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YEAST VITALITY MONITORING DURING THE FERMENTATION PROCESS OF BEER PRODUCTION

Linda Luarasi[1*], Rozana Troja[2], Luljeta Pinguli[2]

[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Boulevard Zogu I, 1000 Tirana, Albania
[2]Department of Industrial Chemistry, Faculty of Natural Sciences, University of Tirana, Boulevard Zogu I, 1000 Tirana, Albania

[*]e-mail: linda.luarasi@gmail.com

Abstract

The yeast performance, during the alcoholic fermentation, directly depends on its activity - a function of the vitality and the physiological state of the viable cells. In order to undertake corrective actions before the inoculation process, it is important to predict the yeast vitality. Yeast vitality is the life span of the vegetative cells and their ability to reproduce.

The most used method is the selective staining of the cells and then the averaging of results obtained in over 10 microscopic plots. The most used stain colors are methylene blue or methylene violet, which penetrate the damaged membranes of dead cells becoming stained, unable to penetrate the impermeable membranes of viable cells which remain transparent. The accepted level of vitality is 80% of viable cells, meanwhile the second and the third generations, which are highly adaptive rather than the first generation, may reach a vitality level up to 90%.

A vitality analysis was carried out for the bottom-fermentation yeast *Saccharomyces cerevisiae* sp. *carlsbergensis*, in a private brewery company in Tirana, during 2015 (January - December), considering the generation I up to generation IX. In the first three generations the average vitality was almost the same, 85%. There was a slight decline from the generation III to generation VII, 85% to 81%, and a sharp increase to generation VIII and IX, 87% and 86%, respectively.

As a conclusion, there are different levels of vitality among the generations, but the difference is not significant, which means there isn't noted any important changes in the biotechnological abilities of the fermentation yeast strain.

***Key words**: Vitality, Yeast, S. cerevisiae, Fermentation.*

1. Introduction

Reproducibility from one batch of beer to another is achieved through use of a constant amount of pitching yeast of a high viability and vitality, a constant fermentation period at a controlled temperature, and a maturation period in the presence of the yeast.

The aim of yeast propagation is to produce as much yeast mass as possible from the wort whereas in the beer fermentation, increase in yeast mass is a waste of carbon that could have been turned into the major product, ethanol.

The modern practice is to replace the yeast culture at regular intervals by a freshly grown culture from the yeast propagation plant. A refrigerated, or perhaps lyophilized (freeze-dried), laboratory stock culture is grown through stages of increasing volume, ultimately to provide the replacement culture for another series of 10- 20 successive fermentations, according to the brewery's practice (Priest, [6]).

When yeast cells are introduced into a nutritious aqueous medium, such as wort, with a temperature range between about 5 ^{0}C and 35 ^{0}C, the cells begin to grow and continue to do so until one of the essential nutrients is exhausted. Each cell repeats an obligatory series of events known as the cell cycle (Walker, [8]).

In some brewing strains cell separation may be defective so that short chains of cells form. In the brewery context, yeast cells are grown for two different purposes. First, in yeast propagation the aim is to produce large quantities of yeast from tiny amounts, i.e. from laboratory stock culture to pitching yeast. The cells are maintained in well-oxygenated nutrient medium through several batches of increasing volume to allow as many cell cycles as needed to attain the desired quantity of yeast. Second, in fermentation the mass

conversion of wort to beer in a single operation is the important task (Priest, [6]).

Fermentation in many ways presents more challenges as there is more than one aim - an economical conversion of wort to beer, delivery of the desired beer qualities of pH, ethanol content, and profile of flavor-active compounds, and the generation of a crop of yeast cells with sufficiently high viability and vitality to be used as pitching yeast in subsequent fermentations (Boyd, [2]).

The normal definitions of these two terms are that viability refers to cells that are alive rather than dead, whereas vitality is a measure of the health or living cells vigor. For pitching yeast both viability and vitality should be as high as possible and are very critical properties in determining subsequent fermentation performance. However, how these two properties can be measured is more problematic and a great number of methods have been proposed (Briggs *et al.*, [1]).

There are two aspects of pitching yeast quality: the yeast must be (a) in an active state and (b) free from beer-spoilage bacteria and wild yeasts. Various methods are available for assessment of yeast viability and the rather more exacting concept of vitality, but the simplest method, which is sufficiently accurate for most purposes, uses methylene blue stain to detect dead cells microscopically. Because that test can be carried out in about 15 minutes, there is no excuse for neglecting to confirm a high-percentage viability, preferably 99 - 100%, but certainly at least 95% before deciding to use the yeast. Yeast-pitching rate is usually in the range of 1 - 2 x 10^7 cells/mL (higher for high-gravity worts), but on a production scale, initial measurement of the yeast by weight is often more convenient (Carvell, [3]).

Although the viability and/or vitality of yeast can, and should, be measured before each fermentation, examination for contaminants is a more lengthy process and it is impracticable to test yeast by standard microbiological culture methods prior to reuse. In lager fermentations, yeasts are harvested on settling out at the end of the fermentation. Usually a cooling jacket is fitted to the cone to maintain the settled yeast in good condition. It is accepted that the viability of the yeast will fall by several percent as a result of the long exposure to the ethanol and other metabolic by-products in the beer, but there may also be the incidental protective effect that bacteria and wild yeast contaminants are selectively killed (Smart, [7]).

A number of times yeast can be reused depends on a variety of factors, but mainly on: the individual strain, quality of the cropped yeast, original wort gravity and company policy. There is a big variation in a number of yeast re-pitching among the breweries. In some breweries a lager brewing yeast culture is used 2 - 3 times while in others even 7 - 9 times for fermentation of wort at similar original gravity. It has also been reported that lager yeast culture can be reused even up to 20 times (Kordialik-Bogacka, [5]).

2. Materials and Methods

The monitoring of yeast viability was performed at a private brewing company in Tirana, Albania. Brewer's yeast taken into consideration was lager strain *Saccharomyces uvarum* (*carlsbergensis*) W34/70, a bottom fermenting yeast. The culture was maintained by subculturing on wort agar slopes at 4 ^{0}C, and the propagation process was carried out in laboratory fermentation trials with an 11°P of malt wort.

A total of 95 samples were analyzed over a 12-months period, and 231 samples during the next 12 months, belonging to generations I - XI. Samples were all taken from storage tanks. Generations I to XI made up 8%, 10%, 13%, 16%, 20%, 19%, 5%, 2%, 0.8%, 0.8% and 1.7% of samples, respectively.

The method of methylene blue staining is described in Analytica Microbiologica - EBC Method 2.2.2.3. [4]. The method aims to provide an estimate of the percentage of viable cells present in a sample of yeast. The methylene blue 0.01 g was dissolved in 10 mL distilled water and later 2 g of sodium citrate dehydrate was added to the solution. Yeast samples of 1 mL were diluted in 9 mL of distilled water. Solution of methylene blue and diluted yeast cells were placed on a glass slide under a coverslip.

Cells are counted under the microscope using 100x magnification and viability is assessed from the ratio of colored (dead in the case of methylene blue) to colorless cells (alive in the case of methylene blue).

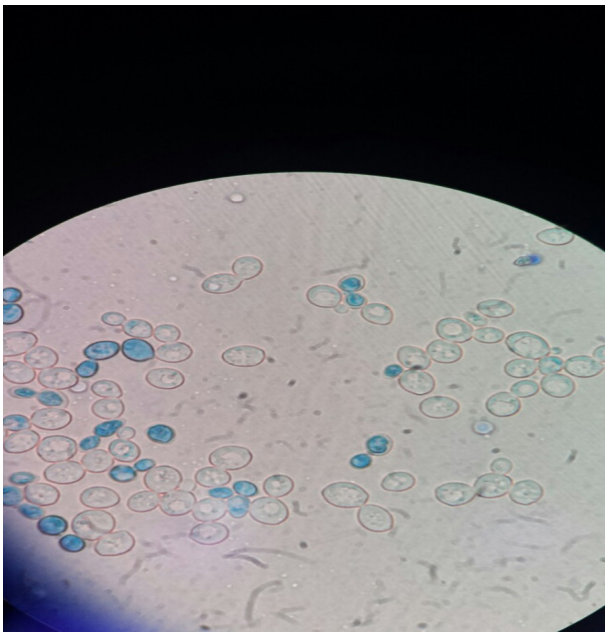

Figure 1. Staining method using methylene blue, where the stained cells are the dead cells

The test is quick but errors can arise from presence of cells with intermediate degrees of color, and there can be a considerable degree of investigator interpretation.

$$Viability\ \% = \frac{total\ counted\ cells - total\ counted\ of\ dead\ cells}{total\ counted\ cells} \cdot 100$$

3. Results and Discussions

The viability of yeast cells after successive fermentation showed some significant changes among the generations. The amount of viable cells ranged from 70% (at the sixth generation) to 99% (at propagation time). The following Figures (from 1 to 4), have shown the fluctuation of the viable cells from generation I to VIII.

During the monitoring of yeast viability for a 12 month period, the first and the second generation has been used, respectively, 8 and 10 time in the beer fermentation. As it appeared in the above figure, at the first generation, viability has fallen under 80%, and at the second generation, the level has remain above the threshold.

The yeast generations III and IV have been used in nine successive fermentations, and the level of viable cells has never fallen under the threshold.

The yeast generations V and VI have been the most used starter cultures in fermentations, and the level of viable cells has resulted in significant changes, being above and under the accepted threshold.

The yeast generations VII has been widely used in fermentations, where it has shown different levels of viability, reaching a peak of 86% and the lowest level of 75%. Concerning the VIII generation is has been used only in five fermentations, but the amount of viable cells have been at high levels reaching 91%.

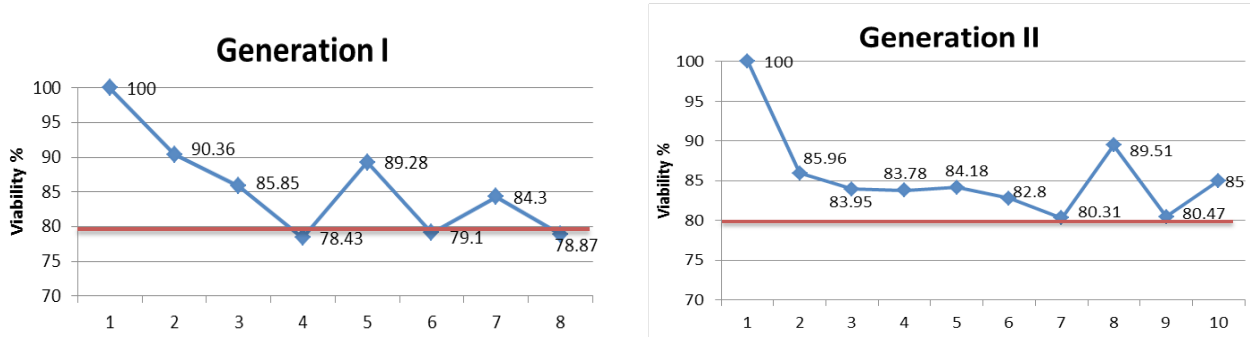

Figure 1. The percentage of yeast viability for the 1st and 2nd generation

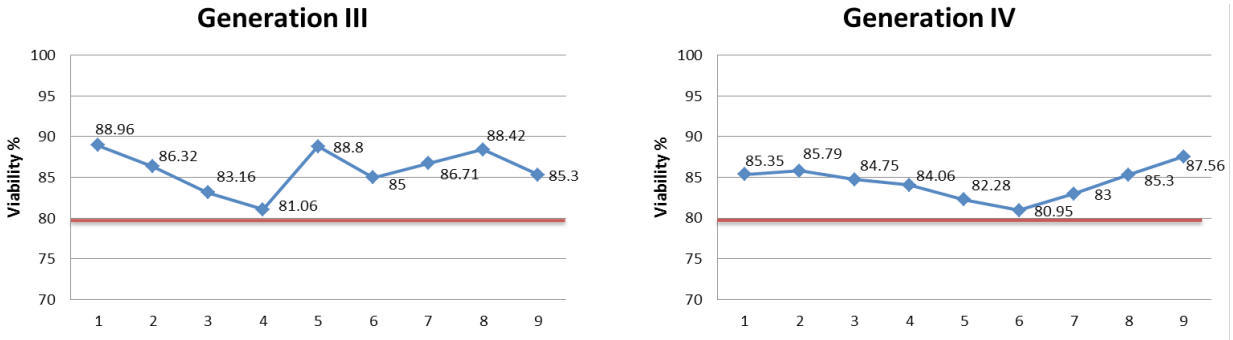

Figure 2. The percentage of yeast viability for the 3rd and 4th generation

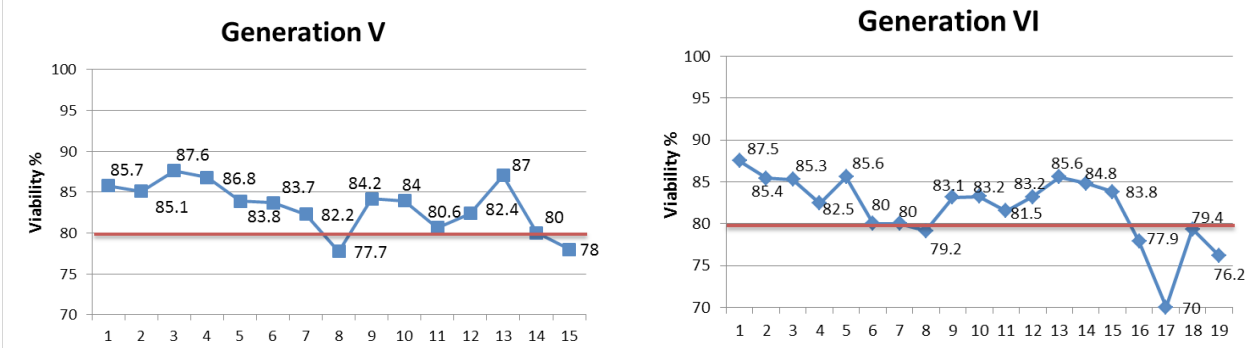

Figure 3. The percentage of yeast viability for 5th and 6th generation

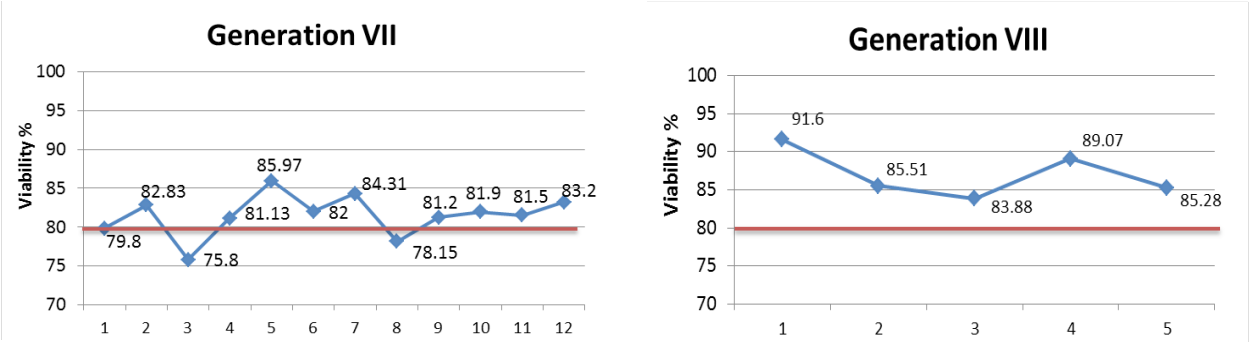

Figure 4. The percentage of yeast viability for 7th and 8th generation

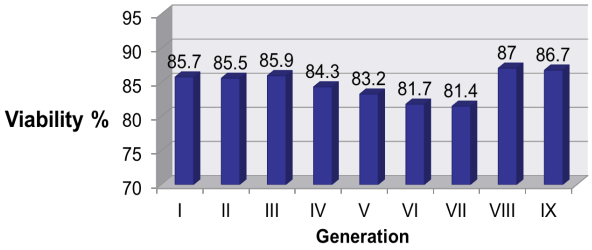

Figure 5. Viability level at different yeast generations

The above Figure 5, summarizes all the average values of the yeast generations that have been used, from I to IX, showing that the mostly used generations that breweries operate, never exceed to VII, and generations III and IV are the most preferred for the fermentation process.

4. Conclusions

- The evaluation of yeast viability during the fermentation process of beer using the staining method showed different levels of viability among different yeast generations.

- In the first four generations viability remains approximately constant at 85%. The successive generations showed a slight decrease of the average value of viability (81%), respectively, at generations V, VI and VII, viability falls below 80% in some cases.

- There is an increase of viability at yeast generations VIII and IX (87%), considered as an unstable increase, which is followed by a slight decrease, and this is the reason why the yeasts belonging to these generations are not re-pitched in fermentation process.

- During the monitoring of yeast viability, different levels of viability resulted among the generations, but the difference is not significant, which means there isn't noted any important changes in the biotechnological abilities of the fermentation yeast strain.

5. References

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