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BEER FERMENTATION TANK MODULE

Yeast Propagation and Validation of the Fermentation Process

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ABSTRACT

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The possibility of providing an excellent product relies almost completely on the equipment being used for its production, especially when dealing with the fermentation-related industry of brewer's yeast. This is why through a site-specific research done at Kahakka Brewery, a yeast propagation module was designed by giving an extensive look at the microorganism's environment for a healthy reproduction, detailing factors which may jeopardize the overall quality and how automation can be applied to facilitate the brewer's life; among validating the fermentation processes, the customizable characteristics of this module will be justified by analyzing fermentation stages of beer. In theory, correct yeast propagation can be done without automation if enough care is taken during the process but decreasing the human factor during brewing (in general) adds a safer route to consistency.

The first chapter deals with the basics of the yeast cell and its favorable environment, leading to an insight of beer production, thoroughly analyzed in the second chapter. Focus is given to the handling and hazards regarding yeast propagation from a laboratory scale up to a 1000 liters batch. The final chapter deals with the theoretical design of a yeast propagation vessel customized for this production facility. The life cycle of different types of yeast used at Kahakka Brewery will be studied as reference.

The conclusion yields the creation of a module, that by attaching it to a production tank would provide the healthiest environment for yeast to multiplicate, increasing its biomass and allowing proper fermentation of industrial size batches. Besides yeast and its reproduction environment, a deeper examination is done regarding the materials of such a vessel and measuring samples related to yeast and microorganism's management.

Key words
Yeast, yeast propagation, microbrewery, food production, fermentation

CONCEPT DEFINITIONS

Wort: Collected extract (sugar) from grains and water mixture

Cast-out: End of wort production

Yeast-off: Removing flocculated yeast from fermentation vessel

Primary fermentation: First 168 hours of fermentation since yeast is added into wort

Secondary fermentation: Final fermentation stage

Maturation: Includes secondary fermentation and any added time for beer to mature

FVs: Fermentation Vessel, tank used for primary fermentation

BBTs: Bright-Beer-Tank used for secondary fermentation and later maturation

CCVs: Cylindroconical Vessel, refers to the shape of the tank where fermentation occurs

Starter: Mini batch of beer, ensuring healthy yeast and propagation

Pitching yeast: Tossing-in/adding yeast into wort

Yeast propagation: Increase in biomass by multiplying yeast cells in natural environment

CIP: Cleaning In Place, cleaning procedure for sanitation of production tanks

hl: Hectoliter, 100 liters

Amino acids: Organic compounds playing important role in final product when brewing

Lag phase: Fermentation stage, often referred as 15 hours of fermentation since pitching the yeast

Nucleotides: Basic building blocks of DNA and RNA inside cells

Spunding/bung-up: CO_2 created during the fermentation is not released from tank.

ABSTRACT
CONCEPT DEFINITIONS
CONTENTS

1 INTRODUCTION 1

2 GENERAL REMARKS AND BASIC CONCEPTS OF BREWERY YEAST 2
2.1 Microbiological and biochemical fundamentals of yeast multiplication 3
2.1.1 Moisture content and dry matter 3
2.1.2 Yeast cell structure 6
2.2 Yeast propagation 7

3 YEAST HANDLING AND PROPAGATION AT KAHAKKA BREWERY 10
3.1 Key considerations 11
3.2 Production settings and recommended environment for yeast pitching 12
3.2.1 Wort production 12
3.2.2 Fermentation 13
3.2.3 Pitching calculations 15
3.3 Batch analysis 16
3.3.1 Fermentation control through temperature and pressure 17
3.3.2 Fermentation control through laboratory methods 19
3.3.3 Cell counting and viability test 21
3.3.4 Evaluation of the results 27

4 YEAST PROPAGATION MODULE 28

5 CONCLUSIONS 30

REFERENCES 32
APPENDICES 33

FIGURES
FIGURE 1. Yeast cell features 6
FIGURE 2. Typical laboratory propagation for ale yeast 8
FIGURE 3. General yeast propagation procedure at Kahakka Brewery 10
FIGURE 4. Simplified flow diagram of beer production at Kahakka Brewery 12
FIGURE 5. Plato° and pH during primary fermentation of batch #33 18
FIGURE 6. Plato° and pH during primary fermentation of batch #46 21

PICTURES
PICTURE 1. Different yeast packages used for fermentation. 11
PICTURE 2. Dry yeast sachet pitched at Kahakka Brewery 16
PICTURE 3. Dilution steps 22
PICTURE 4. $1mm^2$ grid at 100x magnitude 23
PICTURE 5. $0.2mm^2$ top left corner cell at 400x magnitude 24

TABLES
TABLE 1. Yeast dry matter composition 5
TABLE 2. Batch #33 following of fermentation 18
TABLE 3. Batch #46 following of fermentation 20

TABLE 4. Cell counting results, 6 days fermenting........25
TABLE 5. Cell counting results, 10 days fermenting before adding flocculant........25
TABLE 6. Cell counting results, day after adding flocculant........26
TABLE 7. Cell counting results, end of primary fermentation........26
TABLE 8. Billion cells/ml and viability results........27

1 INTRODUCTION

Yeast pitching requires a constant supply of the same high-quality yeast mass which will help to ferment sugary worth. The life cycle of yeast during fermentation can be divided into aerobic and anaerobic conditions, the former to promote cell multiplication and the latter for producing ethanol from fermentable sugars.

In this research yeast is analyzed and tested under laboratory conditions to justify the design of a yeast propagation vessel, given that previously there is no documentation regarding fermentation and final maturation of beer production in the local Kahakka Brewery. It was concluded that such an analysis can facilitate the decision-making during production and brightening of the beer. This conclusion should be integrated into a production schedule, optimizing time and processes, thus improving the overall quality of the products. To conclude the parameters to control and the hazards involved in microorganism's manipulation, first, research literature will provide the parameters for optimal yeast handling and the yeast strains used at Kahakka Brewery will be specified. Second, an examination will be done at the production, handling and fermentation control regarding the yeast environment in the brewery. Third, by providing a comparison of literature and production at Kahakka Brewery, the characteristics and details of a yeast propagation module will be justified. Main topics will be the technology applied to control the yeast propagation, and what is required to produce healthy yeast mass. Besides an inside on yeast biology and environment, food technology and process optimization will be reviewed.

The research indicates that procuring a stress less environment will propagate the healthiest yeast, when, among the whole brewing process and later maturation of the beer, key elements as temperature, pressure, oxygen content and enough nutrients are supplied. Allowing the yeast to complete its life cycle the best way possible was determined to be performed by the utilization of a propagation-only module that by attaching it to fermentation tanks at place would manipulate the aforementioned parameters.

2 GENERAL REMARKS AND BASIC CONCEPTS OF BREWERY YEAST

The present chapter begins by describing the basics of yeast and fermentation. Concepts about yeast biology, what microorganisms are created during fermentation and how does yeast behave on a microscopic scale will be familiarized. The aim is not to create a biology book, but it is necessary to understand how this organism has such an important effect on the beer production.

Yeast is a single-cell organism, meaning it has no skin as a form of protection like other organisms but compensate in numbers and rapid cell replication. A single yeast cell is about 5 to 10 microns in size and circular-to-ovoid shape. Yeast cells are ten times larger than a microbe and a visible yeast colony in a petri dish contains at least 1 million cells. There are more than 500 species of yeast and between each there is thousands of different strains. Multiple yeast forms are found everywhere in the world, for example outside breweries, living in soil, on insects and crustaceans, on animals and plants, basically settling on every surface they reach. (White & Zainasheff, 2010) Uncontrolled yeast, referred to as wild yeast, is a common problem inside breweries, given the easiness they possess to find a suitable environment and multiply. Most breweries do not want wild yeast fermenting their products. (Kunze 2014).

Annemuler, Manger & Lietz (2011, 313), lists the following operations as general remarks and basic concepts regarding yeast management; the isolation and selection of brewing yeast strains, the propagation of pure culture yeast in the brewery laboratory, careful storage and manipulation of yeast strains cultures, propagation of yeast in the brewery, pitching and control of fermentation, the treatment and storage of yeast, and the recovery of beer from tank bottoms and the utilization of surplus yeast.

Yeast is commonly described as one of the most important ingredients when brewing, especially in fermentation related processes. There exist dozens of different yeast strains which affect differently the end-product, and it is important to know which type of yeast to use depending on the desired beer type. That is why brewers, winemakers and distillers use specific species of yeast for their products. (White & Zainasheff 2010.) Previously in the brewing industry, a considerable difference was pointed between two main yeast species used in the past, namely ale yeast and lager yeast, both coming from the same "sugar fungus". The former is also referred as "top-fermenting" type Saccharomyces cerevisiae, producing more fruity and malty beers, while the latter is referred as "bottom-fermenting" type Saccharomyces carlsbergensis, giving a characteristic sulfurous aroma. (Goldammer, 2008.)

Thanks to the diligent work of brewers from the past who did an arduous work trying to avoid contamination of their crops and harvests, the genetics of the saccharomyces cerevisiae are well known and easy to recognize inside forensic laboratories, and it is easy to ring alarms once wild yeast has appeared at any controlled process. It was also this craving for consistency and the negation of wild yeast mating with the crops which eventually modified the yeast cell genes to the point where they lost the ability to form spores and mate. This evolution assures even more consistency from batch to batch. (White & Zainasheff 2010.)

2.1 Microbiological and biochemical fundamentals of yeast multiplication

To properly assess the yeast metabolism quantitatively, it is necessary to consider the fundamental microbiological and biochemical compositions of pure yeast cultures in order to later determine the nutrients requirement, to calculate an accurate yield percentage and to design propagation plants. It must be noticed that the chemical composition of yeast can vary widely depending on the breeding conditions, intensity of multiplication, the age of the cells and the nutritional state of the cell. (Annemuller et all 2011) The aims of the brewer are different of those of the yeast; thus, a brewer must know how to "steer" the fermentation process by manipulating the substances that yeast communicates with.

2.1.1 Moisture content and dry matter

The moisture content of a yeast cell varies from different sources. Kunze (92, 2014), indicates that the yeast cell consists of 75 % water against Annemuller et all (2011, 63) giving between 65 to 67 % true moisture content, while Briggs, Boulton, Brookes & Stevens (2004, 387) approximates it to 80 %, meaning that about 20 to 35 % is dry matter (YDM); roughly keeping a ratio of wet to dry of 5 : 1, being Carbon the most abundant element, accounting for just below 50% of the dry weight (Briggs et all, 2004, 387). Yeast dry matter varies its composition between proteins, carbohydrates, fats, and minerals as phosphates, potassium, sodium, calcium, magnesium and zinc. Other major elements inside the cell are oxygen (30 to 35%), nitrogen and hydrogen (5%) and phosphorus (1%) (Briggs et all, 2004, 387). Yeast also contains vitamins such as thiamine (B1), riboflavin, nicotine acid, folic acid, pantothenic acid, pyridoxal and biotin (Kunze, 2014). Moisture content inside the cell allows the metabolism of the yeast to work properly, by keeping the proteins and cell elements well hydrated.

The chemical composition is a good insight regarding the nutritional requirements of the yeast for its multiplication. While sources such as Annemuller et all (2011, 65) indicate that the metadata regarding

chemical composition of baker's yeast, referred to as "molformulas", can be used for understanding and manipulating brewing yeast, Kunze (2014, 93) and White et all (2010, 18) provide a total description and characterization of the brewing yeast. There will be no discrimination between them when describing the cell unless it is necessary. Molformulas list the macro elements required for yeast multiplication, these being many substances as proteins (being the most abundant, 40 to 45% of cell dry weight), carbohydrates (30 to 35%), nucleic acids (6 to 8%), and lipids (4 to 5%) (Briggs et all, 2004, 387), which are formed by a large number of single components. These also work for crop and pure cultures of brewing yeast.

During fermentation, yeast produces many "by-products" which are not cataloged in the same group of substances but provide great influence on the taste and characteristics of beer (Kunze 2014). Briggs et all (2004, 387) indicate an approximate 5 to 10% of the cell dry mass to be of mineral content. The smaller group present in the cell are vitamins which relate to the nutrient requirements for growth-promotion (Annemuller et all, 2001). The precise composition of macro elements inside the yeast cell and their percentage varies between yeast species and other reasons previously mentioned, thus Briggs et all (2004, 387) indicates that is not possible to provide a more generalized composition. Besides these, each yeast cell consists of the cell plasma and cell membrane, both discussed more on detail in the next section. Table 1 summarizes the composition of the dry matter for Saccharomyces yeast.

TABLE 1. Yeast dry matter composition (adapted from Annemuller et all 2011)

Components by classes	**Subgroups**
Crude protein 45…60% *40…45%	Proteins + aminoacids Albumine Gobuline Phosphorproteids Nucleic acids Nucleotides Peptones polypeptides
Carbohydrates 15…39% *30…35%	Glycogen Yeast mannan Yeast glucan Trehalose
Crude fatty substances 2…12%	total fatty substances neutral fats phosphatides lipoidsymplexes sterols cerebrines
Ash 6…12%	P_2O_5 K_2O MgO CaO Na_2O SiO_2 Fe_2O_3 SO_4 Cl^-
*source: Briggs et all (387, 2004)	

2.1.2 Yeast cell structure

The main features of a typical yeast cell are those of a single-cell organism, oval shaped that occurs in single, pairs, chains and cluster conglomerations. (Stewart & Russel 1998 [Hills 2015].) Each cell consist of the cell wall, composed of three distinguishable layers. It is mostly made of carbohydrates and grants around 25% of the dry weight. The inner layer is a chitin layer mostly of glucans, the outer layer is mostly mannoproteins and the intermediate is a mixture of both. The plasma membrane lays between the cell wall and the inside of the cell and due to its constituents, it allows the formation of a daughter cell when budding, among several other roles (Hills 2015). Inside the cytoplasm, series of organelles are responsible for the metabolic reactions. Figure 1 features organelles inside the yeast cell.

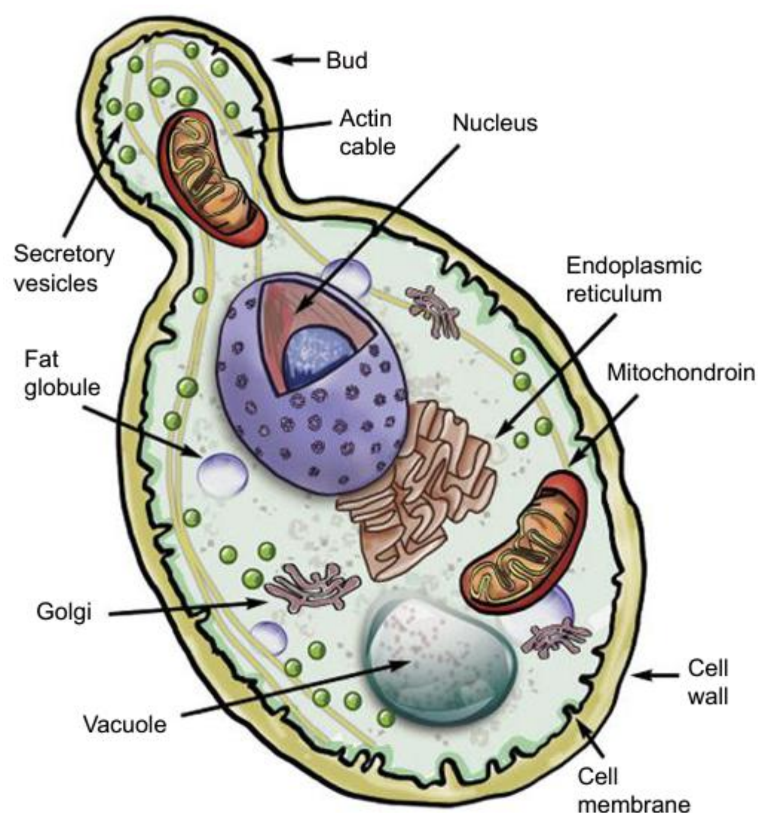


FIGURE 1. Yeast cell features (Stewart & Russell 1998 [Hills 2015]).

The genes that control all metabolic processes from which the cell acquires its own construction plan are encoded in the DNA, located in the nucleus, where the core matrix and the chromosomes communicate with the cell through pores, even that the double nuclear membrane is closed (Kunze 2014).

2.2 Yeast propagation

Yeast has the possibility to grow in quantity from small sized into commercially pitchable volumes. This task requires extreme attention to sanitary practices and yeast health, as well as increased oxygen input (White & Zainasheff 2010). Propagating is not only about growing yeast mass, but rather about growing the healthiest yeast possible. The brewer’s community refers to the beginning of propagation as “starters”. Scaling-up of yeast introduces active growing cells to fresh nutrients to harvest a healthy crop of yeast in the best physiological state. (Goldammer 2008.)

White & Zainasheff (2010, 126) divide propagation as a two-stage process, starting in the laboratory from a plate culture and cultivated until a size the brewery needs to manipulate. The acquisition of propagated yeast can start at the same brewery if it possesses the laboratory equipment and can provide a successful propagation, or it can be grown and bought from a yeast pitching manufacturer. The initial cultures to propagate from are inoculated from a “master” culture and propagated until their size is substantial enough for pitching the desired batch, meaning the number of transfers depends on the final size, and the higher the amount required, the higher the risk of contamination. Initial laboratory scale multiplication is small to ensure quality overall (Goldammer, 2008).

White & Zainasheff (2010, 127) included in the keys for a successful propagation the following requirements: An aseptic technique, to ensure the purity of the culture; a sterile growth media to avoid amplifying any contamination from the previous step; avoiding to exceed appropriate increments in step-up volume, to ensure healthy growth rates; a safe aeration method; and temperature which enhances growth rate. Once a successful laboratory scale propagation has been obtained, careful transfer between small size batches are done until a plant phase is achieved. During propagation, temperature is maintained at a set level and the yeast is intermittently aerated and pitched into a fermentation vessel or production tank. This step is repeated until the size is enough for a commercial batch. Optimum temperatures at these early stages vary depending on the source, but they can be at the same temperature for cast-out, about 25 °C when the wort is cooled down after boiling, previous to start fermentation, lower or higher, to prevent temperature shock or to stimulate growth. (Goldammer 2008.) Propagation plants are usually situated on different rooms as those of production, to avoid contamination risks and consist of one or multiple propagation vessels, usually stainless-steel tanks of increasing volume. A common laboratory propagation may look like in Figure 2.

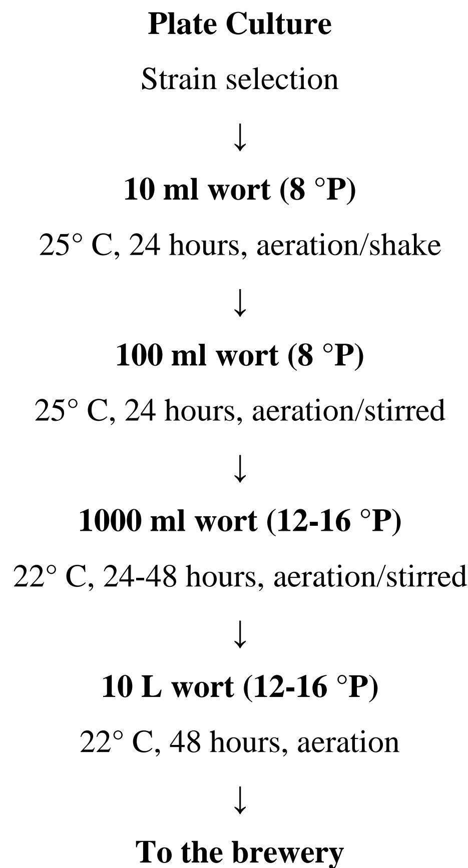


FIGURE 2. typical laboratory propagation for ale yeast. White & Zainasheff (2010, 128)

At Kahakka Brewery, yeast propagation starts by adding 200 grams of dry malt extract into 2,5 liters of water and let to boil until the volume of liquid is 2 liters. Once the mix is cooled down and transferred to an Erlenmeyer flask, 40 ml of yeast is added. The flask is tightly sealed to avoid any contamination and left stirring for 24 hours on a stirring plate. The next step is to divide the starter into 2 different pots, tossing 1 liter of starter into each pot and adding another mix of 4 liters with 500 grams dry malt extract, making a total of two 5 liters pots, each being aerated for 1 minute. Once aeration is complete, both 5 liters are mixed into one 10 liter pot which is pitched into a propagation tank and mixed with 100 liters of fresh wort which later will be used for the fermentation of a 1000 liters batch, as shown in Figure 3 b2. If enough precaution is achieved, there should be no risk of infection while making the starter; the problem is that the brewery does not have a dedicated place for keeping the starter in a contamination-free zone. It must be said that the most hazardous parts of this process are the handling and risk-free manipulation of the 2- and 5-liter starters. Once it reaches the propagation tank, contamination hazards drop considerably.

The sole purpose of acquiring a yeast propagation module would be to prevent handling errors, achieved by automating the process and recreating a contamination-free, closed system for the multiplication of a pure culture. It is preferable to install fixed piping and avoid the usage of hoses or fittings. If automation will be utilized, it is recommended to add no manual handling at all. A fully automated yeast propagation plant would meet the requirements as; fixed piping, double-seat piping with valve seat lifted by compressed air, the capacity to store worth to keep its contamination-free condition, avoiding unnecessary fittings, pumps and pipes that fulfill its function, and an incorporated CIP (Cleaning in Place) system (Annemuller et all 2011).

In Kahakka Brewery, there is one dedicated tank for propagation. So far, it is only used for the propagation of one yeast strain and this yeast is used for the fermentation of one type of beer. To begin propagation, two basic elements are needed. First, the yeast to propagate and second, fresh wort as the propagation medium. The yeast can be obtained from two sources, the first type is collected as biomass from an already existing fermenting tank, by a process known as “yeast-off”, on which flocculated yeast is collected from the bottom of a fermentation vessel, and the second method of preparing the yeast is by making “starters”, which means propagating a small yeast culture from laboratory quantities (2 liters) to batch-size quantity (100 liters). This production process at Kahakka Brewery is explained in chapter 3. Fresh wort is collected after boiling the wort, before transferring to primary fermentation, in a process referred to as cast-out.

3 YEAST HANDLING AND PROPAGATION AT KAHAKKA BREWERY

The present chapter will provide an insight into the fermentation process at Kahakka Brewery, where yeast takes special participation. Although wort fermentation is the most important part of brewing, the beginning of fermentation (hot part) will be mentioned to allow a general picture of the process, how these two parts relate to each other and how yeast is introduced. Two types of yeast are used for fermenting wort at Kahakka Brewery. First type is dry yeast which needs to be rehydrated and the second type is ready to pitch yeast (liquid form) which can be propagated. Both initial amounts need to be increased in order to utilize for industrial batches. Figure 3 illustrates the yeast propagation practices used at the beginning of this research.

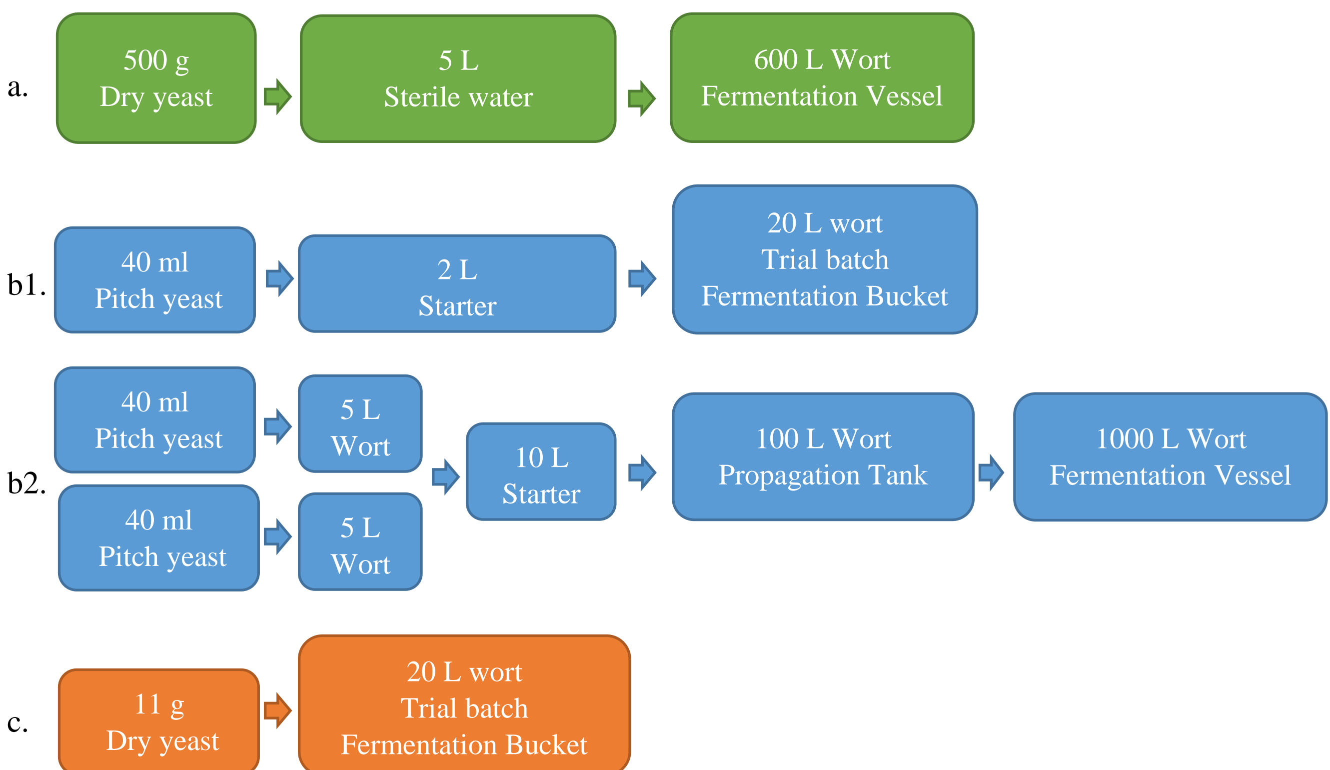


FIGURE 3. General yeast propagation procedure at Kahakka Brewery

Given the many occasion were jeopardizing the propagation is possible, and that multiple times it did happen that a test-batch or "starter" got ruined because of an infection, most of these practices were changed and the utilization and/or propagation of small yeast amounts were cut to a minimum, promoting the industrialization of beer batches regarding quantity and yeast pitching rates, trying to minimize yeast handling as much as possible. This translated to the use of new packages of fresh yeast for every batch. Resent practices, including the Standard Operational Procedure (S.O.P.) make use of multiple new dry

or liquid yeast packages pitched directly into the fermenter tanks while casting out. Picture 1 shows the different strains pitched during production.

PICTURE 1. Different yeast packages used for fermentation.

3.1 Key considerations

The largest wort volume needed to ferment is about 1400 liters, and the methodology used at Kahakka Brewery indicates that 0,5 liters of tick slurry (biomass) is needed for each hectoliter of wort. While propagating, 5% of sugar content will become biomass in a normal fermentation. If 100 liters are propagated, 5 liters of tick slurry are obtained, which is enough to ferment 1400 liters as mentioned above.

During production conditions, about 587 kJ per kg of extract are produced (Kunze, 2014, 423). This extract is then two-thirds fermented during the main fermentation. One hectoliter of pitched wort contains about 12 kg of extract, two-thirds of it would be about 8 kg, which is fermented during primary fermentation, consequently 4696 kJ are produced for each hectoliter of wort which must be cooled down, mainly through cooling jackets with a mixture of glycol as the cooling-media.

3.2 Production settings and recommended environment for yeast pitching

The whole process of brewing one batch of beer can be divided in two: the hot part refers to the wort production and the cold part refers to the after-boil fermentation and maturation of the beer. To have a clear understanding of the yeast environment in the brewery, the basics of a batch produced at Kahakka Brewery will be mentioned. The process begins by milling the malt grains and mixing the grits with hot water inside the mash tun. The sugary liquid obtained from this process is referred to as liquor.

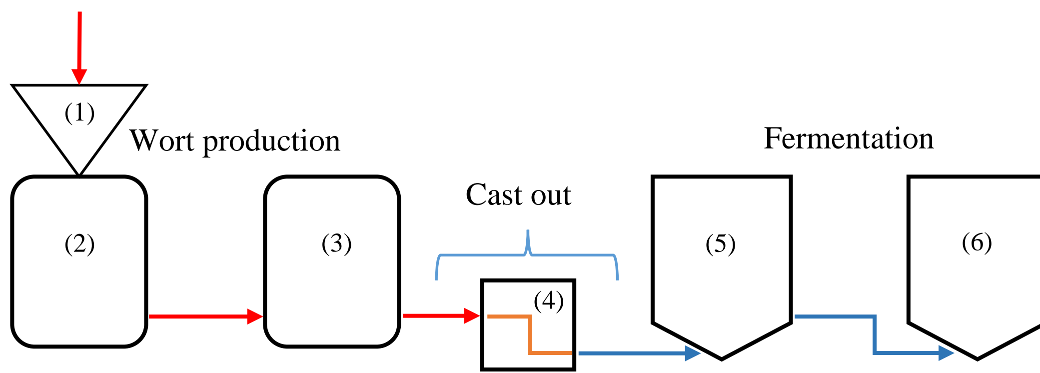


FIGURE 4. Simplified flow diagram of beer production in the brewery. Red and blue arrows represent the hot and cold parts of beer production respectively. (1) malt grits, (2) mash tun, (3) boiling kettle, (4) heat exchanger, (5) fermentation vessel, (6) bright-beer-tank.

The liquor is then collected and boiled inside the boiling kettle, were final ingredients are added and the hot part of a batch production comes to an end. After the liquor is chilled to a desired temperature not to shock the yeast, the cold part of the production begins, yeast is pitched, and fermentation should start. The following pages will describe in more detail the production steps, illustrated on Figure 4.

3.2.1 Wort production

According to Kunze (2014, 201), the most important process in beer production is the fermentation of the sugars contained in the wort to form alcohol and carbon dioxide. The whole purpose of wort production is to generate the conditions for the dissolving of malt components which are originally insoluble, in particular, the production of fermentable sugars with the aid of enzymes. Wort production is the beginning of fermentation and starts by milling the malt grains into a suitable size. In the

brewhouse, the malt grits are mixed with water inside the mash conversion vessel (mash tun) to obtain as much soluble extract as possible. Once this process is complete, the wort is separated from the spent grains and transferred to a boiling kettle. The wort is then boiled, and hops are added for the taste characteristics in the beer. Solids are collected inside this tank by means of a whirlpooling effect, making a cone in the center where solids get trapped. Once the whirlpooling is over, the liquor is transferred through a heat exchanger into a fermentation vessel, in a process referred to as cast-out, lowering the temperature of the worth since the next fermentation steps must be performed at lower temperatures, not to affect the yeast. Once cast-out is done, the cold part of the process begins.

3.2.2 Fermentation

To ferment wort, the sugars must be fermented by the yeast into ethanol and carbon dioxide, among other flavor compounds (Kunze 2014; White & Zainasheff 2010). The result of this process also brings the formation of by-products which affect the taste, aroma and different properties of the beer, all related to the yeast metabolism. Kunze (2014, 385) indicates that the reactions occurring during primary fermentation can be differentiated from those occurring during secondary fermentation and maturation, but the processes overlap. These must be considered as continuous processes. White & Zainasheff (2010, 65) mention that experts divide fermentation into four or more phases: lag, growth, fermentation and sedimentation, but agree with Kunze about processes overlapping. This is due to the fact that the activity affecting yeast varies on each individual cell through fermentation and is preferable to understand the on-going process through "hours after adding the yeast". White & Zainasheff (2010, 66) simplify the fermentation process by the following three phases: lag phase from zero to 15 hours, exponential growth phase for one to four days, and stationary phase for three to ten days.

Wort must be pitched immediately with yeast and distributed uniformly. The cell concentration while pitching influences the speed of the fermentation reaction, as well as the wort temperature must be controlled, not to shock the yeast. Favorable temperatures for lager yeasts range from 7 to 15 °C when pitching, growing slower than ale yeast and settling to the bottom of the tank, thus referred as bottom-fermenters. Ale yeasts' temperature for pitching ranges from 10 to 25 °C and rise to the surface while fermenting, thus being referred as "top-fermenting". (Goldammer 2008.) If the wort is not pitched with yeast immediately, wort bacteria would reproduce fast enough to consume vitamins and growth substances which are necessary for a positive fermentation. During cast-out, wort must be aerated to increase yeast propagation, increase the fermentation intensity, and different favorable influences in the taste of the final beer, as long as the oxygen saturation is not excessive. (Kunze 2014.) In the brewery,

oxygen is introduced through an aeration stone right after the wort is cooled before entering the fermentation vessel, at a rate of 5-3 l/min.

Once the yeast is introduced to its new environment, the cell needs to get used to the new temperature, pH value, and higher sugar concentration. During the process known as “lag phase”, yeast cell will excrete amino acids and nucleotides that will soon reabsorb. At this point, before it gets closer to the new medium, yeast cells will draw its reserve substances that provide the initial energy, lowering the glycogen content. Right after, given the abundant amount of fermentable sugars in the wort, it will quickly start to break down the sugar by respiration and fermentation. (Kunze 2014.) A drop in pH value is also a mark for the lag phase, given the utilization of phosphate and reduction of oxygen (Goldammer 2008). The same sugary content slows respiration rate and intensifies fermentation through a process called “Crab-tree effect”. Simultaneously, yeast forms new cells logarithmically, through asexual reproduction by means of buds, a form of cell division. The whole process of wort production (hot part) is to achieve and ensure that the necessary structural elements are present for the desired products to react. Kunze (386, 2014) refers to these structural elements as: amino acids, to produce new yeast substances; phosphate for the bond in ATP; sugars; salt and trace elements (zinc); and sufficient oxygen.

If any of the previous elements is not abundant enough, fermentation may fail, even though yeast can synthetize these structural elements itself. It is during this logarithmic phase (log phase, also named growth phase or respiration phase) when yeast is the most active and builds a reserve of carbohydrates to compensate any energy recovery in the case of nutrient deficiency (Kunze 2014). It is glycogen, one of the intracellular reserves that acts as energy for the early cell activity, being broken down into glucose which the yeast cell uses for its main concern, reproduction (Goldammer 2008). Respiration phase results in a foam layer covering the wort surface due to carbon dioxide being released and happens once enough glycogen reserves were created. This phase is the most important because the yeast cell uses the oxygen in the sugary media to oxidize acids compounds and the wort flavor disappears, being replaced for what is called “green beer flavor”, referring to the beer not being mature enough.(Goldammer 2008).

The log phase then comes to an end when the supply of fermentable sugars has been reduced (drop in Plato reading) and almost zero fermentable elements remain. Commercial batches provide one to three multiplication in the cell number but is concluded that the amount of yeast growth is proportional to the nutrients in the wort (Goldammer 2008). Fermentation follows as anaerobic process once the oxygen supply is scrubbed. During this stage, yeast is mostly in suspension converting the last fermentables into carbon dioxide, ethanol and by-products (Goldammer, 2008). Once the fermentation is complete (about

3 to 7 days), yeast starts to flocculate, there is no multiplication, and alcohol and CO_2 inhibit the cell. Since there is no more action inside the tank (intensive bubbling has stopped) the yeast cell sinks to the bottom of the tank where it can be harvested (Kunze 2014). At this point yeast will search any means of survival, primary trying to feed on maltose and maltotriose, using its own metabolisms as means of energy to move. The cone in which it sank would prevent it from moving, causing it to rapidly deteriorate. As it dries up its own reserves, the cell beings to excrete metabolic products and enzymes that have a detrimental effect on the foam and flavor, the pH of the beer increases, and the dissolving enzymes being excreted are a form of nutrition of contaminants, for this reason, yeast has to be harvested (removed) repeatedly on time, through a process known as "yeast-off" (Kunze 2014). The harvested yeast can be stored cold or dumped. At Kahakka Brewery, primary fermentation of the beer is completed in cylindroconical vessels referred to as fermentation vessels (FVs) and later maturation of the beer is performed in bright-beer-tanks (BBTs).

3.2.3 Pitching calculations

Besides giving especial attention to the stability and health of the yeast when fermenting beer, the amount of yeast to be used must be calculated, and these deal with amount of yeast cells per liter of wort, meaning how many yeast cells are needed to pitch-in to properly ferment the quantity of liquor on the tank. There is no exact number of yeast cells for number of liters, being a home brew or a commercial batch, because of the many factors the calculation depends on, but most sources agree on the safe zone for a pitching rate being 1 million viable cells per degree of Plato per ml of wort (Goldammer, 2008; Daniels, 2000). Other factors affecting pitching rates are yeast strains, fermentation capacity of the yeast, yeast viability, flocculation characteristics, even the history of the yeast batch, and of course the desired beer flavor characteristics. (Goldammer 2008).

Daniels (2000, 118), mention an "absolute minimum" of pitching quantity to use for home-brewing batches size, or the minimum pitchable quantity possible to acquire commercially, some 10 to 20 billion yeast cells for 5 gallons of wort, about 20 liters, referred by him as the homebrew pitching rate. In Kahakka Brewery, this same amount is used for test batches of 20 liters brew, pitching about 11 grams of dry yeast (recommending 0,5-1 gram per liter of wort). Pitchable yeast used locally is either dry or liquid in the form of biomass.

PICTURE 2. Dry yeast sachet pitched at Kahakka Brewery (11g), for test batches.

High or low pitching rates have multiple effects on beer. Lower pitching rates can favor the formation of byproducts leading to a deviation from the desired yeast effect on the product, like the formation of higher alcohols, esters and diacetyl, or even increment the sulfur dioxide level of the beer. Extreme cases of low yeast count can even stuck the fermentation altogether (Goldammer 2008). Higher or excessive pitching rates promote off-flavors on the beer, and can cause “hot spots” during fermentation, deviating even more the flavors of the beer and affecting the stability once the product is bottled. Also, high pitching yeast can lead to an aging on the yeast population, avoiding the creation of new cells that is why some brewers pitch at lower rates to promote the reproduction of new yeast.(Goldammer 2008).

3.3 Batch analysis

Over a period of three months, the fermentation of two batches will be analyzed mainly through the sugar content of the liquid and its pH. The present analysis would focus on what is mentioned as “steering fermentation” by Annemuller et all (2011, 363) and “conditioning” by Goldammer (2008, 255), in which the required time and quality of the final product are influenced not only by the quality of the yeast and of the wort, the pitching process and of the equipment, but most importantly by the conditions prevailing during fermentation. These can be temperature settings, pressure inside the tank and yeast harvesting. The standard approach to monitoring fermentation according to Bamforth (2002,

118) is to follow the drop in specific gravity, referring to sugars converting into ethanol. Other measurements as the CO_2 content, pH drop, or yeast multiplication can also be utilized for validation of the recipe or technical improvements. These favorable parameters are often already adjusted at the beginning of fermentation and is the case at Kahakka Brewery. The Plato gravity scale used for fermentation control analyses refers to the concentration of dissolved solids in the wort. Degrees Plato (°P) quantifies the extract (sugars) obtained from the malt grits as percentage by weight (Philliskirk). Samples are taken by directly opening the sample port of the tank, filling a two-liter jug, which is taken into the laboratory for analysis. Before proceeding, enough liquid is shaken by splashing it between two small plastic cups, to remove the CO_2 avoiding any false reading. Once the liquid has been splashed for 5 minutes, the last step before measuring is to use an ultrasonic wave machine to remove as much gas as possible from a 50 ml sample. After 7 minutes in the ultrasonic bath, the sample is measured. The tool utilized for Plato° readings is a handheld density meter DMA 35 Basic, from Anton Paar, and the pH is measured using a beer pH-meter HI 99151 from HANNA instruments.

3.3.1 Fermentation control through temperature and pressure

The first analyzed batch is a lager, which uses a yeast strain S-189 provided by FERMENTIS, pitching-in 3 "active dry-yeast" packages of 500 g. At cast-out, the Original Gravity (OG) was 11,1 °Plato and a pH value of 5,4. At the beginning of fermentation, the tank is set to 12 °Celsius. After 4 days in the Fermentation Vessel (FV) the temperature is changed to 17,5 °Celsius to avoid an early CO_2 dissolving into the beer. The day after, the Fermentation Vessel is bunged-up (Huolihan 2018), or spunded (Kunze 2014), to disallow the liberation of CO_2 and carbonate the beer, thus rising the pressure in the tank (APPENDIX 1). Six days after cast-out, a process known as Yeast-Off is performed by removing the flocculated yeast collected at the bottom of the tank. Ten days after the beginning of fermentation, the temperature is dropped to 2,5 °C and a Flocculant is added into the tank to help "clear" the beer and compiling the yeast at the bottom. After almost 5 weeks inside the Fermentation Vessel, the product is transferred into a Bright-beer-tank (BBT) to achieve final fermentation and maturation, where the expected Final Gravity (FG) of 2 °Plato is obtained. A second yeast harvesting could be completed before transferring but given that there is no use yet for the collected yeast at the bottom of the tank, transfer is realized and the yeast is left in the FV, removing it once the tank is rinsed and cleaned. The summarized information regarding fermentation analysis of this batch is in Table 2 and Figure 1.

TABLE 2. Batch #33 following of fermentation; Temperature and pressure changes

Fermentation Time (days)	Temperature (°C)	Notes
0	12	Wort taste
2	12	Intense bubbling (APPENDIX 2/1)
3	12	Bubbling ceases (APPENDIX 2/2)
4	17,5	Temperature rise
6	17,5	Spunding (APPENDIX 1)
7	17,5	Yeast off
8	17,5	Plato/pH stabilized
11	2,5	Flocculant added + temperature change
20	2,5	
34	2,5	Transfer to BBT. Final gravity reached
38	2,5	Bottled

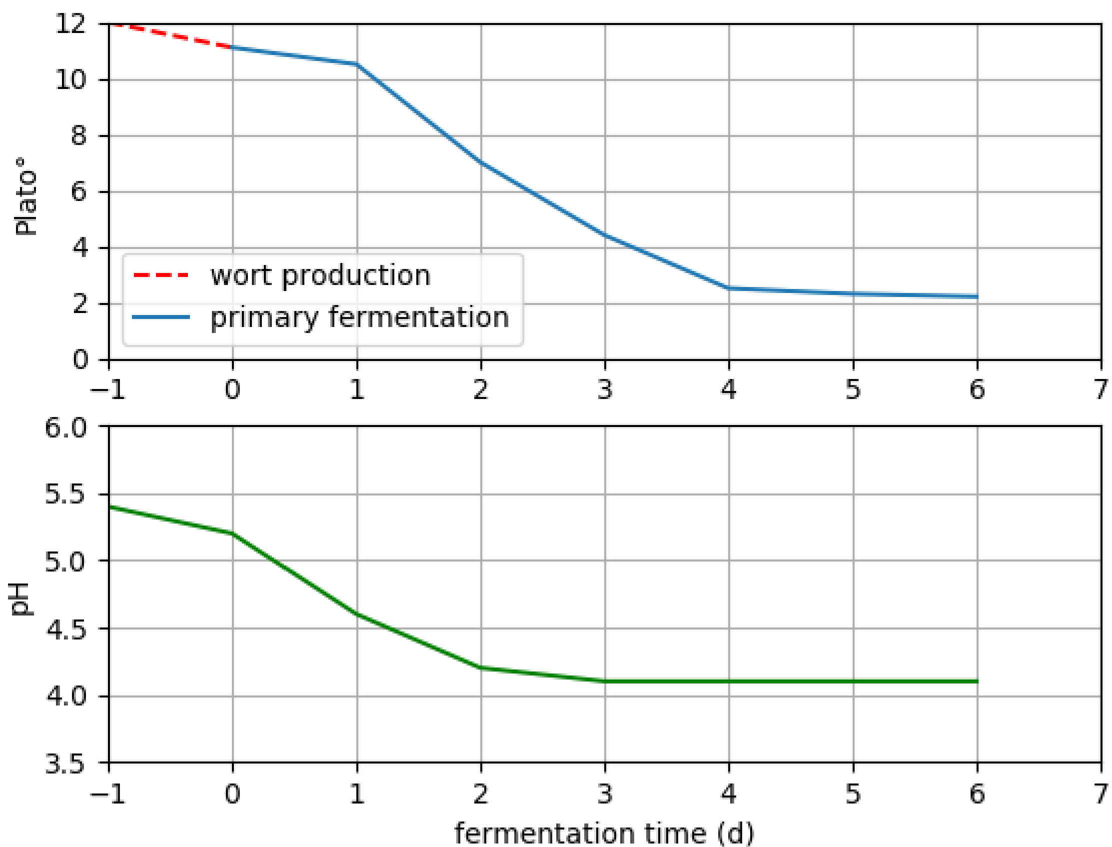


FIGURE 5. Plato° and pH during primary fermentation of batch #33

3.3.2 Fermentation control through laboratory methods

During fermentation, a microscopic examination of the yeast can provide a good representation of the yeast health. Stressed yeast can be recognized through abnormal showing or irregular shaped cells. Foreign particles that should not be around at this stage as trub, grains or diatomaceous earth can also be detected. Even though a microscopic view is a good indicator about the wort composition, aeration, yeast handing and fermentation conditions, it is not particularly delicate when detecting common brewery contaminants (Goldammer 2008). If a deeper analysis is required, further tests must be supplemented. Pellettieri (2015, 75) provides examples regarding viability tests versus vitality tests, both which can be performed to predict not only if the cell is still alive, but also the metabolic activity. The former tests are in the form of slide culture; Brightfield Methylene Blue or Violet stain, and Fluorescent dye. The latter deal with CO_2 generation or O_2 uptake, acidification, magnesium release or intracellular pH and Flow cytometry. Pellettieri notes that vitality tests are still controversial and subjects of ongoing research (2015, Figure 6.2). Besides the aforementioned tests, breweries can measure the cell density through fermentation with the aid of a hemocytometer, which can provide data to improve yeast harvesting scheduling. Annemuller et all (2011, 342) describes two control methods to determine the yeast concentration in wort or yeast suspension: using a hemocytometer or an automated, intelligent particle counter. The present analysis is performed with a professional lab testing kit from White Labs and a suitable microscope.

The cell density test is performed by homogenizing a sample of slurry yeast and diluting it to thin enough level to count (Pellettieri 2015). Stirring is important in the same way described for the Plato° measurement, because the sample can have gas within it. Once a consistent, homogenous mix is achieved, dilution is the next critical stage to avoid errors in cell counting, given that an extra drop can contain millions of yeast cells (Pellettieri 2015). When proper dilution steps are taken, a drop is carefully placed under a cover slip and the counting chamber is filled. The counting chamber is a three-dimensional area with a precise amount of fluid in it. The conversion is from cubic centimeters to ml. Pellettieri (2015, 76) provides the math of the hemocytometer to result in concentration cells/ml following the multiplication of total number of cells in the counting chamber times 10,000. Another direct approach of the same calculation can be equated as:

$$Concetration\left(\frac{cell}{ml}\right) = average\ cell\ count * dilution\ factor * area\ under\ the\ converslip.$$

(Pellettieri, 2015)

Pellettieri (2015, 77) lists the common mistakes that jeopardize the proper analysis when utilizing a hemocytometer as: wrong cover slips, which are properly sized and weighted; poor filling technique; too little liquid in the counting chamber; poor counting technique; and an overcrowded camber.

The sample to analyze is a Pale lager, pitched with a mix of two different yeast strains. For a batch of 1400 liters, one kilo of yeast strain S-23 and 500g of S-189 are added, both provided as active dry yeast from FERMENTIS. The Cast-out temperature is 21 °C, lowering it down to 13 °C once fermentation is recognized to go on through active bubbling coming from the spundling valve (24 hours after pitching). At this point the tank is not yet bunged-up and will be pressurized once the active bubbling slows down. Five days after pitching, the tank is bunged-up and the temperature is set to 20 °C. At this point the Plato has lowered considerably and is close to Final gravity. A synthesis of the fermentation temperature, pH values and extract content of the batch is shown in Table 3, Figure 2.

Fermentation Time (days)	**Temperature (°C)**	**Notes**
0	21	Wort taste
1	13	Intense bubbling
3	13	Bubbling ceases
4	20	Spunding (pressurized)
5	20	
6	20	Yeast collection for lab testing
7	20	
8	-1	Flocculant added + temperature change
10	-1	
11	-1	
12	-1	Transfer to BBT

TABLE 3. Batch #46 following of fermentation, temperature and pressure changes

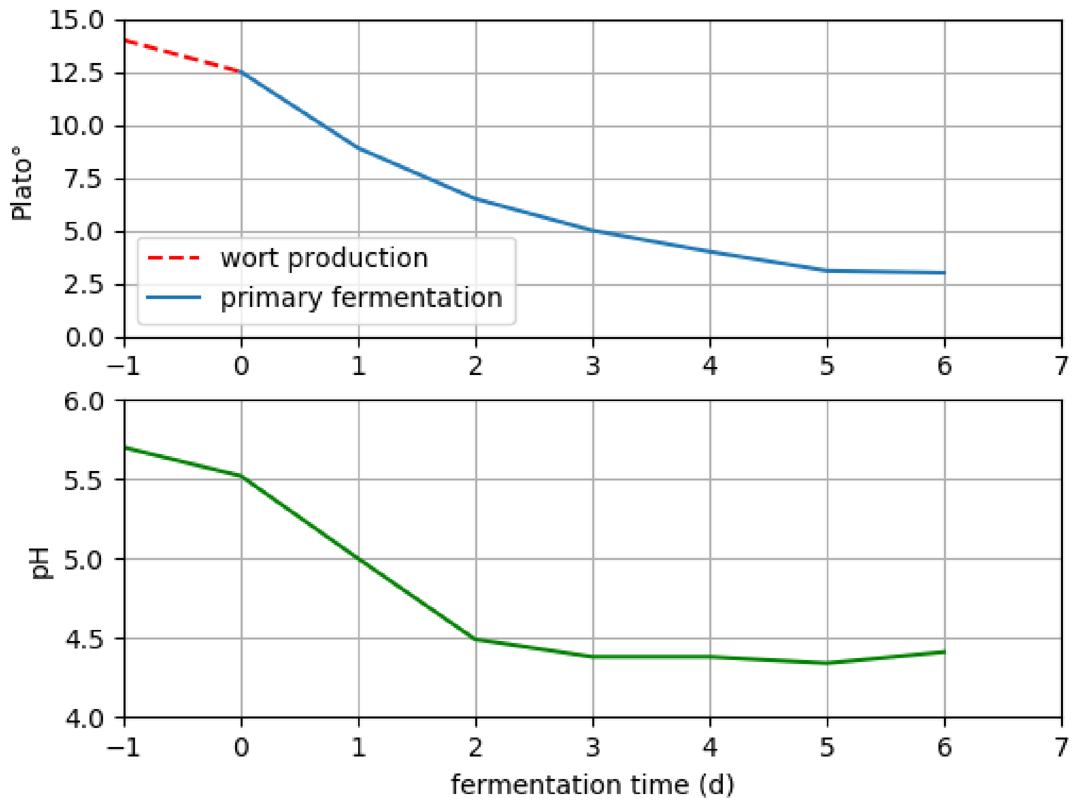


FIGURE 6. Plato° and pH during primary fermentation of batch #46

3.3.3 Cell counting and viability test

The cell counting analysis was performed by utilizing a microscope kit for professional lab testing, which supplemented almost all the required elements to successfully use the hemocytometer, listed below is the equipment used for analysis:

- Methylene blue stain solution
- Hemocytometer
- Handheld counter
- 10 µl transfer pipette
- 1 ml pipette
- Microscope with 400x capability
- Stirring plate and magnet
- Yeast slurry sample
- 50 ml beaker
- Distilled water

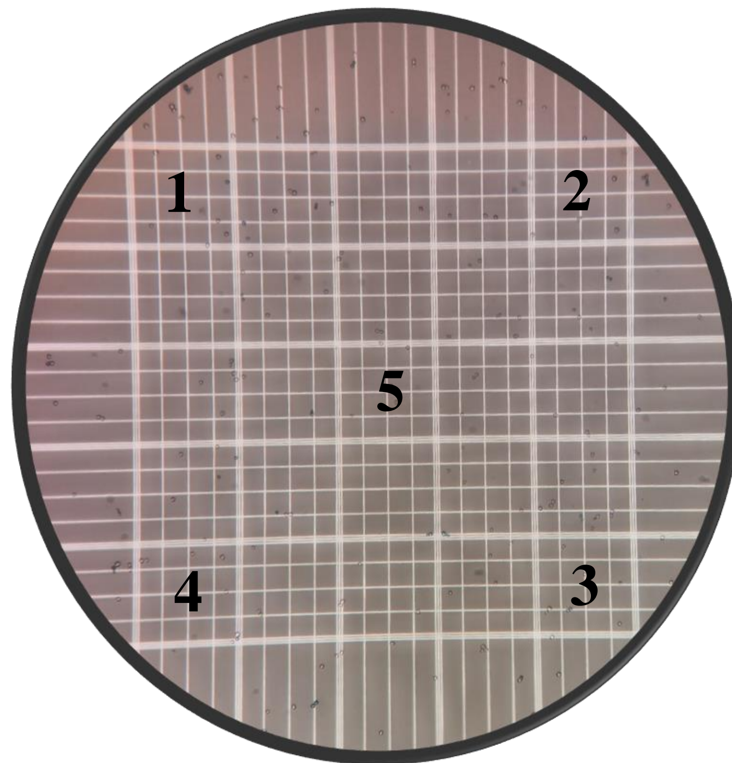
- Conical and sample tubes

To prepare the sample, thick slurry is taken from the dump valve of the Fermentation Vessel one week after pitching the yeast and transferred into the laboratory in a sterile plastic container. In this case, thick slurry needs to be diluted before counting in order to get a more precise result. This dilution factor is accounted for when calculating the result. In the laboratory, sufficient quantity of biomass is magnetically stirred for five minutes in a 100 ml beaker using a stirring plate and a magnet, to homogenize the biomass and remove any gas which could create bubbles when counting yeast cells. The dilution is done by first taking 1 ml of homogeneous slurry and mixing it with 9 ml of distilled water in a conical tube, diluting with a factor of 1:10. For the next step, 1 ml of this first dilution is mixed with 9 ml of distilled water once more, making a second dilution factor of 1:100. The final step before filling the hemocytometer is to mix 1 ml of diluted slurry with 1 ml of methylene blue stain, making a 1:2 final dilution and facilitating the performance of a viability test by allowing to detect dead cells (Picture 3). The overall dilution factor is 1:200.

PICTURE 3. Dilution steps

Making sure that the hemocytometer is clean, around 10 µl (or a drop smaller than a pea) of diluted stained mix is placed in the middle of the counting chamber, and the cover slip is positioned so it covers both grids. Once the sample is ready for counting, the hemocytometer is carefully located on the microscope stage.

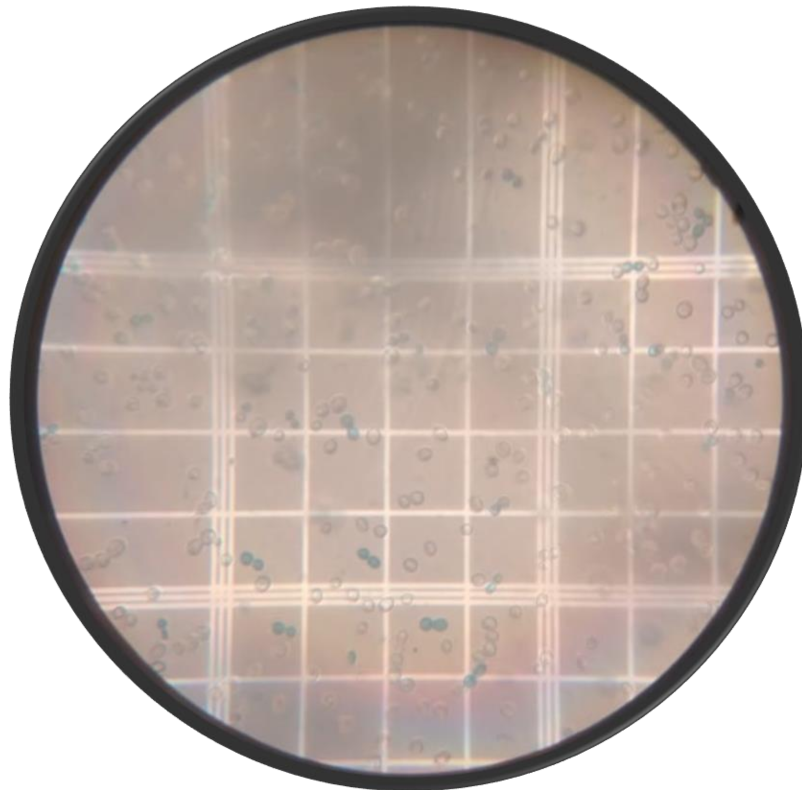
The first approach is 100x to allocate the central grid in the hemocytometer, which is a 5 by 5 square of total 1 mm sides. Each square is divided in 4 by 4 inside squares which are used for counting. Once the main helping lines are found, a 400x lens can be used. From the 5 by 5 grid, only 5 chambers are selected, being the four corners and the center square (Picture 4).



PICTURE 4. $1mm^2$ grid at 100x magnitude. Cells are counted on each numbered square.

At this point certain considerations must be taken, given that the yeast cells are easily seen all around. An example is to follow a protocol as in this case cells lying in the bottom and right border lines are not counted, bud cells are counted only if they are at least half the size of the mother cell, and dark blue cells, as seen on Picture 5 are not counted because that means they do not have the metabolic capability to expel the intruding dye (non-viable), meaning they are dead (White Labs 2019). The results of each test are shown on Table 4 to Table 7, page 25.

The calculations with the results below take the following factors: cells counted; squares counted; dilution factor and volume of the chamber, which is a constant. The viability calculation is completed following the equation: number of live cells / total cells counted x 100 = % Viability (Pellettieri 2015). From tables 4 to 7, boxes which are marked with a symbol (*) are not counted, because they differ from the average and skew the results.



PICTURE 5. 0.2mm^2 top left corner cell at 400x magnitude. Cells inside the 4 by 4 square are counted

TABLE 4. Cell counting results. 6 days fermenting

Chamber	Counting result, 1st time		Counting result, 2nd time	
	Alive	Dead	Alive	Dead
1st	80	32	66*	11
2nd	82	9	84	8
3rd	85	7	77	10
4th	64*	15	76	12
5th	77	15	72	15
Sum	324	63	309	45

6 days after fermentation started the main reactions of the yeast cell that define the beer's profile occurred. It is the initial condition at these stage that define the final product.

TABLE 5. Cell counting results. 10 days fermenting before adding flocculant

Chamber	Counting result, 1st time		Counting result, 2nd time	
	Alive	Dead	Alive	Dead
1st	111*	22	29	3
2nd	92	25	31	3
3rd	90	26	27	2
4th	91	24	31	8
5th	80	42	17*	1
sum	353	117	118	17

Before adding the flocculant, yeast is harvested from the fermentation vessel aiming to maximize the flocculation of solids inside the tank once it is added, lowering the haziness of the beer.

TABLE 6. Cell counting results, day after adding flocculant

Chamber	**Counting result, 1st time**		**Counting result, 2nd time**	
	Alive	**Dead**	**Alive**	**Dead**
1st	29*	9	45	5
2nd	36	5	39	13
3rd	43	5	42	14
4th	39	5	39	4
5th	45	9	41	6
sum	163	24	206	42

The flocculant efficiency is highest at 24 to 48 hours of activity, thus the tank is given 24 hours before removing flocculated solids and 48 hours before transferring the content to a bright-beer-tank for maturation.

TABLE 7. Cell counting results, end of primary fermentation

Chamber	**Counting result, 1st time**		**Counting result, 2nd time**	
	Alive	**Dead**	**Alive**	**Dead**
1st	45*	17	55	14
2nd	60	12	49	18
3rd	56	16	42	16
4th	61	12	41	14
5th	63	12	54	12
sum	240	52	241	74

Once primary fermentation has ended, the green beer is ready for maturation in the bright-beer-tank, where the amount of yeast cells should be close to zero. Most of the fermentable sugars have been converted into alcohol, and the beer is allowed to rest at low temperatures. Cell density is calculated for each day a sample was obtained through the hemocytometer and microscope methods previously described. Results are shown in Table 8.

TABLE 8. Billion cells/ml and viability results.

Fermentation time (days)	Billion cells/ml (1st – 2nd sample)	%viability
6	3.2 – 3.1	83-87
10	3.5– 1.2	89-87
11 (flocculant added)	1.6 – 2.1	87-83
13 (end of primary fermentation)	2.4 – 2.4	82 - 82

3.3.4 Evaluation of the results

The cell counting experiment indicates that there is no considerable difference regarding the harvesting quantity of biomass during primary fermentation at most until the end of it. The overall amount of biomass that is possible to collect will vary over this period, but the practice indicates that the total collected at least one week after pitching would be enough to harvest and utilize for a second batch, without larger consequences. Annemuler et all (2011, 368) indicates that harvesting can be performed either once the total fermentable extract has been utilized or at the end of primary fermentation, like is the case at Kahakka Brewery. The added value is that this is the first time this experiment is performed, accrediting to schedule further similar analysis, allowing to compare beer profiles, thus improving the overall quality of the product supplied by the company.

Hills (2015, 12) synthetizes the multitude of factors that control the overall yeast performance during the brewing process as; The yeast strains used, the condition at which yeast is pitched and through fermentation, the wort sugar spectrum, the wort gravity when pitching, and the geometry of the fermenters used, being a cylindroconical fermentation vessel in this case. It must be considered that at the present stage of the brewery it is not possible to provide other mentioned technicalities as the concentration of free amino nitrogen, ions concentration, the tolerance of yeast cultures and individual cells to stress, the wort dissolved oxygen (DO) level, or the flocculation characteristics of the culture when fermenting, thus they are specified but out of the scope of the module’s reach and further design. These and the previous conditions regarding yeast life cycle will be taken into consideration in the next chapter, when justifying the aspects to work with.

4 YEAST PROPAGATION MODULE

After the laboratory propagations, yeast multiplication at the brewery occurs in yeast propagation plants, generally consisting of multiple size vessels each larger than the other until the cells amount is enough for pitching a cylindroconical vessel to ferment an industrial batch. Two points are sacred regarding a proper multiplication of yeast: sterile air input and a controlled sterile environment. The former is required to fuel the production of new cell substances (Bamforth 2002) and the latter because contaminating organisms cannot be removed given that they utilize the same propagation conditions as yeast (Kunze 2014.)

Most yeast propagation plants consist of two vessels, one as a wort stabilizer and the second as a propagator where the wort is fermented and cooled down again. This feature is not mandatory, as one tank is also used where only the propagation occurs. It would be favorable that the propagation plant has the capacity to stir the contents with an agitator and use an injection system for aeration and CIP (Kunze 2014). Still important would be the capacity to vary the temperature of the tank, even that not much fluctuation is needed at this stage. The wort that will be used for multiplication media is already at a favorable temperature and the settings of the plant would only be necessary to moderate the speed at which propagation is happening (increasing or decreasing heat). At this stage, most considerations are taken to avoid stressing the yeast. (Hill 2015.)

To counter the lacking of proper propagation methods at Kahakka Brewery, and the need for the same quality yeast to use in production, the result is the combination of the propagation plant units discussed above, but instead of dedicating one tank only to propagate, a personalized module will be attached to an existing cylindroconical fermentation tank, that will provide the proper environment required without the need to “losing” one tank only for propagation purposes. The characteristics of such a module will be explained in the following paragraphs.

The fermentation system already in place is a cylindroconical fermenter with a capacity of 1400 liters and a conical base, allowing easy yeast collection and CIP sanitation. These type of fermentation vessels (FV) are capable of double functions as primary fermentation and later conditioning and maturation but commonly used only for the former. FV’s range their size between 100 and 7000 hl, and a height to diameter ratio from 1:5 to 3:1, with a working pressure from 1 to 1,3 bars above atmospheric pressure (Goldammer 2008). The vessel is equipped with pressure and vacuum release valves to avoid any explosion or implosion created during the emptying, filling or pressurization of the vessel. The most

important characteristic of the vessel is the gradient at the bottom, designed to aid yeast runoff to the bottom of the tank, for easy collection and removal, leaving the beer as free of yeast as possible. The recommended angle of the conical base is about 70° (Goldammer 2008), which correlates with Annemuller et all (2011, 371) indicating an angle between 60° to 70° and not exceeding 90°.

Other characteristics about these unit tanks is the foam production, for which “headspace” is calculated and a common practice by breweries is to leave 15 to 25% of head space to accommodate the foam produced at this stage (Goldammer 2008). Cooling jackets are used for temperature control. Given that these units come with temperature probes already installed, it is easy to regulate temperature by automatic electrical switches that allow a glycol mixture to circulate through the vessel’s jacket. Similar hygienic and maintenance practices as those for the already existing tanks in production are to be joined to the module.

5 CONCLUSIONS

The instructor for this thesis is a brew master, and former head brewer at Kahakka Brewery. During this research he mentioned that there is no “risk-free” method of manipulating yeast and that a minimal hazard of yeast contamination is always present, no matter how much equipment is applied into it. After testing and handling yeast in the laboratory, I would agree that achieving a hazard-less environment would be far from a (micro)brewery and close to a yeast propagation plant or a yeast growth laboratory. Again, this does not mean that safe yeast handling is impossible with low budget equipment but would totally not recommend trying without necessary critical control points.

A general fermentation analysis can be carried through by following only three parameters, these being temperature, pressure and extract content of the beer. Combined with controlling such parameters, it is recommended to obtain a deeper knowledge regarding the yeast’s life cycle, at least to a basic level, granting the brewer further interaction with the fermentation reactions, thus being able to calibrate the final product attributes even more. That is why part of the main research was a connection with the yeast’s elements to manipulate during fermentation and the brewing style at Kahakka Brewery, particularly the yeast manipulation during production.

Besides maintaining a constant fermentation profile, a secondary motivation for a brewery to propagate its own yeast would be the reduction of costs in production, since the price of a fresh package of yeast is by no means cheap, negating the option for microbreweries of obtaining yeast in larger quantity batches. Economic decisions as these need to be weighed against the cost of contaminating a batch of beer through poor yeast handling, if in-site propagation is practiced. Independent of biomass multiplication being used or not, cell analysis in the laboratory is totally recommended, for any brewery size. The method in this research was chosen because it did not require a high-tech apparatus and is a practice which condition the employee to make use of sanitary working habits. It has been concluded that maintaining positive sanitary practices at these early stages can be expanded into later “contamination-free” yeast manipulation. It is this same reason why such a specific propagation module is not required inside each brewery but having one would sustain that sanitary practices are adequate. Must be noted that the design of such equipment will vary for each brewery.

For assuring a healthy propagation, the predominant material to use is stainless steel, but certain properties must be taken into consideration as for example, corrosion resisting and the involved temperature, pH-values, installation conditions and costs; given that there is no “universally usable”

steel (Annemuller et all, 2011). If total automatization is employed once the module is connected to the tank, rotating piston, eccentric screw pumps or membrane pumps shall be utilized, with a flow velocity ≤ 20…30 hl/h to avoid any "funneling" effect on the yeast allowing beer to flow through it. These pumps should be constructed according to the European Hygienic Equipment Design Group (EHEDG) and 3-A-Standard 74-00 (US) and same standards should be utilized in all other components of the module. Annemuller et all (2011, 371) recommends the yeast cropping to be carried out in repeated steps.

With the actual setup, the eventual yeast-off procedures have provided about 40 liters of healthy reusable biomass. At Kahakka Brewery, this collected biomass is almost always dumped, just for fermentation conditioning purposes; and in few particular occasions, it has been collected and pitched again into another batch, thus it is well expected that if the tank/batch sizes are kept constant, there should be no lack of biomass amount to pitch, given that if required, about 10 kilos of yeast would be needed, being well below the amount that can be harvested from the cylindroconical vessel. Given this factor, the module would not need to hold much biomass or wort quantity, but instead it would circulate the biomass through itself and the FV, while aerating and controlling the temperature, promoting healthy yeast reproduction environment. The propagated yeast would be then kept inside the FV tank, until further utilization is required. It is recommended to fully automize the module through electrical switches, being that the purpose of acquiring such an equipment is to decrease the human factor in production. Similar fittings as those already existing on production tanks and CIP station of the brewhouse are to be installed on the module, offering a multiuse in case heating, steering or aerating is needed. Nonetheless the utilization of the module for different procedures than propagating is not recommended at all. As conclusion, the next step for this production facility is to improve its quality laboratory equipment by the purchase of a microscope and scheduling regular biomass density calculations. As for production, it is recommended to promote sanitary task-specific equipment usage.

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APPENDIX 1