In this era of accountant-led brewing companies it is perhaps sometimes forgotten that, along with the workforce, yeast is the single most precious asset that the company owns. Of course, all stages of the brewing process contribute to the final product; nevertheless, it is a sobering thought, or at least it should be, that without the transforming abilities of yeast we would all be left with nothing much more than very sticky fingers and a lot of unhappy customers.

There may be some shortcuts that can be applied to the brewing process; however, these do not extend to cavalier treatment of yeast. How does this stack-up against the backdrop of modern brewing practice? Well, brewing yeast is a very versatile organism. It possesses a genome that allows it to adapt rapidly and thrive under a wide variety of environmental conditions. It has an armoury of mechanisms for dealing with applied stresses. This is just as well since the brewery yeast cycle is characterised by step changes in external conditions and each step imposes stresses which need to be tolerated if the process is to perform to specification (See Table 1).

Much of the modern large capacity brewing industry is understandably focused on identifying methods for reducing revenue and Capex costs. In the fermentation stage this has resulted in ever larger batch sizes, very concentrated worts and commonly elevated fermentation temperatures. The effect of these trends has been an exacerbation of most of the effects listed in Table 1 and a common observation is that the yeast is beginning to show signs of buckling under the strain.

The data in Figure 1 shows a Cusum plot of average pitching yeast viability for production scale high-gravity lager fermentations over a period of several months. Predictably the reduced yeast viability was accompanied by the other undesirable markers of compromised yeast health such as elevated beer pH and poor foam performance.

**Current best practice**

The intention of this article is to describe how fermentation management and yeast handling needs to be adapted to avoid common pitfalls and meet the needs of the modern industry. Those suppliers that are contributing to these needs are highlighted.

The aims of fermentation are to produce green beer of consistent and desired analysis at maximum yield and within a consistent and preferably short cycle time. In order to support this, yeast handling must deliver pure pitching yeast of the correct strain at the right time, in the desired quantity and in an appropriate condition. These requirements can only be met if all the links in the chain that lead from wort to green beer are robust and subject to continual scrutiny.

Yeast handling in the brewery must be underpinned by a robust yeast supply system. Whether master cultures are held by the brewery or supplied by a third party, the gold standard method of preservation is storage in liquid nitrogen. Recovery of cultures from cryogenic refrigerators, generation of working cultures and assessments of strain purity is perhaps one of the last areas in the brewery where automation has not yet been proven able to dispense with skilled operators.

The principal task of the yeast supply

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**Table 1: Conditions and potential stresses to which yeast is exposed in normal brewing practice**

<table>
<thead>
<tr>
<th>Brewing stage</th>
<th>Implications</th>
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<tbody>
<tr>
<td>Storage tank as slurry in beer</td>
<td>Low temperature</td>
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<tr>
<td></td>
<td>Starvation</td>
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<td></td>
<td>High ethanol</td>
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<td></td>
<td>Anaerobiosis</td>
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<tr>
<td>Post-pitching</td>
<td>Up-shift in temperature</td>
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<td>Shift to aerobiciosis</td>
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<td></td>
<td>Osmotic shock</td>
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<td></td>
<td>Exposure to nutrients</td>
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<tr>
<td>End-fermentation</td>
<td>Starvation</td>
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<td></td>
<td>Anaerobiosis</td>
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<td></td>
<td>High ethanol</td>
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<tr>
<td></td>
<td>Elevated pressure</td>
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<tr>
<td></td>
<td>Warm temperature</td>
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<tr>
<td>Cropping and return to storage</td>
<td>Down-shift in temperature</td>
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<tr>
<td></td>
<td>Potential for selection of variants or undesirable portions of crop</td>
</tr>
<tr>
<td>Propagation</td>
<td>Growth on wort under warm, aerobic but repressed conditions</td>
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</tbody>
</table>
system is to deliver periodically to the brewery a pure culture of the correct strain. In large part this requires the use of classical microbiological techniques; however, the only definitive method of strain assurance is one based on genotyping. This remains a somewhat onerous task requiring expensive and specialised equipment and skilled operatives. The majority of brewing laboratories are not equipped to perform these tests and will usually depend on the services of third party suppliers such as Cara Technology (www.cara-online.com).

The importance of assuring that the correct strain is available for brewing cannot be over-emphasised. It is well-attested that the modern brewing process may cause the formation and selection of genetic variants. It is important to recognise this and where non-standard performance is observed and in the absence of other more obvious causes a check on strain identity is recommended.

Yeast propagation
The requirements of propagation can be defined as the need to introduce into the brewery, in a timely fashion, a fresh culture of guaranteed identity and purity, of consistent and defined physiological condition, with a high viability and in a quantity sufficient to meet the specified pitching rate of the first production scale fermentation. Preferably the first generation fermentation will have a standard cycle time and yield in-specification beer which will not require blending.

The modern brewing process places great demands on propagation plant. In view of this it is perhaps surprising that many breweries are happy to invest capital in new fermentation plant but not to upgrade already stretched propagators. The stressful conditions to which yeast is exposed as a consequence of modern fermentation practice has already been alluded to. One of the responses to this has been to place ever lower limits on the maximum number of time yeast cultures can be serially re-pitched and less than ten generations is now commonplace.

Couple this with the requirement for greater quantities of yeast to cater for large capacity vessels fermenting high gravity worts and it may be appreciated why proper attention needs to be paid to this area of practice.

There remains a lack of agreement as to what the physiological condition of newly propagated yeast should be. Perhaps it should mirror pitching yeast which may be defined as stationary phase, sterol and unsaturated fatty acid-depleted and with a ‘fermentative’ phenotype.

Without undergoing a cycle of fermentation it is not possible for newly propagated yeast to duplicate this precisely. A practical approach to propagation is to ensure that conditions are repressing by using wort as the feedstock and terminating the process before full derepression can occur but to maximise cell yields by providing continuous aerobic conditions and short cycle times by using a relatively warm temperature. Terminal cell counts of 180 – 200 x 10^6 cells per ml may be achieved within 24 – 36 hours using continuously aerated malt wort and an incubation temperature of 25°C (See Figure 2).

Aside from the requirement for scrupulous attention to be paid to hygiene, the key aspect of a modern propagator is the requirement to deliver a continuous supply of dissolved oxygen to the growing yeast cells. This requires the plant to have a means of efficiently transferring oxygen from the gas to liquid phases. In practice this means supplying pure and sterile gaseous oxygen at an appropriate rate and then providing high rates of agitation to ensure that the gas is dispersed in the liquid in the form of very small bubbles in order to maximize the surface area of the gas liquid interface.

Companies such as ScandiBrew (Alfa Laval) provide propagation yeast equipment where they have paid proper attention to the critical issue of oxygen transfer. Using similar equipment it is possible to use step-up ratios of around 12:1 for individual stages of the plant and a final stage with an operating volume of approximately 150hl would be capable of supplying sufficient yeast to pitch around 2000hl of high gravity wort.

Nevertheless, using propagation plant of the type described it remains a common observation that the first generation fermentation is slower than standard and produces beer that requires blending; and this despite the fact that correct pitching rates are achieved and the yeast being of high viability and vitality. It appears that the timing and control of conditions at the end of propagation might be influential and work continues to identify what these should be.

It has been suggested that fed-batch propagators might be applied to the supply of pitching yeast. This approach requires a highly efficient system of aeration and a separate supply of sugar which is fed into the culture at an exponentially increasing rate. This ensures that at any given instant the sugar concentration is very low and in consequence conditions remain de-repressing. The result is to produce very high yields around five-fold greater than can be achieved with a conventional propagation plant. This approach is already in use for the production of active dried yeast. There is no reason why it should not be applied in a brewery albeit with some Capex spend and the requirement to provide a suitable feed-stock. Yeast cultivated in this manner has a very high sterol / unsaturated fatty acid content and in consequence should have no requirement for wort oxygenation. Whether or not yeast grown in this way will perform in a satisfactory manner remains open to question but undoubtedly the advantages of this method of propagation merit further investigation.

Initiating fermentation
Very large capacity fermenting vessels require to be managed with great care in order to obtain predictable performance and a consistent product. It is usual to describe fermentation control in terms of the pitching rate, dissolved oxygen tension, initial wort concentration and temperature. In a modern brewery all of these parameters can be controlled with adequate precision and reproducibility. With regard to the control of pitching rate the introduction in the 1990s of the Aber in-line yeast biomass monitor (Aber
Instruments) produced a demonstrable step-change improvement in fermentation consistency (Figures 3,4). It remains current best practice.

Despite the advances that have been made in regulating the essential fermentation control parameters, the commonly encountered imbalance between the brewhouse and fermentation plant introduces many pitfalls that require careful negotiation. Where filling times are prolonged and require several individual batches of wort yeast will move out of the lag phase before all the wort is collected (Figure 5). Thus, at typical fermentation temperatures yeast will move out of lag phase in around 6–8 hours. Although superficially little activity may be evident in the lag phase, in actuality it is a period of intense change to which subsequent rates and extents of growth and the formation of flavour-active yeast metabolites are inextricably linked.

The practical significance of this is that the yeast genome will react as soon as it is removed from storage and exposed to the relatively warm and oxygenated wort. It follows that in order to obtain a predictable and consistent response it is necessary to ensure that the genomes of all the yeast cells are simultaneously triggered into action. This requires all the yeast to be pitched at the same time and preferably dispersed rapidly.

By way of illustration it is illuminating to consider the reaction of pitching yeast to exposure to oxygen Figure 6.

The figure shows that during the first 2–3 hours after exposure to oxygen the rate at which yeast consumed oxygen increased by more than four-fold. In the case of yeast being pitched over a prolonged period of time it may be appreciated that the later pitched yeast would not be likely to compete for oxygen with that added earlier. Bearing in mind the role of oxygen in such biochemical processes as sterol and unsaturated fatty acid synthesis as well as the formation of esters it may be appreciated that this is likely to be undesirable and a potent source of inconsistency. It is likely that similar observations would be made with regard to other essential nutrients.

**Active fermentation**

During active fermentation it is essential that all yeast cells have equal opportunity to interact with the external environment; in other words, to be exposed to similar conditions of temperature, pressure and other pertinent parameters and ability to assimilate nutrients and export end-products of metabolism. A pre-requisite is that conditions be homogenous throughout the fermenter. In work described previously in this publication (The Brewer and Distiller International, July 2007 and May, 2008) it has been shown that using traditional methods of management for much of active fermentation conditions are distinctly heterogeneous.

This is understandable in that it is usually considered necessary to allow yeast crops to form at the appropriate time. However, it can be demonstrated that for many large-capacity fermenters sedimentation of yeast begins much earlier than is usually imagined. The consequences are that fermentation rate and extent is to a greater or lesser extent impeded and that premature sedimentation results in an increase in temperature in the cone, losses in viability and a potential for deleterious effects on beer foaming potential and flavour. These effects can be ameliorated by the application of mechanical agitation. Application of agitation ensures that yeast dispersion is rapid and efficient immediately after pitching and it prevents premature sedimentation.

A suitable system based on a pumped loop and a high-efficiency but low-shear rotating jetter head is supplied by IsoMix (part of Alfa Laval) or the static GEA Brewery Systems Ecoferm unit. Using these systems at the production scale, dramatic improvements in fermentation consistency coupled with reduction in cycle times by up to 30% have been observed (See Figure 7).

**Yeast cropping**

Undoubtedly the conditions experienced in the cone in a large fermenter are hostile to yeast. Apart from attenuation being difficult, ethanol concentration is high as is hydrostatic pressure. Early cropping is desirable and provides demonstrable benefits in terms of yeast health (See Figure 8).

The cropping process requires careful setting up in order to ensure efficient separation of yeast from green beer. Production constraints demand that the process is completed in a timely fashion; however, if pumping is too vigorous the centre of the yeast plug may be pulled out first with the result that the desirable middle cut may be contaminated with cold break. Pumps should be low shear types to prevent possible damage to yeast. Although several types of pump may be used the peristaltic types supplied by companies such as Watson-Marlow (www.watson-marlow.co.uk) have been shown to be very effective. Control of cropping can be controlled very effectively using the Aber yeast monitor. Coupling process flow to the output of viable yeast concentration provides a practical method for ensuring that the best
Yeast handling

Figure 8: Cusum plot showing the trend in average viability of yeast at pitch for production scale lager fermentations with respect to time. The arrow indicates the point at which a system was introduced for early and warm yeast cropping (cf. Figure 1).

fraction of the crop is retained for re-pitching purposes.

End of fermentation
For most lager fermentations the end point is defined in terms of the achievement of a specified VDK concentration. Typically this will be linked to the timing of cropping in order to ensure that sufficient yeast is available to remove diacetyl and possibly the application of cooling.

The dynamic of this passive approach is changed where a system of mechanical agitation is available since the brewer has the choice of when to discontinue stirring and allow the yeast to crop (Figure 9). It may be seen that when the agitation was switched off, at around 95 hours, the yeast very rapidly dropped out and accumulated in the cone, as evidenced by the increase in yeast concentration measured by the cone probes and the concomitant decrease in that from the body probe. Interestingly, the formation of the yeast crop and presumed depletion in the suspended yeast count had no apparent effect on the observed decline in total VDK concentration.

Storage of pitching yeast
In the interval between serial fermentations pitching yeast slurries must be stored in a manner that preserves viability and minimizes physiological change. The key parameters are maintenance of low temperatures (2-4°C) prevention of oxygen ingress (and microbial contamination) and limiting the storage time to no more than one or two days. Storage vessels with jacket cooling require gentle agitation to aid atemperation and ability to apply top-pressure with an inert top gas. It is essential that the capacity is sufficient to service the fermenters such that the whole of the slurry required to achieve the desired pitching rate can be accommodated. Where warm cropping is practised it is desirable to have an in-line cooler between the fermenter and storage vessel. Alternatively, if the yeast is to be re-pitched within a few hours storage at fermentation temperature is acceptable and probably less stressful to yeast.

Design and operation of plant clearly must go hand in hand. Care is required where process changes are introduced. Dedicated pitching vessels are commonly used and these are often provided with facilities for acid washing. The latter practice, which might be considered treatment of a symptom rather than a cause, requires very careful control to avoid damage to yeast. As with storage tanks, pitching vessels must have an appropriate capacity. Where pitching is controlled by an Aber system it is better to locate it between the storage and pitching tanks. This avoids having to dispose of a surplus after sufficient has been dosed into wort by the pitching system. It is not unknown for this additional yeast to be simply pitched as a method of ‘disposal’, a quick way of negating the benefits of a costly yeast handling system.

How many generations?
Limits which are placed on the maximum number of times a yeast culture can be serially re-pitched are arbitrary. Probably they result in perfectly good yeast being sent to waste and do not prevent potentially unsatisfactory yeast from being used. It can be demonstrated, as described here, that many of the stresses to which yeast is exposed as a result of current practice can be managed such that high viabilities are maintained. Providing that this is so, the major remaining threats are that cropping procedures may select for an ageing (deteriorated) population or a mutant strain. It is true that crops from agitated fermentations may have different inhomogeneities compared to those which form naturally in unagitated vessels.

Nevertheless, it is likely that non-yeast solids and larger (older) cells will still form the first cut of a crop and this can be discarded. Despite the plethora of published vitality tests which can be applied to yeast, none appears to offer much useful information over the simple methylene blue staining test. There remains a need for a simple, rapid and preferably inexpensive genotyping test. If such were available it would be possible to confirm the identity of pitching yeast and, providing previous performance and viability were satisfactory, re-pitching should be possible irrespective of generational age.

Batch sizes – is big always beautiful?
The productivity of a batch process can be improved simply by increasing the batch size. In the case of fermentation vessels this makes sound economic sense since the cost of the additional stainless steel for a larger tank represents a comparatively small proportion of the total outlay. However, the installation of bigger tanks requires that all the component parts of the yeast handling plant are sized accordingly.

Where piecemeal upgrading of facilities has occurred some degree of mismatching is inevitable and severe discrepancies are responsible for many of the inconsistencies in overall performance which have been touched on already in this article. Wherever possible it makes sense to treat fermentation vessels and all of the ancillary plant as an integrated whole and make sure that all parts are upgraded accordingly. This applies not only to obvious components such as the yeast storage and propagation tanks but also to the pumps, valve blocks and pipe-work which support activities such as pitching and cropping. Companies such as GEA Brewery Systems (www.gea brewery.com) excel in this regard.

Batch sizes are usually regulated by production demands. Many small tanks make sense where the portfolio is diverse and variable. Bigger vessels are more suited to the brewing of one, or only a few, brands. The actual capacity of fermenters, not to mention the aspect ratio, is still usually dictated by more obscure drivers such as tank farm footprints and the ease of transport of large loads from the fabricator to the brewery. Very little attention appears to have been paid to the optimum requirements for yeast cells to convert wort into beer in a consistent and predictable manner. Perhaps this is a subject worthy of more fundamental research.