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Propagation of Beer Yeast

MIJE REÇI[1*], NAIM TAHIRI[2], ISMAIL FERATI[3]

[1]Faculty of Natural Sciences and Mathematics, Study Program of Biology, University of Tetova, Tetovo, North Macedonia

[2]Join Stock Company "Birra Peja", Peja, Republic of Kosovo

[3]Faculty of food technology and Nutrition, State University of Tetovo, Republic of N. Macedonia

*Corresponding author; E-mail: mije.reci@unite.edu.mk

Abstract

Nowadays, many breweries aim not only to have quality yeast, but also to characterize the quality of the beer. Yeast plays the main role in the fermentation process of the sweet solution (wort), so to have the best possible fermentation process, the yeast must be pure (pure culture), active yeast cells and not vacuolated, uninfected with bacterial flora or wild yeast. To this end, many breweries have developed the technological capacity to cultivate yeast from pure culture. Even "Birra Peja" has raised the technological capacities for the propagation of yeast. Propagation of yeast is a very sensitive process and requires that, in addition to the methodology used, the main role is that all stages of propagation are carried out under sterile conditions, which is the main prerequisite to achieve the desired success. The laboratory process takes a central place, therefore the staff who carry out the propagation must be well prepared. The apparatus used from the cultivation of the pure culture to the final propagation process must be sterilized and the manipulations made during the work must be microbiologically sterile from a professional point of view. The system is based on a controlled fed-batch yeast fermentation, which can produce pitching yeast in as little as 24h. So, we will present the process of pure culture propagation from mother cell to the yield of 60 hl of yeast.

Keywords: propagation; yeast; fermentation; pure culture; mother cell.

1. Introduction

Traditionally brewers re-use yeast cropped from fermentation to pitch subsequent brews [4]. In many breweries, this process cannot continue indefinitely due to the occurrence of spontaneous mutants and the increased risk of contamination. Some brewing yeast strains are susceptible to such genetic drift; however, others are more resilient and can remain stable over extended periods of time. While the negative effects of serial re-pitchings have been reported by researchers (e.g., petite generation and flocculation mutations), others have indicated little change in lager yeast serially re-pitched up to 135 times [11], [17], [15], [16]. Also, it was noted that extents of deterioration can vary between yeast strains [9]. Where such introductions are necessary, they generally happen after approximately ten fermentation cycles [3].

In brewery fermentations, the yeast undergoes a lag phase during which little yeast growth takes place. This is followed by a vigorous growth phase where yeast reproduce and finally a fermentation phase, where growth slows down and the sugars in the wort are fermented [5]. For a successful beer fermentation, the yeast must attain sufficient cell numbers in order to convert the sugar in the wort to alcohol. Brewer's yeast is capable of growth under strictly anaerobic conditions only when there is a supply of sterols and unsaturated fatty acids [18], [2]. In wort, this is not usually the case. Unsaturated fatty acids are produced from saturated fatty acids in the presence of oxygen and sterols are produced from squalene in the presence of oxygen and sufficient oxygen is generally supplied to result in approximately a 3 to 4 doubling of the pitching yeast numbers [13]. Oxygen is therefore added for yeast cell reproduction and so that the cells can produce stable cell walls. A high level of expression of genes involved in fatty acid and ergosterol biosynthesis has been

*Corresponding author: Mije Reçi; E-mail: mije.reci@unite.edu.mk
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shown to occur within this period and unexpected near complete repression of many genes involved in early glycolysis and alcohol metabolism [8]. Pitching rates for beer fermentations range from 5–20 million cells/mL wort [8]. For example, should 10×106 cells ml−1 be pitched into air-saturated wort, then the maximum yeast count attained would be of the order of 80–100×106 cells ml−1. This maximal yeast count is normally achieved 18 to 24h after pitching for ale yeasts [7]. The yeast from this propagation vessel is transferred to the primary beer fermentation when it reaches exponential phase, however the beer produced from this first-generation yeast would usually be blended with beer having the correct flavor profile. The yeast crop from the second generation would however produce beer with a typical flavor [7].

Particular yeast strains produced by batch fermentation with continuous aeration of wort can be grown to cell densities upwards of 300 × 106 cells ml−1. Yeast propagadet in breweries is normally aerated intermittently in wort and generally reaches a cell density of approximately 100 × 106 cells ml−1 in an attempt to limit the production of off-flavours in the propagated yeast. As during yeast propagation, the aim is to obtain maximum yield of yeast but also to keep the flavor of the beer similar to a fermentation with the correct flavor profile, so that it can be blended into the production stream [7].

In some cases, the need for "blending-off " of the beer produced from first generation yeast is no longer required. Wackerbauer et al. [1] have used acetaldehyde as a marker compound for successful propagation [20]. A low level of acetaldehyde at the end of propagation is desirable as this compound is an off-flavor in beer and it is not desirable that it be carried over to the beer fermentation. The formation of acetaldehyde is most likely due to the Crabtree effect in brewing yeasts. It is thought to be due to the limited respiratory capacity of yeast cells, this leads to an overflow of reaction from pyruvate, through acetaldehyde and on to ethanol and carbon dioxide [22], [19]. The term overflow metabolism has been used to describe this seemingly wasteful strategy in which cells incompletely oxidize their growth substrate [19]. The specific growth rate should ideally be maintained at maximum oxidative growth rate in order to maximize biomass yield and productivity in fed-batch yeast fermentations [6]. Propagation in wort with a high sugar concentration does not allow full respirative yeast metabolism [10]. In contrast, fed-batch systems used for baker's yeast production maintain the sugar concentration in the growth medium at a low concentration to ensure that the yeast are maintained in a respiratory metabolism [12].

Even in the best-managed brewery, the production environment provides opportunities for the introduction of contaminants to bulk yeast. These may be in the form of bacteria or wild yeast. The consequences of contamination with wild yeast can be significant, resulting in process changes (floccu-lation, fining) or flavour changes (phenolic, medicinal character) [14].

Continued serial re-pitching of yeast may be associated with gradual deterioration in yeast condition, which can result in a decline in fermentation performance. This is unlikely in the case of rapid top-cropped fermentations where there is an opportunity to ensure that the fraction of yeast retained is that which is produced when fermentation is at its most vigorous. In addition, such cropping regimes are to some extent self-purifying. In the case of bottom-cropped fermenters, particularly large-volume cylindroconicals, there is opportunity for high levels of contamination of yeast with trub. The possibility of selecting for non-standard yeast variants has been alluded to already. The use of very large vessels and the tendency towards high-gravity brewing has undoubtedly increased the stresses to which production yeast is subject. It has been demonstrated that senescence of yeast cells may be associated with declining performance of yeast since some cropping regimes may select for larger and therefore possibly older cells within yeast populations [1].

The frequency of introduction of newly propagated yeast into the brewery is a decision for the individual brewery since there are no immutable rules. A typical regime in a modern brewery built and operated to high standards of hygiene would be to introduce propagated yeast every 15-20 generations. However, some breweries would consider this excessive and only allow 5-10 generations to elapse before introducing new yeast.

On the other hand, if a particular yeast line is performing satisfactorily and is microbiologically 'clean', there will be a natural desire to continue brewing with such yeast, even though it may have completed its allotted number of generations. This highlights the need for methods of testing the yeasts' physiological condition, which are predictive of subsequent fermentation performance.

Continuing to use yeast that is performing in a satisfactory manner has other advantages. There is an economic cost to propagation. Apart from the capital investment, the revenue costs are obviously proportional to the frequency of use. In addition, it is commonly observed that the first generation fermentation using newly propagated yeast is atypical. In consequence, the first generation beer has to be blended. Occasionally, the non-standard behaviour may be extended over the first few fermentations. Clearly, this would tend to mitigate against frequent propagation.

The aim of the laboratory phase of fermentation is to generate a pure yeast culture of sufficient size to provide an adequate pitching rate for the first stages of brewery propagation. It is sensible to limit as far as possible the number of aseptic transfers, since these represent the points of greatest risk of contamination. In general, a volume scale-up factor of about 1:10 is satisfactory.

In our research was used *Saccharomyces cerevisiae* yeast. The purpose of this research was based on the description of the technological process of beer production and yeast propagation. The aim of yeast propagation is to obtain maximum yield of yeast, while ensuring the flavor of the beer is similar to a normal fermentation so that it can be blended into the production stream. Since beer fermentation is a rich source of vitamins (especially an excellent source of B vitamins), proteins, minerals (especially silicon, which significantly prevents the possibility of osteoporosis), therefore beer is a healthy and nutritious product which it is consumed a lot in the Kosovo's market.

2. Material and Methods

In this research, the propagation of yeast through the propagator was carried out and the technological process of beer production in the "Birra Peja" factory in the city of Peja was described. The yeast used was *Saccharomyces cerevisiae*. The combined quantitative-qualitative methodology was applied, as well as laboratory analyses. The samples used for the preparation of this paper were chosen in order to apply a comparative method between them.

2.1. Yeast propagation process

Yeast is a single-celled fungus used to ferment beer. To ensure a good supply of yeast, brewers propagate or cultivate their own yeast cultures. Yeast propagation is the process of growing yeast cells from a single cell. Yeast propagation can be done in different ways. The device that performs this process is called a propagator. The yeast propagation process is described as follows over four days:

First day

1. 5-6 liters of cider are taken from the propagator (2 large Erlenmeyer flasks).
2. 50 ml of cider are put in Frehindrov containers.
3. 1 L of cider is placed in the Pasteur flask (it is measured exactly with a ruler).
4. The Frehindrov container and the Pasteur flask are put into the autoclave for sterilizing the cider. Along with the Frehindrov containers and the Pasteur flask, accessories such as: air filters (after they have been filled with cotton), air and fermentation latex tubes, the stopper and the valve in the Karlsberg container can also be sterilized.
5. After sterilizing the Frehindrov container and the Pasteur flask with cider solution, it is cooled in the refrigerator to room temperature.
6. The pure yeast culture is taken from as deep as possible in test tube no. 1 (because we usually have two test tubes with pure culture), in sterile conditions with a glass rod and transferred to Frehindrov's bottle 1 and 2 (which has cider sterilized).
7. It is mixed several times to homogenize all the yeast. After complete mixing, the Frehindrov containers are placed at room temperature for 24 hours.
8. During the 24-hour stay, it is good to be tempted several times.

Figure 1. Erlenmeyer

Figure 2. Frehindrov containers

Figure 3. Pasteur flask

Figure 4. Autoclaves

Second day, after 24 hours

After stirring the yeast solution in the Frehindrov containers (in order to homogenize and empty all the yeast), it is placed in the Pasteur flask (always under sterile conditions, near the burner) and the yeast is homogenized in the Pasteur flask by stirring. From the residue in the Frehindrov container, a sample is taken for counting yeast cells concentrated in 1 ml. The Pasteur flask is placed at room temperature for 24 h. After sterilizing the Karlsberg container, it is placed on the scale measuring the dry weight of the Karlsberg container and filling with cider, the Karlsberg container is filled with 20 L of sterilized cider (from the large Propagator where the sterile cider is located). The Karlsberg container is sent to the laboratory in order for the cider to be at a temperature below 30^{o}C.

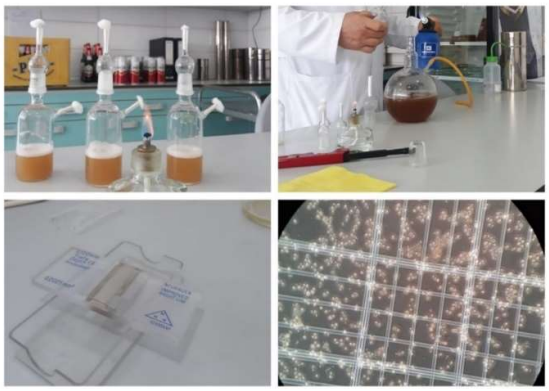

Figure 5. Second phase

Third day

From the Pasteur flask (after shaking the bottom well until the entire solution is homogenized), in sterile conditions, through the special valve in the Karlsberg container, the solution is emptied into the Karlsberg container. From the rest of the solution in the Pasteur bottle, a sample is taken for counting the concentration of cells in 1 ml. The valve seat is closed in the Karlsberg container with the sealing cap. The Karlsberg container in the homogenized solution is sent

to the Yeast Propagation department. Procedure in the propagation department:

1. Sterilization with flame and alcohol (to cool the valve) of the air connection.
2. The air purity sample is taken (lasts 5 minutes).
3. The valve is sterilized again with flame/alcohol.
4. The sterilized air filter is opened from the foil.
5. The latex tube is placed on the long side of the air filter and on the other side with the air valve.
6. The other latex tube is placed on the short side of the filter and on the other side in the Karlsberg container (in the sampling valve).
7. The air is released to flow (and since the apparatus does not show the pressure, we must see from the outgoing air flow how much air is flowing).
8. The time at which the aeration procedure was completed is marked. 50 ml of cider is placed in Frehindrov containers.

Figure 6. Third phase.

The fourth day

Steps taken on the fourth working day:

1. The valve is sterilized in the 1000 l propagator (where cider must be injected by drying with alcohol-flame-alcohol).
2.The latex tube is removed from the long part of the air filter and it is placed in the air filter in the Karlsberg container. (Caution: sterilization of valves at each step).
3. The air hose is removed from the short part of the air filter and it is placed on the 1000 L Propagator valve.
4. The air is released all the way and stays released in the Karlsberg container for a few seconds (in order to obtain a greater driving force for the must/yeast solution to pass through the propagator).
5. The valve of the Karlsberg container and the valve on the Propagator (handle) is opened.
6. The solution is mixed by shaking the Karlsberg container so that all the yeast passes into the propagator.

When the weight of the Karlsberg container is subtracted (since all the time during the transfer of the solution the Karlsberg container is placed on the scale, in order to know the amount of solution passed in the propagator), the following actions are performed:

a) The propagator valve is closed.
b) The valve in the Karlsberg container is closed.
c) The air valve is closed.
d) After the end of the transfer, the lid is opened and a sample is taken at the bottom of the Karlsberg container to count the concentration of yeast cells in 1 ml.
e) The pipes and the Karlsberg container are well cleaned.
f) Notes are taken when the transfer starts until it ends (usually the transfer should be done in 20 min.).
g) In the 1000 l propagator, the fermentation stays for 48 hours.
h) Transfer from the 1000 l propagator to the 5000 l propagator takes place after 48 hours.
i) In the propagator 5000 l plus 1000 l = 6000 l, the fermentation stays for 48 hours.

Figure 7. Fourth phasePropagation of beer yeast

3. Results and Discussion

The process of yeast propagation was carried out in the "Birra Peja" factory and we got the results during four days. The type of yeast was SCVC-K 02/23 (Saccharomyces cerevisiae var. carlsbergensis Cultivation- 02/23). Based on these results, we can say that the fermentation does not have any serious deviations from the normal course, but there are some small differences from sample to sample which are briefly commented on in the following table or diagram.

3.1. Propagation systems.

The use of pure culture plant in brewing is, of course, not new and the first yeast propagators were introduced by Hansen in 1883. The process, therefore, has a long history and several distinct systems have been developed. Nevertheless, all propagation regimes basically consist of a sequence of yeast cultures of progressively increasing volume, starting in the laboratory and culminating in a terminal stage which contains sufficient yeast to pitch the first production scale fermentation. Variations on this theme are possible, such as semi-continuous systems, which maintain cultures at the small brewery scale and thereby reduce the requirement for repeated laboratory propagation.

The aim of the laboratory phase of fermentation is to generate a pure yeast culture of sufficient size to provide an adequate pitching rate for the first stages of brewery propagation. The terminal laboratory culture must be held within a container, which will allow transfer to the brewery under conditions of asepsis, and there be transferred into the brewery propagation vessel under aseptic conditions.

Laboratory propagation uses standard microbiological apparatus. It is sensible to limit as far as possible the number of aseptic transfers, since these represent the points of greatest risk of contamination. In general, a volume scale-up factor of about 1:10 is satisfactory. A typical laboratory propagation regime is shown in table and figure 1.

Table 1. Yeast propagation *Saccharomyces cerevisiae var. carlsbergensis,* cultivation- 02/23 2023 (SCVC-K 02/23).

Number of propagation	Start date	Equipment	Number of yeast cells	Extract %	Time	Notes
02/23	27.03.2023	Frehindrov container			13:30	It was inoculated from the pure culture in the Frehindrov dish for 24 h
02/23	28.03.2023	Pasteur flask	10.98 x10^6/ml After 24 hours of inoculation in the Frehindrov container		07:00	It was inoculated from Frehindrov's container into Pasteur's flask for 24 h
02/23	29.03.2023	Karlsberg container	10.76 x10^6/ml in Pasteur flask		13:30	It was inoculated from Pasteur's flask in container of Karlsberg for 48 h
02/23	31.03.2023	Propagation 1000 L	24.37x10^6/ml in Karlsberg container		13:50	Transferred from the Karlsberg container for 48 h to the 1000L propagator at 13:50
02/23	01.04.2023	Propagation 1000 L	2.39 x10^6/ml	12.6	07:00	
02/23	01.04.2023	Propagation 1000 L	7.78 x10^6/ml	11.8	19:00	
02/23	02.04.2023	Propagation 1000 L	12.68 x10^6/ml		07:00	Moved from 1000 L propagator to 6000 L propagator at 2:00 p.m.
02/23	03.04.2023	Propagation 6000 L	12.12 x10^6/ml	11.7	07:00	
02/23	03.04.2023	Propagation 6000 L	16.19 x10^6/ml	10.9	13:30	
02/23	04.04.2023	Propagation 6000 L	22.66 x10^6/ml	9.0	07:00	
02/23	04.04.2023	Propag. 6000 L	30.93 x10^6/ml	8.3	13:45	
02/23	05.04.2023	Propag. 6000 L	45.5 x10^6/ml	6.8	07:00	Increased propagation, carried over to FCK (Cylindrical Conical Fermentor) 9

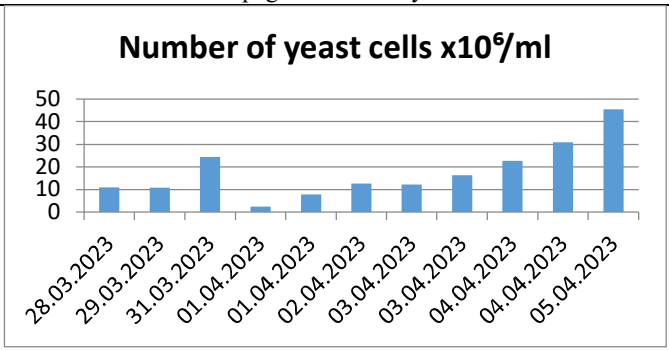

Figure 8. Number of yeast cells x10^6/ml.

According to the results presented in table 1 and graph 1 during propagation, we are describing below for all days of yeast propagation. Since we previously worked the pure culture in the laboratory, then:

On the first day, the inoculation was done from the test tubes containing the pure culture in the Frehindrov container and the incubation continued at room temperature for 24 hours.

On the second day, after 24 hours of incubation, close to the flame, after the homogenization of the solution was done with cider and yeast in the Frehindrov container, then the entire amount was emptied into the Pasteur flask, which had within 1 L cider, and continued to remain in incubation for 24 hours. Then, a quantity of the residue was taken from Frehindrov's container and it was put under a microscope, in order to observe how much fermentation has developed in Frehindrov's container. The number of yeast cells in 1 ml should be from 8-12 million cells/ml.

On the third day, the inoculation was done from the Pasteur flask in the Karlsberg container. The entire solution of yeast and cider is emptied into the Karlsberg container. The number of yeast cells should be 10-12 million cells/ml. In the Karlsberg container, the imbibition lasted 48 hours at room temperature.

On the fifth day, the solution was transferred from the Karlsberg container to the 1,000 l Propagator, where the propagation lasted 48 hours. After transferring the yeast from the Karlsberg container to the propagator, the yeast was taken from the bottom of the Karlsberg container for microscopy. Usually here the yeast multiplies to a concentration of 20-25 million cells/ml. In the 1,000-liter propagator, propagation lasted 48 hours. Usually every 12 hours the concentration of yeast cells and % of the extract was measured. After 48 hours of propagation, the solution was transferred from the 1,000 L propagator to the 5,000 L propagator, which actually becomes 6,000 L of solution. After the transfer, samples were taken and the yeast cells were counted, which is usually a satisfactory result if we have 10-12 million cells/ml.

On the seventh day, upon completion of the propagation process in the 6,000 L propagator, the solution (must + dried) was fed to the Conical Cylindrical Fermenters for additional multiplication to obtain a larger amount of yeast. The number of yeast cells in the 6,000 L propagator is between 45-50 million cells/ml.1.

4. Conclusions

The requirements of a propagator are summarized as follows:

(1) Hygiene is of prime importance and the design and operation of the propagation plant must ensure that a pure yeast culture is generated. Since the propagator is to supply yeast for brewing it is essential that it is not a source of contamination. This is an obvious requirement of propagation but one that is not always adhered to.

(2) The terminal cell count must be adequate to achieve the desired pitching rate in the first generation fermentation.

(3) The yeast must be of high viability (>95%).

(4) The physiological condition of the yeast must be consistent and appropriate for subsequent fermentation.

(5) The cycle time of propagation should be as rapid as possible, both for economy and to minimize the risk of contamination, and should use the fewest possible number of vessels.

(6) Terminal cell counts from the final propagation stage should be as high as possible so as to allow high step-up ratios and minimize the effects on the first generation fermentation of the 'barm ale' introduced with the propagated yeast.

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