CDR Paper 1804

The importance of proper yeast recovery

Methods for determining the quantity of live cells (viability) and their vitality

Simone Bellassai Chemist - Enologist and Food&Beverage analysis expert at CDR – **Lisa Mearelli** Researcher at CDR Chemical Lab "Francesco Bonicolini"

During the fermentation phase of the beer production process, the action of yeast is essential for the transformation of sugars and amino acids in must into alcohol.

The addition of yeast is essential when looking to achieve optimal fermentation, because yeast is naturally present in raw materials and is fully denatured during the crushing phase.

Why reuse yeast

Yeast is a raw material which must be purchased. The cost of yeast increases in reverse proportion compared to the quantity of beer being produced. Therefore, and particularly in the production of craft beer, purchasing yeast significantly affects production costs and some brewers reuse yeast in order to cut costs.

The re-usage of yeast may also result in better and more interesting types of beer.

Indeed, given the chance, yeast reproduces several times and evolves into a population of cells that is actually "different" from the one used in the initial inoculation, resulting in significant changes to the beer's organoleptic qualities, with excellent results.

How to obtain yeast suitable for reuse

In order to obtain yeast suitable for reuse, it is necessary to recover fermentation residue which deposits on the bottom of the vat (slurry), containing yeast, coagulated proteins, hop remains and impurities removed from beer must during the fermentation process.

Two procedures are used for the effective removal of all impurities from yeast, once the slurry is separated from beer must: *rinsing* and *washing*.

In *rinsing,* yeast cells are freed from hop residue, coagulated proteins and remaining impurities. This is thanks to the dilution of the residue with water exploiting the different weight of slurry components.

In *washing*, acid or other similar chemical compounds are used to reduce the number of active lactic bacteria normally present in a recovered yeast sample thus presenting a serious threat to beer must, even before fermentation has begun. The *acid washing* procedure has different effects on various yeast strains and it may even reduce performance.

A yeast ready for immediate use

In addition to previously mentioned positive aspects in terms of cost reduction and improved beer quality, the yeast recovery, renewal and reuse process also presents advantages in terms of the condition of yeast after recovery. For preservation purposes, commercial yeasts are dried so they require rehydration for a few hours before resuming metabolic activity. Yeasts recycled from previous processes are already hydrated and above all, they are active, ready to

resume their metabolic state as soon as they come into contact with a suitable substrate.

Attention to the efficiency of recovered cells

The reuse of yeasts requires particular attention to recovered cell efficiency, in order to ascertain just how much sediment is required to achieve suitable fermentation for the type of beer you are looking to produce.

Indeed sediment contains live yeasts, ready for use, as well as dead or dying yeasts which are no longer usable and make up what is essentially waste material. It is not actually possible to ascertain the ratio of both types of yeast cells.

Live cell count, or viability

Determining viability, the number of live cells in yeast sediment available for reuse, is a solution to this problem.

Correct fermentation requires a viability of no less than 90%, although some brewers do use samples below this threshold, compensating for a lack of vital cells by adding a greater quantity of recovered yeast.

This may indeed be a winning strategy, but it can also cause problems during fermentation. The overall health of inoculated yeast cells may be so poor as to preclude successful fermentation, resulting in unpleasant aromas and undesirable characteristics for the beer, such as pronounced acidity or unexpected flavours.

This happens because increasing the quantity of reused yeast added to must may also result in the addition of dead or dying cells (as of now it is not possible to separate them from live ones), which negatively affects beer characteristics.

Increasing the percentage of dead cells compared to live ones may also prolong fermentation time, leaving beer exposed to a greater quantity of polluting agents.

Yeast Vitality

In order to resolve these issues, it is important to assess recovered yeast in terms of VITALITY.

Vitality analysis indicates yeast cell health, enabling us to ascertain to what extent cells are capable of feeding and reproducing so that alcoholic fermentation can take place. In short, Vitality is a parameter which measures the metabolic activity of yeast. If yeast is healthy, strong and ready for fermentation, then the sample is said to have a high level of vitality. This parameter is crucial because it is indicates fermentation potential.

A sample of recovered yeast in an excellent state of health presents a vitality level in the range of 2 to 2.7.

To date, methods recognised and used for testing viability and vitality are based on three general principles: loss of yeast cell replication capability, loss of metabolic activity and cell damage.

The method currently used for measuring viability is based on vital colourants, like methylene blue: yeast cell wall integrity is measured according to impermeability to the colourant. Cells are checked under an optical microscope in a successive phase: a lack of colour indicates good viability.

The recognised method for testing vitality, the *acidification power test* (AP TEST), is based on pH changes to the solution containing the yeast being tested. Here follows a description of the procedure:

1. Calibrate the pH-meter before taking each series of readings;
2. Adjust pH of deionised water to 6.5;
3. Pour 15 ml of deionised sterile water into a conical 50 ml centrifuge test tube containing a conical stirrer;
4. Monitor water pH while shaking the test tube for approximately 5 minutes;
5. After five minutes measure and record pH value (AP0) and add 5 ml of pre-washed and concentrated recovered yeast residue ($1x10^9$cells/ml) to the centrifuge test tube.

CDR BEERLab®

6. Shake for 10 minutes and record pH value (AP10);
7. Immediately add 5 ml of 20% glucose solution;
8. Shake for 10 minutes and measure final pH value (AP20); Acidification power is obtained by calculating the difference between AP20 and AP10 values;
9. Repeat the entire procedure twice more to confirm results.

It is obvious from reading the AP TEST procedure that this method requires specific equipment which can only be found in a well-equipped laboratory, as well as trained personnel. It is labour intensive and time-consuming.

This test requires a lot of equipment, including a pH-meter, which must be calibrated and thoroughly cleaned every single time before use, not to mention meticulous and extensive maintenance operations.

Breweries are unlikely to have all of this, which is why they often turn to external chemical laboratories, inevitably incurring costs, in order to check whether they can reuse yeast recovered from previous processes.

This is why CDR has developed an alternative to the AP test, for an altogether simpler and faster analysis procedure, based on the same chemical-physical principle.

Determining vitality using the CDR BeerLab® method

The test developed by CDR involves assessing pH variation of the solution to which the yeast sample is added, with the use of reagents containing chromogenic beer substances. This chromogenic substance changes colour when pH varies, thus enabling the measurement of pH by means of Spectrophotometric readings taken using the CDR BeerLab® system.

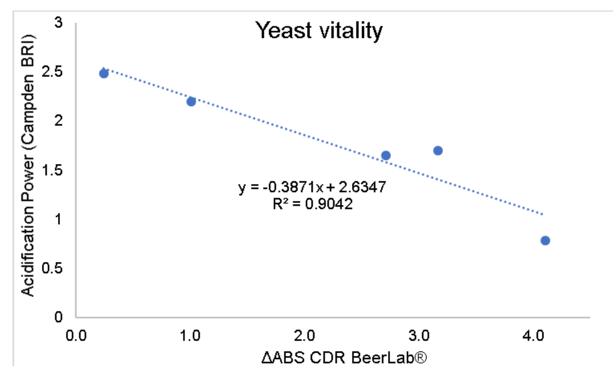
CDR BeerLab® provides test results in *Acidification Power*, thanks to the calibration curves it is equipped with.

Unlike the AP test, the CDR method does not require any calibration or use of specialised laboratory personnel. Test tubes are pre-filled with reagents in required quantities, ready for use. The result is obtained by simple photometer readings.

Correlated with the A*cidification Power test*

The CDR method ensures an optimal level of correlation with the recognised method for measuring vitality, the *Acidification Power test*, as established by the international laboratory of reference, Campden BRI.

Below is a calibration curve of measured results obtained using the CDR method and those obtained using the *Acidification power* method from Campden BRI laboratory.



In the study carried out by the reference institute, 5 slurry samples were triple analysed using the CDR BeerLab® and the reference method, in order to assess the performance of the CDR BeerLab® method.

The Campden BRI study established that for the determination of yeast vitality, CDR BeerLab® results are comparable to those obtained using the reference method (correlation R^2 = 0.90), confirming that the CDR BeerLab® method is precise and repeatable.

CDR FOODLAB®
Quality control systems for food and beverage

Conclusions

The reuse of yeast is useful as it enables considerable savings and can actually improve the organoleptic properties of beer.
When reusing yeasts it is important to pay close attention to recovered cell efficiency, which is why determining viability is so useful.
However, counting cells or determining viability is not sufficient for determining whether recovered substrate cells are capable of enabling alcoholic fermentation.
Therefore, yeast vitality must be determined.
The procedure for determining this parameter according to the recognised method, the *acidification power test* (AP TEST), is laborious and time consuming, it requires specific equipment requiring regular calibration, or which is only available in well-equipped chemistry laboratories used by trained personnel.
This is why the CDR BeerLab® has been developed for determining *vitality*. This method does not require any calibration or use of personnel specialised in laboratory techniques. Test tubes are pre-filled with reagents in required quantities, ready for use. The result is obtained by simple photometer readings.

The method is strongly correlated with the AP TEST, as established by the study carried out by the international reference laboratory Campden BRI, and provides results in *acidification power*.

Therefore the CDR BeerLab® method for determining is simple to use, provides rapid results, is reliable and usable by any operator directly at the brewery on the production line.

Bibliography

[1] Haddad, S., and C. Lindegren. “A Method for Determining the weight of an Individual yeast Cell.” *Applied Microbiology* 1, no. 3, (1953), 153-156.
[2] Lenoel, M., J.P. Meuier, M. Moll, and N. Midoux. “Improved System for Stabilizing Yeast Fermenting Power During Storage.” Proceedings of the 21st European Brewing Congress, 1987, 425-432.
[3] Nielsen, O., “Control of the Yeast Propagation Process: How to Optimize Oxygen Supply and Minimize Strees.” MBAA *Technical Quarterly,* vol. 42, no. 2 (2005), 128-132.
[4] Fernandez, J.L., and W.J. Simpson. *Journal of Applied Bacteriology* 75 (1993), 369.
[5] Kara B. V., Simpson W.J. and Hammond J. R. M., *Predinction of the Fermentation Performance of Brewing Yeast with the Acidification Power Test,* in «Journal of the institute of brewing», no. 94, 1988, pp 153-158
[6] Gabriel P., Dienstbier M., Matoulkovà D., Kosař K., and Sigler K., *Optimised Acidification Power Test of Yeast Vitality and its Use in Brewing Practice,* in «Journal of the institute of brewing», no. 114(3), 2008, pp. 270-276.
[7] Mearelli L. “La Fermentazione della Birra Artigianale: Studio del Metabolismo dei Lieviti e Messa a Punto di un Metodo Rapido per la Misura della Loro Attività Metabolica.” 2018, pp. 14-16.

Link

[a] Yeast Vitality determination
[b] CDR BeerLab® the Beer and Wort analysis
[c] Evaluation of new features (VDK, yeast vitality) of the CDR BeerLab® Analyser – Camden BRI
[d] Campden BRI