide variety of different sources, from beer, brewing yeast, empty bottles etc. Figure 3 is a photomicrograph of wild yeast.

![Figure 3](image)

**Figure 3.** Photomicrograph of (A) wild yeast, and (B) brewing yeast culture contaminated with wild yeast.

In addition to various *Saccharomyces* species, species of the genera *Brettanomyces, Candida, Debaromyces, Hansenula, Kloecckera, Pichia, Rhodotorula, Torulaspora and Zygoschcharomyces* have been isolated. The potential of wild yeasts to cause adverse effects varies with the specific contaminant. If the contaminating wild yeast is another culture yeast, the primary concern is with rate of fermentation, final attenuation, flocculation and taste implications. If
the contaminating yeast is a non-brewing strain and can compete with the culture for wort constituents, inevitably problems will arise as these yeasts can produce a variety of off-flavours and aromas often similar to those produced by contaminating bacteria. Some wild yeasts can utilise wort dextrins (*Saccharomyces diastaticus* has also been discussed), resulting in an over-attenuated beer that lacks body. These yeasts are found as both contaminants of fermentation and at post-fermentation stages of the brewing process. In addition, as previously discussed, wild yeasts often produce a phenolic off-flavour due to the presence of the POF gene. However, under controlled conditions, such as in the production of a German wheat beer or “weissbier”, this phenolic clove-like aroma, produced when yeast decarboxylates wort ferulic acid to 4-vinyl guaiacol (Figure 4), can be a desirable attribute of the beer.

*Figure 4.* Decarboxylation of ferulic acid to 4-vinyl guaiacol by yeast.