YEAST MANAGEMENT – CULTURE HANDLING BETWEEN BREWING FERMENTATIONS

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Abstract

During most fermentation ethanol production procedures, a yeast culture is used only once for a single fermentation cycle. However, in brewing, the yeast culture is harvested at the end of a fermentation for re-use in a subsequent fermentation. In between fermentations, the cropped yeast is stored prior to being repitched. As a consequence of this procedure, the yeast culture must be carefully managed between each fermentation in order to maintain its quality. The steps in this yeast management procedure are discussed.

Keywords: acid washing, cropping, glycogen, high gravity wort, propagation, recycling, storage, trehalose, wort, yeast.

Introduction

The objectives of brewer's wort fermentation are to consistently metabolise wort constituents into ethanol, carbon dioxide and other fermentation products in order to produce beer with satisfactory quality, drinkability and stability. The other major objective is to produce yeast crops that can be confidently re-pitched into subsequent wort brews (Stewart and Russell, 2009).

It is worthy of note that brewing is the only major alcoholic beverage process that recycles its yeast. It is therefore, important to jealously protect the quality of the cropped yeast because it will be used to pitch a later fermentation and will have a profound effect on the quality of the beer resulting from it.Why traditionally breweries recycle their yeast culture, whereas other alcohol fermentation processes such as whisky production do not, is unclear. There is no doubt that fermented whisky wort with the yeast still present does contribute flavour characteristics to the immature spirit during distillation. Recycling of a brewer's yeast culture does simplify the process to some extent and reduces the total amount of yeast required. However, propagation, whether on site in the brewery or off site in another brewery consequently requiring transport between breweries, does introduce a degree of complexity and expense into the overall process.

The collective operations with yeast in between wort fermentations is designated as **Yeast Management** which will be discussed in this paper. It is important to emphasise that although considerable information is available about brewer's yeast fermentation *per se* (for example, Boulton and Quain, 2001; Sofie *et al*, 2010) by comparison, basic detailed information on yeast management processes between fermentations has been lacking. Indeed, although overall fermentation procedures and control have become very sophisticated, yeast management was, until recently, the "poor relation" of the process.

Yeast Management Step by Step

Yeast management can be divided into a number of overlapping procedures:

- Prior to propagation (the production of yeast biomass) after fermentation and cropping most (not all) yeast strains are stored under standard conditions in a brewery or in an accredited culture collection – sometimes both for security.
- Yeast propagation (biomass formation) in wort under aerobic conditions.
- Following propagation the yeast is pitched into wort. This is the first generation (cycle) of a multi-generation procedure.
- At the end of fermentation (attenuation), yeast cropping occurs followed by storage prior to re-pitching.
- In order to eliminate contaminating bacteria the yeast slurry can be acid washed. Also, sometimes, but less frequently these days, the yeast slurry is sieved to remove contaminating trub (coagulated protein-phenol solid material).

Storage of Yeast Stock Cultures Between Propagations

The advent of the use of pure yeast strain fermentation dates from the studies of Emil Christian Hansen (Figure 1) working in the Carlsberg Laboratory in Copenhagen during the latter decades of the nineteenth century. He isolated four separate strains from the Carlsberg lager yeast culture (Holter and Moller, 1976). He studied these four strains from the standpoint of overall brewery performance and only one of them proved to be suitable for beer fermentation. This strain, designated as "Carlsberg Yeast No. 1" was introduced into the Carlsberg Brewery in Copenhagen for use on a production scale on 13 May 1883 and pure strain brewing of lager beer can be considered to have commenced on this date (Holter and Moller, 1976). Carlsberg Yeast No.1 was named *Saccharomyces carlsbergensis* Hansen 1883. This grouping of lager yeasts is now known as *Saccharomyces pastorianus* (Pederson, 1995).

[FIGURE 1 NEAR HERE]

Recently, a research group from Argentina, Portugal and the USA published a paper entitled "Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast." (Libkind *et al*., 2011). This confirmed that *Saccharomyces pastorianus* is a domesticated hybrid yeast species created by the fusion of *Saccharomyces cerevisiae* with a previously unknown species that has now been designated *Saccharomyces eubayanus* because of its close relationship with the wine yeast *Saccharomyces bayanus.* They also reported that *Saccharomyces eubayanus* exists in the forests of Patagonia and was not found in Europe until the advent of trans-Atlantic trade between Argentina and Europe. The Libkind *et al*., (2011) paper contains a draft genome sequence of *Saccharomyces eubayanus* and it is 99.5% identical to the non-*Saccharomyces cerevisiae* portion of the *Saccharomyces pastorianus* genome sequence and supports specific changes in wort sugar and sulphate metabolism compared to ale strains that are critical for determining lager beer characteristics. This study provides us with a great deal of valuable information about the genomic structure of lager yeast strains. However, the proposal that the consistuent species, *Saccharomyces eubayanus*, is originally unique to Patagonia is still questionable!

With the advent of the use of pure yeast strain fermentation in brewing, Hansen soon found it was necessary to furnish the Carlsberg brewery with production quantities of pure cultures of the single lager strain and that it would be more convenient to develop a specific apparatus for the purpose of large scale yeast propagation. Consequently, in association with with a coppersmith (W.E. Jansen), Hansen developed an apparatus specific for the purpose. At the beginning of 1886, this apparatus was effectively working in the Carlsberg brewery and it was also working in a number of breweries including Heineken.

As a result of Hansen and Jansen's efforts, the practice of employing a pure strain in lager production was soon adapted by breweries worldwide, particularly in the USA. Ale-producing regions however, met this "radical innovation" with severe opposition and skepticism! The method was merely regarded as a means of reducing wild yeast and bacterial infection. It was not until the middle of the last century that the pure ale strain methods were adopted. Indeed, a few ale producing brewers have yet to adopt this procedure! Recently, Anderson (2012) has published a paper entitled: "One yeast or two? Pure yeast and top fermentations" which focuses on the reluctance for British ale brewers to introduce pure yeast in the production of top fermentation beers until comparatively recently!

The long term preservation of a brewing yeast strain requires that not only is optimal survival important, but it is imperative that no change in the characteristics of the yeast strain occurs. Hansen's studies resulted in storage of his strains in liquid nutrient media prior to propagation. This evolved into many breweries and independent culture collections maintaining their yeast strains on nutrient media solidified initially with gelatine and subsequently with agar. Some yeast strains are difficult to maintain in a stable state and long term preservation by lyophilisation (freeze drying), which has proven useful for mycelial fungi and bacteria (Kirsop and Doyle, 1990), has been found to give poor survival results with brewing yeast strains (Kirsop, 1955). However, as will be described later, the use of dried yeast for pitching into wort is increasing in popularity.

Storage studies have been conducted with a number of ale and lager brewing yeast strains (Russell and Stewart, 1981). The following storage conditions were investigated:

- Low temperature as a result of storage in liquid nitrogen (-196ºC). With the advent of -70ºC refrigerators in the 1980s, liquid nitrogen has been largely replaced for this purpose with similar results;

- Lyophilisation (freeze drying);
- Storage in distilled water;
- Storage under oil;
- Repeated direct transfer on solid culture media (subcultured once a week for two years);
- Long term storage at 21ºC on solid nutrient medium – subcultured every six months for two years;
- Long term storage at 4ºC on solid nutrient medium – subcultured every six months.

After a two year storage period, wort fermentation tests that included fermentation rate and wort sugar uptake efficiency, flocculation characteristics, sporulation ability, formation of respiratory deficient colonies and rate of survival were conducted and the results compared to the characteristics of the stored control culture. Low temperature storage appears to be the storage method of choice. However, there are capital and ongoing cost considerations connected with this method. Storage at 4ºC on nutrient agar slopes, subcultured every 6 months, was the next method of preference to low temperature storage and this method is simple to perform and relatively inexpensive. Lyophilisation and other storage methods revealed yeast instability which varied from strain to strain. Many breweries today store their strains (or contract store them) at -70ºC. Routine subculturing of yeast cultures on solid media every six months or so although being a less desirable storage method is still is an acceptable method. Freeze drying should be avoided as a storage method (Finn and Stewart, 2008) but its use for a pitching culture is becoming increasingly popular.

Yeast Propagation

The propagation of pure strain lager yeast cultures dates from 1890, has already been discussed in this paper and is an essential part of the brewing process. However, the yeast

propagation process is still not fully understood because of the yeast's facultative nature (ability to metabolise sugars in both aerobic and anaerobic conditions). It is important to be aware that brewing yeast strains do not live for ever and should be replaced with fresh yeast after they have been used to ferment wort a number of times (Nielson, 2005), which will vary depending on the yeast strain and the wort gravity. Compared with fermentation, the propagation of yeast has many similarities with the cultivation of baker's yeast.

A brewer's yeast strain (lager and ale) should possess good fermenting characteristics and also have the ability to grow rapidly. The problem with the growth of brewer's yeast strains is that they exhibit the phenomenon of catabolite repression which is also termed the Crabtree Effect [defined as: "aerobic ethanol formation (not biomass) at high growth rates under conditions of excess fermentable sugars"] which limits the yeast's ability to take up oxygen during propagation in wort. Also, in the last decade or so, there has been growing awareness that various factors during propagation may stress the yeast culture and consequently may stress the yeast and influence yeast vitality/viability and beer quality.

In a brewery, propagation is carried out in a batch reactor, with wort as the medium. This is basically the same medium that will be used later for fermentation into beer. Although wort gravities have been increased for fermentation, weaker worts are still more appropriate for propagation. The propagation medium used to produce yeast for the distilling and baker's yeast industries is usually molasses (sometimes hydrolysed whey) where the major sugar is sucrose plus a nitrogen source. Also, a fed batch reactor with a continuous supply of dilute substrate and intense aeration (oxygenation) is used to produce distiller's and baker's yeast. When propagation in a brewery is carried out in a batch reactor, the use of wort limits aerobic yeast growth in a concentrated sugar solution making it difficult to produce theoretical quantities of biomass. However, the brewing industry has chosen to tolerate this problem,

because optimizing yeast growth in a molasses/nitrogen medium could jeopardize wort fermentation properties and lead to poorer beer quality. Also, brewing focuses on strict sanitary conditions in order to avoid infection (the production of distiller's and baker's yeast is not completely asceptic) and to minimize yeast stress during propagation in order to avoid the negative effects on fermentation. It is worth repeating that brewer's yeast propagation is based on aerobic conditions and the extensive use of air or oxygen throughout the process. It differs extensively from brewing fermentations where oxygen is only required at the beginning of the process in order for the lag phase cells to begin to synthesize from glycogen (Figure 2) unsaturated fatty acids and sterols (Figure 3) which are important membrane constituents.

[FIGURE 2 NEAR HERE]

[FIGURE 3 NEAR HERE]

It is interesting to note that oxygen is only required at the following stages in the malting and brewing process:

- During barley germination during malting.
- For biomass formation during yeast propagation.
- At the beginning of fermentation when the yeast is pitched into wort.

At any other point in the process, oxygen can have a negative effect on beer quality particularly when there is dissolved oxygen in the packaged product leading to stale characteristics in the beer.

Yeast Storage

At the end of fermentation, the yeast is cropped for further use employing the flocculating characteristics of the yeast strain or with a centrifuge. However, in this discussion, yeast cropping is considered to be part of fermentation, not yeast management between fermentations. One method of yeast cropping that is increasing in popularity is the use of

centrifuges, although their use has not been without its problems (Table I) (Chlup and Stewart, 2011).

[TABLE I NEAR HERE]

If a cropped yeast culture is not stored properly, cell consistency will suffer and it will adversely effect fermentation and beer quality. Following cropping, the yeast is stored in a room that is conveniently sanitised, contains a plentiful supply of sterile water and a separate filtered air supply with positive pressure to prevent the entry of contaminants and a temperature of 0ºC. Alternatively, insulated tanks in a dehumidified room can be employed. Also "off the shelf" yeast storage facilities are available at various working capacities.

Yeast is predominantly stored under six inches of beer (sterile water has been employed in the past but its use is unpopular these days). When high gravity brewing is practised, it is important to ensure that the ethanol level of the storage beer is decreased to 4-6%(v/v) ethanol in order to maintain the viability and vitality of the stored yeast. As more sophisticated systems become available, storage tanks with external cooling (0-4ºC), equipped with low shear stirring devices, have become popular. The need for low shear stirring systems has been shown to be important. With high velocity agitation in a yeast storage tank, the yeast cell surface can become disrupted and intracellular proteases (particularly proteinase A) are excreted and this can result in unfilterable mannan hazes in beer (Stoupis *et al* 2002) and poor head retention due to protease hydrolytic activity on foam stability enhancing peptides (Cooper *et al*, 2000). There are procedures where the yeast is not stored between fermentations. In this case, the yeast is pitched directly from one fermenter to another. This yeast handling procedure occurs with cylindroconical (vertical) fermenters and is termed "cone to cone yeast pitching". The procedure was employed by some breweries in the 1980s

and 1990s but currently has limited application because of lack of opportunity and time to conduct contamination studies on the yeast between fermentations.

One of the factors that will affect fermentation rate is the condition under which the yeast culture is stored between fermentations. Of particular importance in this regard is the influence of temperature during these storage conditions on the cell's intracellular glycogen level. Glycogen is the major reserve carbohydrate stored within the yeast cell and similar in structure to plant amylopectin (Figure 2). It has already been discussed that glycogen serves as a store of biochemical energy during the lag phase of fermentation when the energy demand is intense for the synthesis of such compounds as sterols and unsaturated fatty acids (Figure 3). Consequently, it is important that appropriate levels of glycogen and trehalose (Figure 4) are maintained during storage so that during the initial stages of fermentation the yeast cell is able to synthesise sterols and unsaturated fatty acids and trehalose. Trehalose is a non-reducing disaccharide (Figure 4) that plays a protective role in osmoregulation, protection of cells during conditions of nutrient depletion and starvation and in improving cell resistance to high and low temperatures and elevated ethanol (Oda *et al*, 1986).

[FIGURE 4 NEAR HERE]

Storage temperature (Figure 5) has a direct influence on the rate and extent of glycogen dissimulation, as might be expected considering the effect that temperature has upon metabolic rates in general. Although strain dependent, of particular interest is the fact that within 48 hours, the yeast stored semi-aerobically at 15ºC has only 15% of the original glycogen concentration remaining. Glycogen reductions to this extent will have a profound effect on wort fermentation (Figure 6).

[FIGURE 5 NEAR HERE]

[FIGURE 6 NEAR HERE]

The number of times that a yeast crop (generation) is used for wort fermentations is standard procedure in a brewery. Typically, a yeast culture is currently used 10-20 times today prior to reverting to a fresh culture of the same strain from the pure yeast culture plant. If a particular yeast culture is used beyond the crop specification fermentation difficulties (for example, fermentation rate and extent are typical examples) are sometimes encountered. An example of this effect is when a brewery increases its wort gravity adopts high gravity brewing procedures. A particular brewing operation, over a 15 year period, increased its wort gravity incrementally. In order to avoid fermentation difficulties, it reduced the number of yeast crops from a single propagation:

12º Plato wort - > 20 yeast cycles

14º Plato wort - 16 yeast cycles

16º Plato wort - 12 yeast cycles

18º Plato wort - 8 yeast cycles

The reason why multiple yeast generations can have a negative effect on a culture's fermentation performance is unclear. However, multiple generations will result in reduced levels of intracellular glycogen and an increase in trehalose indicating additional stress conditions as the cycles progress (Table II) (Boulton and Quain, 2001).

[TABLE II NEAR HERE]

Yeast storage conditions between brewing fermentations can affect fermentation efficiency and beer quality. Good yeast handling practices should include collection and storage procedures including avoiding inclusion of oxygen in the slurry, cooling the slurry to 0-4ºC soon after collection and, perhaps most importantly, ensuring that glycogen levels are maintained because of its critical property at the start of wort fermentation.

Yeast Washing

Acid washing pitching yeast at pH 2-2.2 (with either phosphoric, tartaric, hydrochloric and nitric acid solutions) usually during the later stages of storage just prior to being pitched into wort for fermentation has been employed by many breweries for the past 100 years (and longer) as an effective method to eliminate contaminating bacteria (not wild yeasts) without adversely affecting the physiological quality of the yeast culture. The acid washing regime differs between breweries with some routinely acid washing their yeast after each fermentation, whereas other brewers only acid wash their pitching yeast when there is significant bacterial contamination and some refrain from acid washing completely. Brewer's yeast strains are normally resistant to acidic conditions when the washing procedure is conducted properly. However, if other environmental and operating conditions are modified then the acid resistance of the culture will vary. Simpson and Hammond (1989) demonstrated that if the temperature of acid washing was greater than 5ºC and/or the ethanol concentration was greater than 8%(v/v) acid washing had a detrimental effect on the culture causing a decrease in viability and fermentation performance. The physiological condition of the yeast prior to acid washing is an important factor in acid tolerance with yeast in poor physiological condition prior to washing being more adversely affected by acid washing than healthy yeast.

Acid washing primarily affects the yeast cell envelope with the physiological systems associated with both the cell wall and the plasma membrane, subsequently decreasing yeast vitality as measured by the acidification power test (Kara *et al,* 1988). Studies from this laboratory (Cunningham and Stewart, 2000) have reported that acid washing pitching yeast from high gravity (20º Plato) wort fermentations did not affect the fermentation performance of cropped yeast if it was maintained in good physiological condition. Oxygenation of the yeast at the start of fermentation stimulated yeast growth leading to a more efficient wort

fermentation and equally important in the context of yeast management between fermentations produced yeast that was in good physiological condition permitting it to tolerate exposure to acid washing conditions (phosphoric acid solution at pH 2.2). These data support the findings of Simpson and Hammond (1989) who concluded that yeast in poor physiological condition should not be acid washed.

In summary, the do's and do not's for yeast acid washing listed by Simpson and Hammond (1989) are still appropriate:

Acid washing of yeast can be summarised into the do's and do not's.

The Do's of acid washing are:

- Use food grade acid;
- Chill the acid and the yeast slurry before use to less than 5ºC;
- Wash the yeast as a beer slurry or as a slurry in water;
- Ensure constant stirring whilst the acid is added to the yeast and preferably throughout the wash;
- Ensure that the temperature of the yeast slurry does not exceed 5ºC during washing;
- Verify the pH of the yeast slurry; and
- Pitch the yeast immediately after washing.

The Do Not's of acid washing are:

- Do not wash for more than two hours – very important;
- Do not store washed yeast;
- Do not wash unhealthy yeast; and
- Avoid washing yeast from high gravity fermentations prior to dilution.

There are a number of options to acid washing brewer's yeast:

- Never acid wash yeast;
- Low yeast generation (cycle) specification;
- Discard yeast when there is evidence of contamination (bacteria and/or wild yeasts);
- Acid wash every cycle, this procedure can have adverse effects on yeast; or
- Acid wash when bacteria infection levels warrant the procedure.

Summary

It is important to jealously protect the quality of the cropped yeast between fermentations because it will be used to pitch a later fermentation and will have a profound effect on the quality and stability of the beer produced with it.

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