Modern ways to measure yeast in craft breweries

The market share for craft breweries has grown considerably over the last few years. In addition, craft breweries are known to experiment with uncommon ingredients and techniques, thus guiding innovation in the industry. Market expansion and continuous process innovation underlines the need for more sophisticated yeast management systems.

by Dr Aditya Bhat and Katy Thomson
Aber Instruments Ltd, UK

Many of the breweries expand their capacity by installing an additional large fermenter(s). For yeast management, this might lead to a shortage of yeast brinks or a requirement to adopt cone-to-cone pitching techniques. Another side to expansion is investment in more space, which is not always possible. Therefore, strategies that could reduce variability in fermentation times, and which can help plan more accurately are invaluable.

Yeast pitching basics
Consistent fermentation performance and beer quality are heavily influenced by the accuracy of reliable dosing/pitching the exact amount of live yeast into each fermenter.(1, 2, 3). The beer style and yeast strain dictates the actual amount of yeast the brewer should add. Precise regulation of pitching is the key to ensuring consistent performance in terms of fermentation cycle times, extent of yeast growth, the related efficiency of extract conversion and the formation of yeast-derived beer flavour components.

Over or under-pitching of yeast is one of the major hindrances to success in craft breweries and can lead to high wastage, poor yeast management and increased batch-to-batch variation. Specifying how much ‘live’ yeast should be added accurately and consistently, based solely on know-how and formula tables is extremely challenging. Traditionally, the cell concentration in the pitching yeast slurry is analysed via yeast spun solids measurements (corrected for viability) or is based on cell counts.

Viability correction, if performed, is typically carried out by counting methylene blue stained yeast cells under the microscope. While spun solids estimation is prone to error due to the presence of variable amounts of non-yeast solids, estimation using methylene blue stained cells is highly inaccurate because of the inherent difficulty in manually counting large number of cells. The presence of variable amounts of trub in yeast slurries means that under-pitching is the most likely result. This is a cause for concern, but is probably out-weighted by the poor repeatability of manual analyses. Inter-operator differences with manual counting are well known and in absolute terms, the precision of manual counting methods is no better than +/-20%2,3.

Automation could eliminate problems associated with manual techniques. The Aber Yeast Monitor, an on-line instrument that measures the live yeast cell concentration, can be used to dose exact amounts of live yeast into the fermenter and is known to reduce the error to around +/- 2 to 5%. The Yeast Monitor is useful to monitor and control the exact quantity of live yeast that is added to the fermenter.

Some of the larger craft breweries have created customised yeast dosing systems using the Yeast Monitor. However, this may not always be possible in a typical expanding craft brewery. Firstly, the brewery could have several fermenters and yeast storage vessels located around the site. Secondly, it may not have access to other equipment necessary for automating the operation. Add to that the challenge of taking a representative sample of the pitching yeast from the bottom of the cone of the fermenter, when cone to cone pitching is in play.

Therefore, there is a definite need for a portable yeast dosing skid that:

• can be connected to any yeast storage vessel or fermenter
• has an integrated local PLC and flow meter in its design
• can be connected by hoses to the two cones of a fermenter for cone-to-cone pitching.

Aber Instruments (UK) introduced the first portable skid, the PerfectPitch, in 2014. The skid comprises of a Yeast Monitor, a flowmeter and a mini PLC together with pipework for connection to hoses and a sampling port (Figure 1). The skid is also designed to undergo CIP and is IP65 rated.

How does the Aber PerfectPitch work?
The Yeast Monitor probe works on the proven principle of radio-frequency impedance measurement. The capacitance measured by the Yeast Monitor in the yeast slurry is directly proportional to the amount of live yeast present. This capacitance can be represented either as % live spun solids or as live cells/ml. The output is linear over a wide concentration range.

A preset target concentration is entered into the mini PLC in the form of a recipe. The Yeast Monitor and the flowmeter communicate with the mini PLC to ensure the correct concentration of live yeast has been pitched into the fermenter.

Figure 1: The Aber PerfectPitch yeast pitching skid
A beacon on the skid comes on when the preset target concentration is reached, which can be used as an indicator to switch off the pump, if required.

The first PerfectPitch system was installed at the Meantime Brewery, London (UK) in January 2014. Prior to using the yeast pitching skid, Meantime Brewery dosed the yeast slurry using mass. It was observed that the viable yeast concentration was overestimated when pitching using mass, thus pitching fewer litres than necessary. This led to slower fermentations and inadequate fermentation performance. In contrast, the portable skid estimated the right amount of live yeast, thus pitching the appropriate litres of live yeast necessary for an improved/more consistent fermentation performance (Figure 2).

Laboratory Yeast Counting

Not all craft breweries are either large enough or in a position to adopt automatic yeast pitching systems, but there is still a requirement for accurate and consistent yeast counting and viability estimation in the laboratory. Present methods may include manual cell counting using a haemocytometer or plate counts, which are labour intensive and error-prone. In general, the method used for lab estimation of yeast concentration should:

• be simple and quick to use
• work with safe dyes
• potentially be used by process operators
• be accurate and consistent between operators
• be comparable with traditional microscopic methods.

A number of automated yeast counters are available in the market and each method has its own specific benefits. For craft breweries, some of the instruments are prohibitively expensive – either for the main instrument, the consumables or both. The approach taken by Aber Instruments for laboratory testing is to offer two alternative techniques, both offering unique benefits. The first method uses the principle of radio-frequency impedance in the Compact Lab Yeast Analyser or the CLYA (figure 3). The benefits of the CLYA include:

• no inter-operator differences
• provides live yeast measurement in the form of % spun solids or cells/ml

On the other hand, the CLYA does not provide a % viability measurement. The instrument works well above a few million cells/ml of yeast and there is no upper limit.

Conventional methods to measure viability of yeast samples in a brewery include plating or manual cell counting. Both these methods can be labour-intensive/time consuming and may introduce potential sources for human error through making up serial dilutions, counting cells and distinguishing viable from non-viable cells. Manual cell counting is also heavily subject to inter-operator variability for the same yeast sample.

There is currently a shortage of technologies that can count yeast through automated image analysis without the use of fluorescence. Fluorescent dyes, seen in the majority of automated cell counters, are unfavourable as they are more hazardous to work with and quickly quench. Conventional dyes are more preferable because they are not as toxic, remain stable and are less expensive. In addition, viability estimation using fluorescent dyes tends to be a two-step process, either through analysing two separate samples with one dye, or applying two dyes to the same sample. This means a longer analysis time and higher consumable usage.

The Countstar (Figure 4), the second option provided by Aber instruments, is an automated yeast cell counter that provides users with a % viability measurement, along with a range of other yeast parameters such as live, dead and total cell concentration in cells per ml (cpm), cell size and % aggregate, within seconds. It is a slide-based technology that uses bright field image analysis and conventional dyes with advanced user friendly software to analyse yeast samples.

The instrument is plug-in-and-play and requires no regular maintenance. Individually packaged disposable plastic slides (with five sample chambers) have been designed for the Countstar. Having multiple chambers means each slide can be used to analyse five samples. This is an advantage, as it lowers cost and waste produced for each individual test. Viability of yeast is determined by simply mixing the sample with a cell stain, such as methylene blue, before loading into a chamber. The instrument takes around 10-20 seconds to analyse an image for a variety of parameters, as mentioned before.

Another advantage of using the Countstar is that it uses the same dye exclusion technique as the conventional methods used to determine viability in the majority of brewing labs. This process distinguishes between live and dead cells within a sample, since dead cells take up the stain whereas live cells are left colourless[4, 5]. The intuitive Countstar software is capable of making the distinction between the stained and unstained cells, circling live cells green and dead stained cells yellow, to produce a % viability of the sample for the user.

As the Countstar automates the process of cell
counting, the majority of the previously mentioned problems associated with manual counting are alleviated.

The Aber Countstar has been shown to perform very well in studies to test measurement repeatability. Figure 5 represents the live cells/ml of yeast when measured using manual counts with the haemocytometer, the Countstar and the CLYA. It is apparent that the cell concentration measured by the Countstar was comparable to the haemocytometer, but the former showed a much lower variability (evident from the error bars). This test was performed by a single operator.

Figure 6 highlights inter-operator variability for viability measurement determined using manual counts and the Countstar. It is clear from the figure that a much higher variation was seen when four users perform viability estimation for yeast samples using the haemocytometer. This inter-operator deviation is greatly reduced with the Aber Countstar. Some variation can still be seen, which is attributed to the intrinsic variation within the sample, dilution procedure and efficiency of mixing the solution before loading it onto the slide.

Additional tests demonstrated that the Countstar compares well with manual cell counts, and provides reliable, accurate and repeatable measurement between 5 x 10^4 and 3 x 10^7 cpm, the recommended concentration range for analysis with the Countstar.

Knowledge of yeast cell size in a brewery can be a very handy tool to understand more about the process and quality of yeast. Estimation of cell size using manual methods is even more time-consuming and error prone than determining cell concentration/viability. The Countstar can provide brewers with reliable yeast cell size analysis in a matter of seconds. When performed manually, the cell size result is almost always an average of a few cells, whereas the Countstar expresses a cell size averaged from hundreds of cells within the sample.

The software displays the average cell diameter along with a more detailed diameter histogram of the population. Tests at a reputed brewery (data not illustrated) showed that the cell size estimated using the Countstar was comparable with that recorded manually, while being less labour intensive, quick and more repeatable.

The Countstar also provides the user with other useful information, including percentage of cells in aggregates, which could be useful for understanding the behaviour of yeast during the process better. The Countstar software can automatically save data and images for future reference, perform simple data analyses and export data to Microsoft Excel, making it a valuable tool in the Countstar system.

Summary
Up until the late 1980s, automation options for the brewing industry were sparse. When available, the larger breweries had the necessary resources to adopt automation to monitor and control their process better. Understandably, taking up automation was a challenge for the craft breweries then, primarily due to cost issues. However, the automation options available today, both online and offline are much more affordable for the larger craft breweries to implement and benefit from. Instruments such as the PerfectPitch, the CLYA and the Countstar can go a long way into bolstering the present trend of craft brewing expansion and innovation.

References