CHALMERS

The effect of yeast propagation temperature on diacetyl reduction

An in-process study at Spendrups brewery

Master of Science Thesis in the Master Degree Programme, Biotechnology

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Göteborg, Sweden, 2010

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This Master of Science thesis was performed within the master degree programme of Biotechnology, in the track Food & Health, Chalmers University of Technology, in cooperation with Spendrups bryggeri, Vårby.

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ABSTRACT

Brewing is an ancient art and is one of the oldest biotechnologies utilized by humans. The most characteristic step during brewing is the fermentation of sugars to yield alcohol by yeast. However, it is not the alcohol production which is the time limiting factor during lager beer fermentation but the reduction of diacetyl. Diacetyl is an unwanted flavor compound in beer due to its butter scotch characteristic and it has a low human threshold of 100ppb. The diacetyl must be reduced below the threshold before the first fermentation can be terminated and the beer can proceed to maturation.

The propagation of brewing yeast *Saccharomyces pastorianus* Weihenstephan 34/70 was studied at Spendrups brewery. The temperature of the yeast propagation system at Spendrups brewery was increased from 13°C to 20°C in order to see behavioral changes in yeast growth from the current propagation system. The subsequent fermentations were followed in order to study the diacetyl reduction time.

This study showed that increased propagation temperature yielded an overall higher biomass in shorter time. Each one of the rounds of propagation seemed to yield different amounts of yeast biomass and there was reason to compare the propagation times of each round. It seemed like the yeast growth during propagation depended on what phase the previously propagated yeast was in upon pitching.

The diacetyl reduction capacity by the differently propagated yeasts was compared by measuring the time taken in days to reduce the diacetyl to below the human threshold of 100ppb. The time taken for diacetyl reduction was not significantly altered by increased propagation temperature.

KEYWORDS: Yeast propagation, Diacetyl formation, Diacetyl reduction, In-process brewing

Effeketen av jästpropageringstemperatur på diacetyl reduktion
-En processtudie vid Spendrups bryggeri
LOVISA MARTIN MARAIS
Institutionen för kemi- och bioteknik
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SAMMANFATTNING

Bryggning av öl är en av de äldsta bioteknologiska processer som människan har använts sig av. Det typiska steget vid bryggning är förvandlingen av förjäsbara sockerarter i vörten till alkohol av mikroorganismen jäst. Man skulle kunna tro att alkoholproduktionen är den tidsbegränsade faktorn under fermentationen men så ligger det inte till. Det är nämligen nedbrytandet av ett ämne som kallas diacetyl som begränsar fermenatationstiden. Diacetyl är ett icke önskvärt ämne vid öltillverkning då det ger ölet en smak av smörkola eller honung. Diacetylen måste brytas ned innan fermentationen kan avslutas och ölet kan skickas vidare till lagring.

I denna studie var propageringen av löwenjästen *Saccharomyces pastorianus* Weihenstephan 34/70 undersökt vid Spendrups bryggeri. För att kunna studera och jämfora eventuella ändringar av tillväxten av celler med tidigare propagering vid Spendrups höjdes propageringstemperaturen från 13°C till 20°C. De efterföljande fermenteringarna av varje nysatt jäst följdes och nedbrytningstiden av diacetylen kunde dokumenteras.

Från denna studie visade resultaten att en högre propageringstemperatur gav en högre biomassa på kortare tid. Varje propageringscykel verkade ge olika tillväxter av jästen och det fanns anledning att jämföra propageringstiden i varje cykel. Det verkade som om mängden biomassa berodde på i vilken fas jästen befann sig i den tidigare propageringen.

Den nypropagerade jästens nedbrytningskapacitet av diacetyl jämfördes med den tidigare propageringen vid Spendrups. Nedrytningstiden mättes i antal dagar innan diacetylen hade gått ner under 100ppb. Det visade sig att en ökad propageringstemperatur inte hade stor effekt på nedbrytningstiden av diacetyl.

SÖKORD: Jästpropagering, Diacetyl, Nedbrytande av diacetyl, Bryggning under process

ACKNOWLEDGEMENT

I would like to take this opportunity to thank Spendrups brewery for having me at their facilities while this study was conducted. I direct my special thanks to my supervisor Brewing Master Richard Bengtsson who always was positive to my ideas and helped me keeping my mood up many times. Also, I want to thank the Yeast Master Wolfram Oettel for his enthusiasm of my field of work. It has been an exciting time where many new concepts have been introduced to me which I will bring with me in the future.

I thank Thomas Andlid for accepting the role as my examiner and for his time doing so. Also, I would like to thank Christina Stenqvist, my opponent, for her time and constructive criticism.

Last but not least: Thank you Rudy for all your love and constant support.

Stockholm, June 2010

Lovisa Martin Marais

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1 INTRODUCTION

Even though not known from the beginning, yeast is responsible for one of the oldest biotechnologies in history. A biotechnological process, where living organisms convert a substrate into more beneficial products for mankind, is precisely what beer brewing is all about (Walker, 2000).

Yeast enables breweries to produce a desirable alcoholic beverage thanks to the conversion of fermentable sugars into ethanol. Of course, the art of brewing is ancient and the final beer is a result of a long and careful process beginning with malted barley grains. However, without the fermentation by yeast no beer can be produced (Eßlinger, 2009).

At breweries the yeast lives two different lives. First the yeast grows under aerobic conditions during propagation where the goal is to increase in cell number. Parameters such as temperature, aeration and wort composition plays a significant role for the viability of yeast cells and thereby the ability to grow. During fermentation, which represents the second life and main yeast activity at breweries, the environment is switched from aerobic to anaerobic. The main goal in fermentation is the yeast's capability of producing alcohol and at the same time the yeast growth slows down (Müller and Lösche, 2004). Key parameter for a successful alcohol production is the vitality of the yeast which indicates the rate of metabolic activity by the yeast cell (Guido *et al.*, 2004).

At Spendrups brewery the yeast is grown and propagated from lab into large scale propagation in cylindrical vessels with a conical bottom. The yeast handling has been carried out in the same way for as long as known and probably once incorporated based on trial and error. That is, the temperature setting has never systematically been changed in order to try to optimize the yeast growth. The yeast growth has not been monitored during propagation at Spendrups and therefore the rest extract has been the sole determinant of the state the yeast is in.

Yeast is not only responsible for the alcohol production during fermentation; it also gives rise to some flavor compounds. One of these flavor compounds, diacetyl, is highly unwanted in the finished beer. During fermentation, a buildup of diacetyl occurs as a result from leaked intermediates during amino acid biosynthesis in yeast. Diacetyl is an undesirable flavor compound as it imparts a butterscotch flavor to the beer and needs to be reduced. Both the

production and reduction of diacetyl have been reported to be coupled to the activity of yeast cells during fermentation (Yamauchi *et al.*, 1995; Hansen and Kielland-Brandt, 1996). It has been suggested that in fact, the reduction of diacetyl by yeast is the time limiting factor during fermentation as it needs to be reduced to below 100ppb, the human threshold, before maturation can be initiated (Kobayashi *et al.*, 2005).

1.2 AIM OF STUDY

In this thesis, the propagation temperature was increased in order to study the assumingly increased yeast growth. Also, the behavior of yeast growth during propagation rounds was analyzed. Up until now, no study has been conducted on how the propagation of yeast can affect the diacetyl reduction during fermentation in process production at Spendrups brewery. Therefore this study focused on the reduction time and behavior of diacetyl reduction during fermentation from each round of propagation. The driving force behind this work was the wish from Spendrups brewery to increase their knowledge of yeast behavior during propagation and if time could be saved with a possible faster diacetyl reduction time.

2 THEORETICAL BACKGROUND

In this chapter an overview of some theoretical aspects regarding brewing and the yeast are presented. The yeast handling at Spendrups brewery is outline and how measurements of rest extract and yeast biomass were taken during this study is discussed with regards to the devices used.

2.1 IN PROCESS BREWING

In order to get an understanding for how beer is produced at Spendrups brewery the process is outlined in figure 2.1. The basic steps shows how the process begins with malted barley which after milling undergoes mashing, lautering and boiling. During the boiling step hops is added and then separation takes place. At Spendrups brewery the separation step is performed by a separator instead of a whirlpool as shown in the figure below. After the separation, the collected wort is cooled and aerated before added to yeast in a fermentation tank. The step where yeast is added to the wort is called pitching. As seen in figure 2.1 the yeast is added and recollected after fermentation before maturation begins. Finally the finished beer passes through filtration and packaging is the last step in the beer production line at the brewery (Eßlinger, 2009).

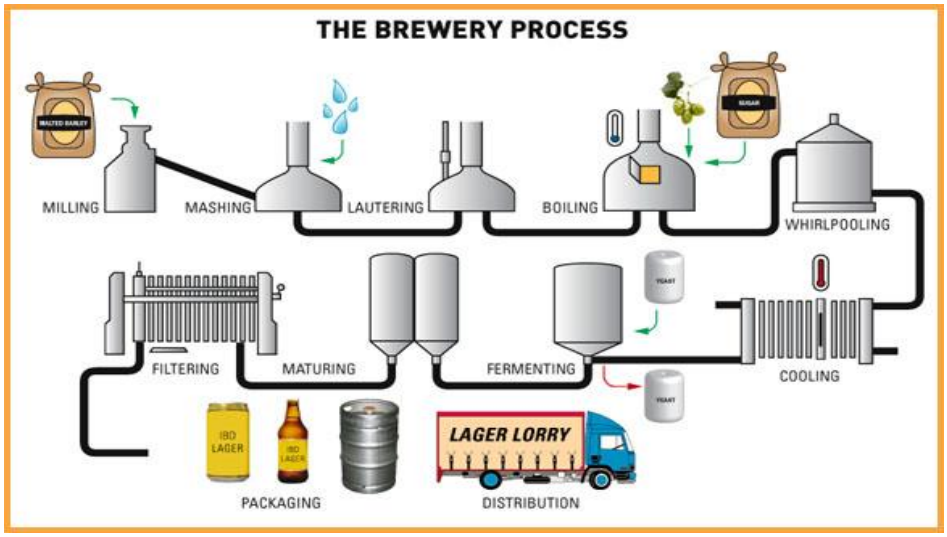


Figure 2.1 Simplified diagram of the in- process production of beer (IBD, 2010).

2.2 YEAST AND ITS PURPOSE AT THE BREWERY

Yeast is a facultative anaerobe meaning that it can survive and grow in both aerobic and anaerobic environments (Walker, 2000). At breweries, the facultative nature of yeast becomes very obvious since it actually grows in both aerobic and anaerobic environments

In the aerobic environment yeast is allowed to grow and increase in cell number by budding. The aerobic growth process at breweries is called propagation and is responsible for the increase of yeast cells before pitching can take place (Müller and Lösche, 2004). Pitching rate is the initial inoculum of yeast cell density in the fermentation step. The recommended pitching rate for large scale fermentations is 10^7 cells/ml (Walker, 2000). In the fermentation step the yeast growth is slowed down and fermentation products, such as alcohol, are instead formed at a higher rate (Müller and Lösche, 2004). The main goal of propagation at breweries is to produce yeast which later on during fermentation efficiently produces alcohol.

2.2.1 PITCHING RATE

It is important for a brewery that the propagated yeast has reached a sufficient cell number before pitching takes place. If the pitching rate is too low the fermentation will take too long time resulting in an inefficient process. If the pitching rate is too high it can lead to decreased viability of yeast which is to be collected and repitched from the fermentation. In addition, loss of bitterness and filtration problems can result from over-pitching (Boyd *et al.*, 2003).

2.2.2 YEAST VIABILITY AND VITALITY

Even if the pitching rate is important, the viability and the vitality of yeast cells are crucial factors for the fermentation performance (Novak *et al.*, 2007). The viability of a yeast cell is described as the yeast's ability to grow and its capability to handle environmental stress. The vitality is defined as the metabolic activity of a cell. That is, a cell can have low viability but still be vital (Walker, 2000). It makes sense that yeast performance during fermentation would be more successful if carried out with vital yeast and it was shown that yeast with higher vitality yielded higher fermentation rates even at lower pitching rates compared to yeast with lower vitality (Guido *et al.*, 2004).

2.2.3 GROWTH TEMPERATURE

Yeast growth depends on a number of different factors for optimal growth and one of the most important physical parameter is temperature. Yeast has so called cardinal temperatures which

indicate minimum, maximum and optimal growth temperatures. The optimal temperature refers to the temperature which allows chemical and enzymatic reactions to occur at maximal possible rates. The maximum temperature indicates the highest possible temperature for which the organism can still grow in, above this temperature enzymes denature and cell death is the result (Madigan and Martinko, 2006).

Most yeast used in industry has an optimal temperature of between 20°C and 30°C but can differ amongst strains. The maximum temperature ranges from 35-43°C for S*accharomyces cerevisiae*, however some strains like *Saccharomyces pastorianus* has shown to not grow at temperatures above 35°C (Walker, 2000).

2.2.4 LAGER YEAST

The strains of *Saccharomyces cerevisiae* used for brewing lager beer differ from strains used for ales. According to the first studies of lager and ale yeast, the lager yeast was said to belong to *Saccharomyces carlbergensis* and ale was produced using *Saccharomyces cerevisiae* strain. Later on it was clear that lager yeast actually belonged to the *Saccharomyces pastorianus* strain after DNA-DNA hybridization studies had been done (Nakao et al., 2009). However, in many texts lager beer is still referred to as *S. carlbergensis*.

The lager brewing yeast strain *Saccharomyces pastorianus* W-34/70 is used for fermentation at Spendrups brewery. The yeast strain is known to be a strong diacetyl reducer and a study conducted by Fix (1993) showed that the strain W-34/70 was the most powerful diacetyl reducer when compared to two other Weihenstephan strains.

Lager brewing yeast strains are often characterized by their low fermenting temperatures and their powdery characteristic. The latter indicating that flocculation only fully takes place after chilling has begun. When the temperature is lowered, the yeast flocculate at the bottom of the fermentation tank which allows for a more successful fermentation with dispersed yeast cells in the wort during the whole fermentation stage. The name bottom fermenting yeast which is often used for lager yeast comes from the fact that it flocculates at the bottom of the fermentation tank (Powell *et al.*, 2003).

2.3 YEAST HANDLING AND PROPAGATION AT SPENDRUPS

The handling of yeast starts in the lab and is subsequently grown into larger quantities until it can be used in large scale process. Figure 2.2 outlines the path of yeast handling from lab to yeast cellar. Figure 2.3 shows how the yeast, when propagated in yeast cellar, is pitched into a fermentation tank in a round and filled up with wort again.

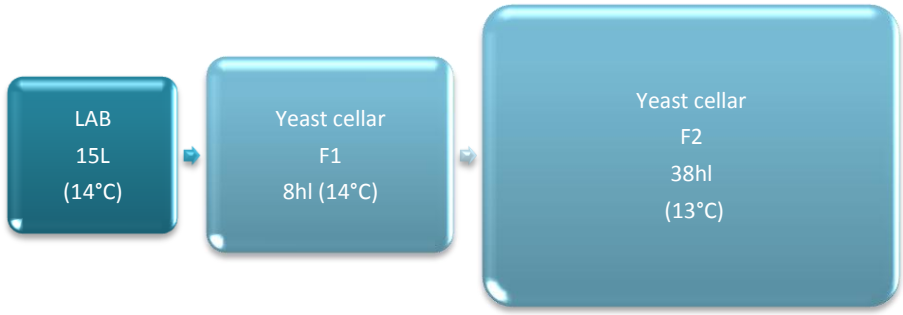


Figure 2.2 schematic illustrations of current propagation at Spendrups brewery.

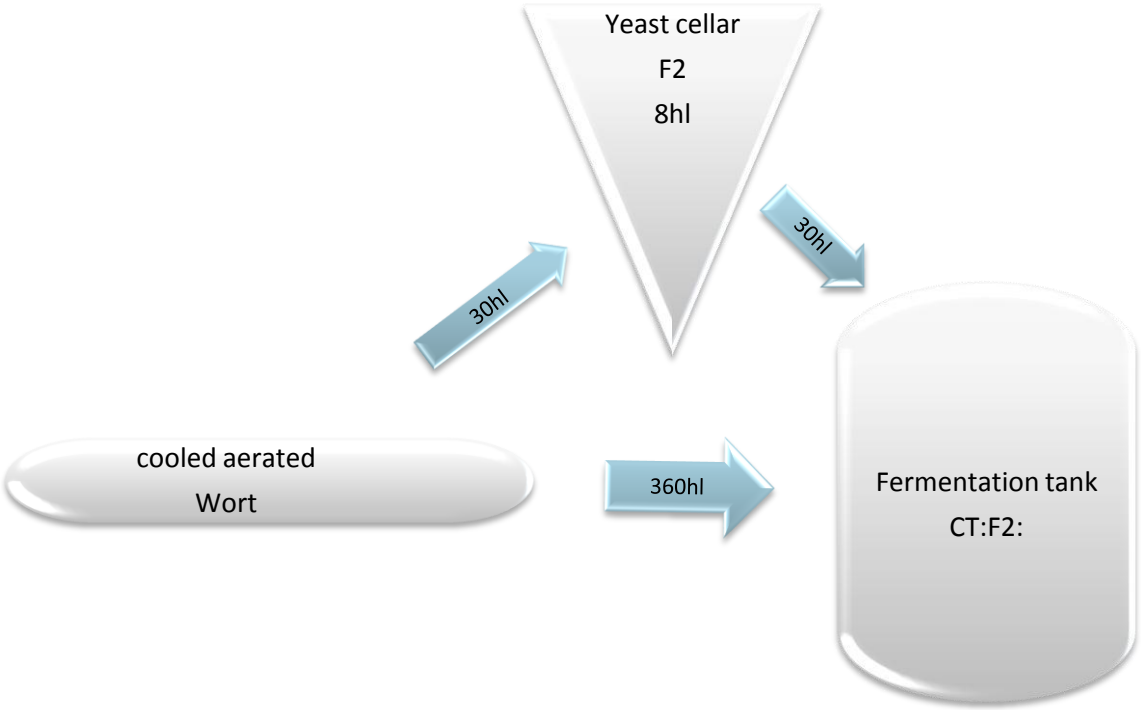


Figure 2.3 Schematic illustration showing the procedure of each round of propagation at Spendrups brewery.

2.3.1 GROWING YEAST IN LAB

The lager yeast *Saccharomyces pastorianus* Weihenstephan is ordered and stored in a refrigerator at 4°C. Yeast is taken from stock and grown in 100ml sterile wort with an extract of about 13°P. Every time new yeast from stock is propagated it is used for three times before new yeast from stock is used again.

The 100ml flask with yeast is thoroughly swirled before it is poured into three 100ml flasks with sterile wort and left to grow for approximately three days. The grown yeast is then transferred into 500ml flasks containing sterile wort and into one 100ml flask which is stored until next propagation in 4°C. The 500ml flasks are transferred into 2000ml flasks which subsequently are transferred into 5000ml flasks all containing sterile wort before it can be handed over to the F1 propagation tank in the yeast cellar.

2.3.2 YEAST CELLAR

The F1 tank is filled up with 15L lab propagated yeast and 8hl sterilized wort .The propagation in F1 takes approximately one week in order to reach a yeast growth good enough for further propagation and a decrease in extract to below 5°P from ca 13.5°P. The temperature in F1 is set to 14°C, the same as the temperature during lab propagation.

Once the propagation in F1 tank is considered finished its content is transferred into F2 propagation tank and 30hl cooled and aerated wort is added. This is the first round of propagation from one yeast batch at Spendrups brewery, see figure 2.3 for illustration of this step.

During propagation the temperature is held at 13°C and aeration is kept constant with 10 minutes aeration following 10 minutes pause. During aeration the yeast solution is mixed due to a stream caused by the incoming air. The proposed time for propagation in F2 tank is two days in order to reach a minimum yeast count of 150 million cells per milliliter. However, at Spendrups brewery the time taken for propagating each round of yeast varies depending on availability for small fermenting tanks and other in-process related deviations.

2.3.3 YEAST COUNT

In the current system at Spendrups brewery the guideline for yeast propagation recommends that a yeast count of 150 million cells per milliliter is reached before pitching takes place. That is, 30

hl of 150 million cells/ml is to be pitched into a fermentation tank with approximately 360hl wort. That would give an approximate pitching rate of 12 million cells/ml. However, the yeast count has never been monitored at Spendrups during propagation hence the yeast growth has been an assumption from measurements of the rest extract.

2.3.4 MEASURED REST EXTRACT

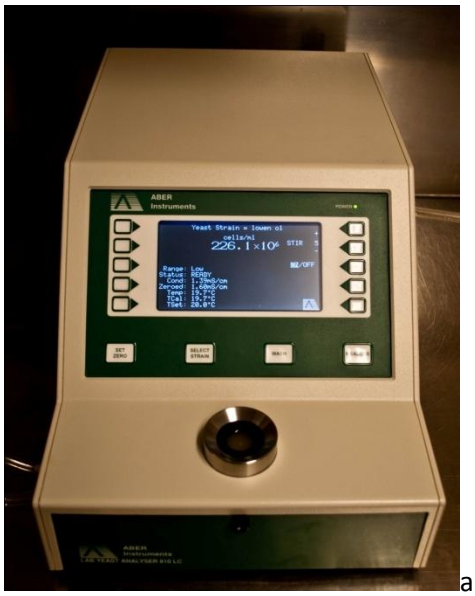
The extract of yeast in propagation is measured using a device called density meter at the brewery, see figure 2.4. It indicates the rest extract by direct measurement of the percent sugar as sucrose by weight in yeast solution. The units are given in degree Plato (°P) (Manning, 1993).

The measured extract serves as a guideline for the amount of sugar consumed which in turn indicates whether or not the yeast is growing over time. The propagated yeast solution is recommended to have an extract reading below 5 °P when the starting extract is approximately 13.5 °P. This guideline originates from in-process observations that the yeast is "thick enough" when the extract has decreased a certain amount.

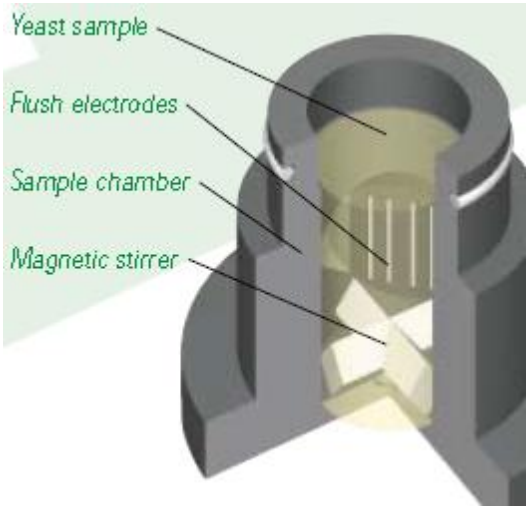
Figure 2.4 Extract measurement device called density meter (Martin Marais, 2010 [Photograph]).

2.4 ABER LAB YEAST ANALYSER

Yeast biomass was measured using Lab Yeast Analyser Model 810LC. Figure 2.5 illustrates the device and where the sample is put.



a)



b)

Figure 2.5 ABER Lab Yeast Analyser (a) (Martin Marais, 2010. [Photograph]); Schematic illustration of the sample chamber of Lab Yeast Analyser (b) (Aber Intruments, 2010).

The viable yeast is measured using a technique based on radio-frequency (RF) impedance. The basic concept is based on the polarization of yeast cells outlined in figure 2.6. The system is based on two outer pins creating an electrical field which causes ions in the sample fluid to travel towards the two differently charged electrodes. Yeast cells become polarized because ions cannot freely move across the non-conducting plasma membrane. The polarized cells are behaving like small capacitors in the sample and the signal can be measured as current by the two inner pins (Carvell and Turner, 2003).

The system is not disturbed by non viable cells or damaged membranes due to the fact that no charge is created across the membrane if the cell is damaged since ions can then move freely across.

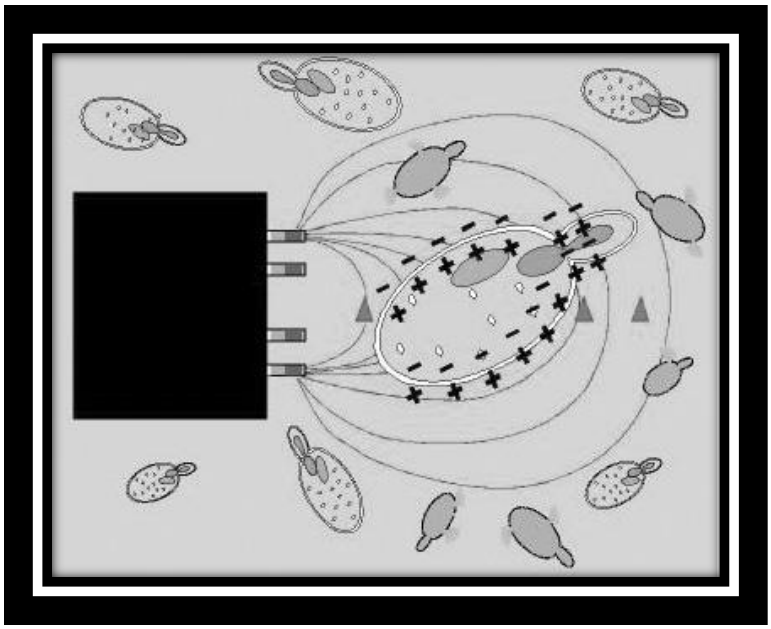


Figure 2.6 The response of yeast cells when placed in a radiofrequency electrical field (Carvell and Turner, 2003).

However, the suspending fluid, the size of the yeast cell, internal conductivity (SI) and the capacitance of the cell membrane (RCM) are factors that influence the results. The device are based on the fact that within a given yeast strain the two factors SI and RCM are almost constant and therefore the only significantly varying factor is the liquid in which the yeast cells are suspended. The conductivity is then measured and the percentage viable spun solids are calculated using the algorithm. From percentage viable spun solids the cells/ml is derived.

Since the suspending fluid is the largest variable during the analysis of yeast content, it is of great importance for the accuracy of the results that the Lab Yeast Analyser is blanked with wort and not with water before proceeding taking measurements.

2.5 DIACETYL

As mentioned in the introduction, diacetyl is an unwanted compound formed during brewing and needs to be broken down before beer can proceed to maturation. Diacetyl imparts typical butterscotch flavor to beer and needs to be reduced to below 100ppb (Kobayashi *et al.*, 2005). Its homologue, 2,3-pentadione, has the same flavor effect as diacetyl but it is not of the same industrial significance as diacetyl due to a ten times higher threshold (Eßlinger, 2009).

2.5.1 FORMATION OF DIACETYL

In order to understand where diacetyl production begins, one should look at the formation of the precursor α-acetolactate, an intermediate in the biosynthesis of valine. In figure 2.7, the biosynthetic pathways of the branched-chain amino acids isoleucine, leucine and valine (ILV) are outlined. It is evident that the pathways of ILV biosynthesis have a lot in common. Not only do they share enzymes in some steps but the flavor compounds diacetyl and its homologue, 2,3-pentadione, are formed similarly (Hansen and Kielland-Brandt, 1996; van Bergen, 2006).

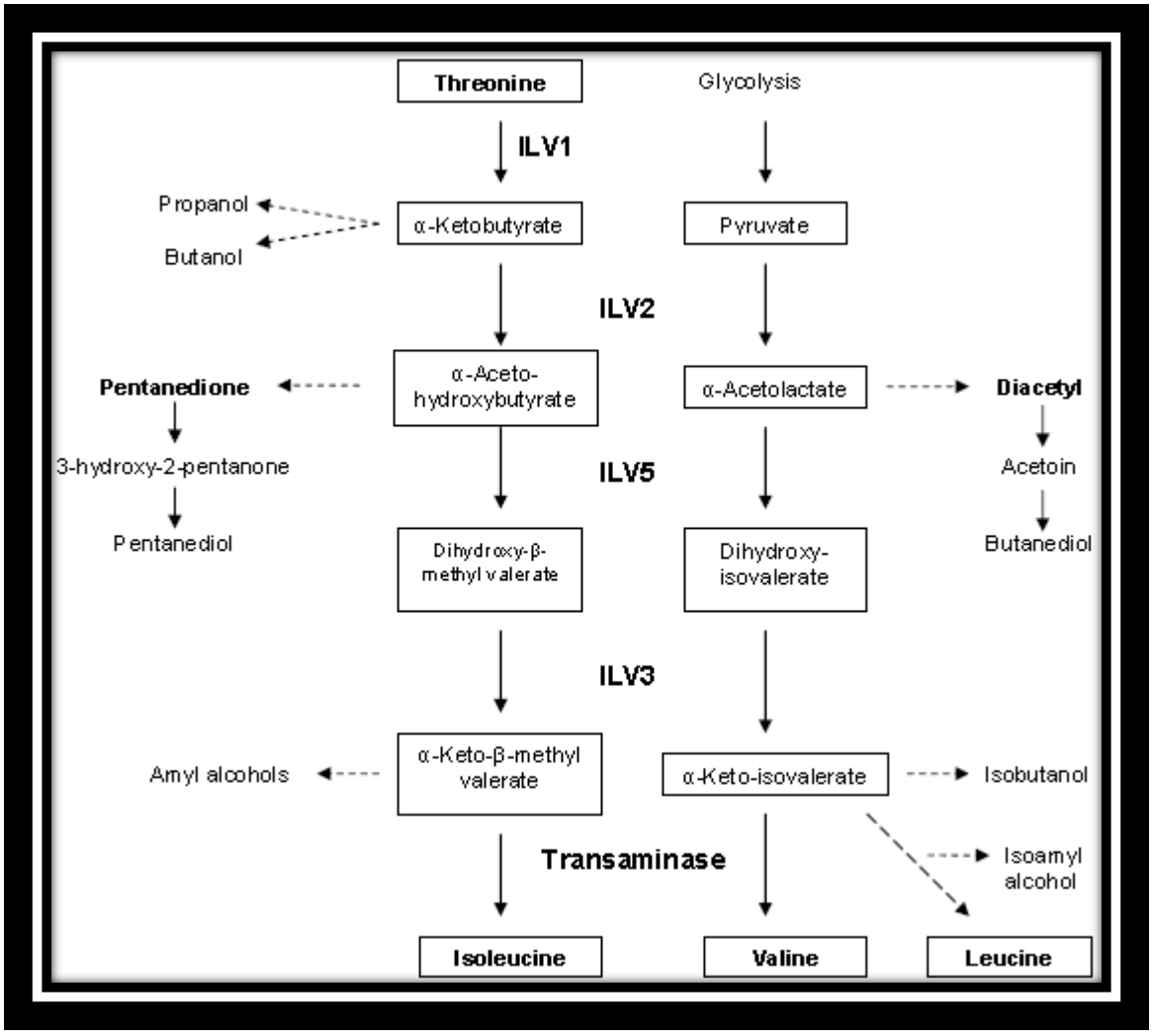


Figure 2.7 The biosynthetic pathways of the branched-chain amino acids Isoleucine, Valine and Leucine in yeast (van Bergen, 2006).

When some of the α-acetolactate leaks out of the yeast cell it undergoes a slow oxidation and diacetyl is formed as a result of decarboxylation (Hansen and Kielland-Brandt, 1996; van Bergen, 2006). In figure 2.8, the extracellular formation of diacetyl is shown.

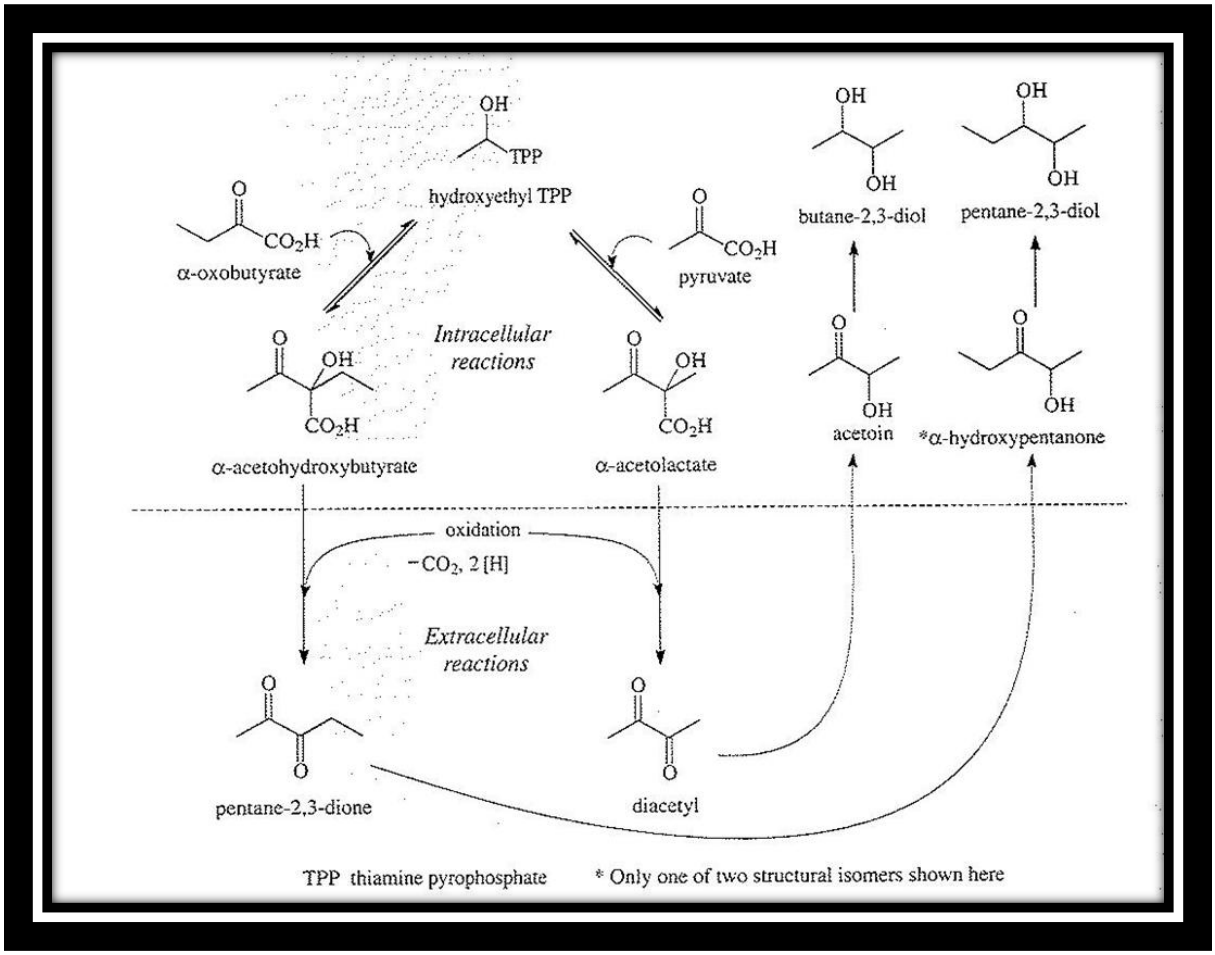


Figure 2.8 Pathway of diacetyl formation from leaked out α-acetolactate (Hughes, 2001).

According to van Bergen (2006), it has been discussed by Lundblad (1995) that a possible reason for the yeast to excrete α-acetolactate is to maintain cellular health. Diacetyl and 2,3-pentanedione are highly reactive dicarbonyls and, if build-up inside the cell, can react with other amino acid residues causing cross-linkage of proteins. This phenomenon has been associated with a number of diseases in higher eukaryotic organisms where diacetyl has shown to play a role in cell deterioration and aging (van Bergen, 2006). However, the real cellular reasons and mechanisms for the diacetyl production are not well known yet.

2.5.2 REDUCTION OF DIACETYL

Reduction of diacetyl takes place inside the yeast cell, see figure 2.8 and figure 2.9, where diacetyl is reabsorbed by the yeast cell and converted into acetoin by enzymatic activity. The assimilation has been shown to be selective for diacetyl and not for α-acetolactate. The assimilation step could perhaps be the rate limiting step for reduction of diacetyl. Once inside the yeast cell, diacetyl is reduced in two steps by two different enzymes. Diacetyl reductase reduces diacetyl to acetoin which is further reduced to butane-2,3-diol by alcohol deghydrogenase. Both acetoin and butane-2,3-diol can escape the cell but have much higher flavor thresholds compared to diacetyl and are therefore not causing any threat to flavor of the finished beer (Yamauchi *et al.* 1995).

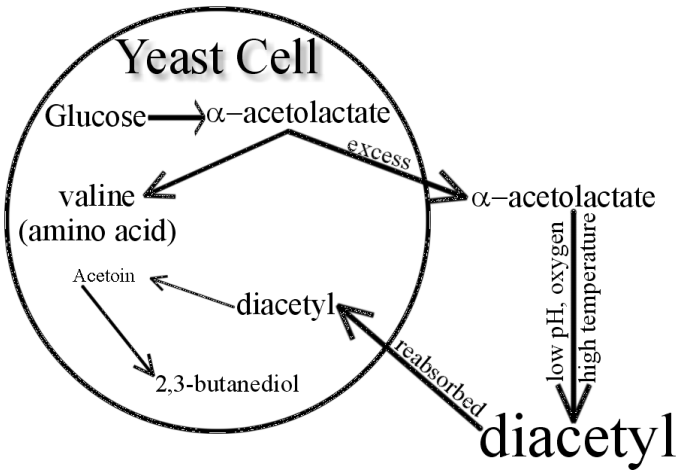


Figure 2.9 Illustration of compounds involved during diacetyl formation and reduction inside and outside of the yeast cell (Stueven, 2003).

2.5.3 FACTORS AFFECTING DIACETYL FORMATION AND REDUCTION

Some factors seem to affect the formation and reduction of diacetyl and include the fermentation temperature, amount of free amino acids in wort, yeast strain and the physiological condition of the pitched yeast.

2.5.3.1 Temperature

The temperature during lager fermentation is normally rather low, between 9-14°C. The oxidation of α-acetolactate to diacetyl increases with increased temperature and when the diacetyl peaks were compared during a study with different fermentation temperatures it was evident that the higher temperature yielded higher peaks of diacetyl. However, the same study

showed that higher fermentation temperature also resulted in higher reduction rate, indicating the role of temperature on the ability of yeast to reduce the carbonyl compound (Mudura *et al*., 2006). The reason for keeping the fermentation temperature rather low during lager brewing is that an excess amount of esters and other aroma compound produced by yeast can cause unwanted character to the beer (Bengtsson, 2010).

2.5.3.2 Wort

Wort contains free amino acids as a result of mashing. Depending on the mashing temperature more or less enzymes are left for cleaving proteins into smaller fragments such as amino acids. If the wort contains a high amount of valine, the yeast is not forced to produce it itself and as a result the amount of produced α-acetolactate is much lower. The lower the amount of α-acetolactate produced by the yeast, the lower the formation of diacetyl outside the cell will be (van Bergen, 2006).

2.5.3.3 Yeast strain

Different yeast strains differ in their ability to reduce diacetyl. In a study performed by Fix (1993) three different lager yeast strains were tested with regard to their strength in reducing diacetyl. The outcome showed that two of three strains yielded only a sixth of the amount of the third strain. Also, the study showed that the strong reducers produced much less diacetyl compared to the third strain. Indeed the genetics behind the reduction capacity plays a large role and it is important for breweries to use the stronger ones.

2.5.3.4 Propagation Condition

Little has been written about the effect of propagated yeast on the reduction of diacetyl. Since diacetyl reduction is somehow an activity performed by yeast during fermentation it is likely that it would be increased by cells with high vitality. Just like the capacity of fermenting alcohol is due to the metabolic activity of the cells (Guido *et al.*, 2004) the diacetyl reduction could be of similar activity. If the yeast is propagated in higher temperature and its viability increases, there could be a possibility of increased vitality showed by faster diacetyl reduction.

3 METHODOLOGY

In this section the methodology used during this study is outlined.

3.1 PROPAGATION

The current propagation system at Spendrups, outlined in section 2.3, was followed and yeast biomass was recorded on a daily basis with Aber Lab Yeast Analyser. The extract was measured using a density meter. The obtained results for nine rounds of yeast batch 1 served as reference for coming yeast batch 2.

In table 3.1, the tests performed on yeast batch 2 are outlined. Even though the temperature was supposed to be the only changed parameter during the propagation of the second batch, there were other unforeseeable changes that were needed to take into consideration. Due to in process testing, parameters may change as a result of accommodation for the instant needs of the brewery. The reason for only testing one other temperature during this study was to try and include possible alterations from round to round. Different temperatures for every round would give highly uncertain results due to changes in yeast.

Table 3.1 Schedule showing parameters changed in each round of propagation of second yeast batch.

Parameters	F2:21	F2:22	F2:23	F2:24	F2:27	F2:29	F2:210	F2:211	F2:212	F2:213
Temp.	11.5	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	11.0
Aeration	Const.	Off 2days	Const.	Const.	Const.	Const.	Const.	Const.	Const.	Const.
Time (days)	10	4	3	4	5	5	5	2	6	2
Start extract (°P)	13.5	13.5	13.5	13.5	18.0	13.5	13.5	13.5	13.5	13.5

3.2 FERMENTATION

The 30hl propagated yeast was put into a small fermentation tank with 360hl wort (approximately 13.5°P) and left for fermentation. Each fermentation tank corresponded to a propagation round and examined thereafter. The diacetyl was measured by the lab using gas chromatography and results were recorded on a daily basis, when possible. Also the extract during the fermentation was measured using a density meter.

Parameters for fermentation were held as constant as possible during the whole study. Fermentation temperature was held at 14°C. Time taken for fermentation was only recorded with regards to diacetyl reduction.

The new temperature was tested in order for yeast to hopefully grow faster and perhaps reach a higher biomass. If the yeast could grow better at a higher propagation temperature it could hopefully result in better metabolic activity and perhaps be expressed by faster diacetyl reduction during fermentation.

3.3 Measurement devices

The devices used for measuring yeast biomass and extract in this study are not regarded as accurate and the results given by them can only be used for indication of extract and yeast biomass behavior during the experiments. For measurements during in-process propagation and fermentation the exact numbers for extract or biomass are not needed and less time consuming measurement techniques are wanted.

4 RESULTS AND DISCUSSION

This chapter presents and discusses the results obtained for this study. This chapter is mainly divided into propagation and fermentation for an easier overview.

4.1 PROPAGATION

The results from propagation are discussed in this section. Results from both yeast batches are compared with regards to biomass and extract drop.

4.1.1 PROPAGATION ACCORDING TO THE CURRENT SYSTEM AT SPENDRUPS: FIRST YEAST BATCH

The first nine rounds of propagation were all from the same initial yeast from the lab. The temperature was held at 13°C and the aeration was constant. The yeast biomass over time was measured using ABER Lab Yeast Analyser LC810. The results were shown in table 4. 1.

Table 4.1 Yeast biomass recorded at each round of first yeast batch at 13°C

day	F2:1	F2:2	F2:3	F2:4	F2:5	F2:6	F2:7	F2:8	F2:9
1			8E+07	5E+07	6E+07	5E+07	4E+07	9E+07	5E+07
2			2.0E+08	6E+07		9E+07		1.6E+08	1.1E+08
3	5.1E+08		2.7E+08	1.1E+08		1.4E+08			
4	3.4E+08	1.5E+08	2.7E+08		2.6E+08		2.1E+08		
5								2.3E+08	
6							2.4E+08	2.4E+08	
7			5.3E+08						

4.1.1.1 Yeast count from propagation rounds of first yeast batch

The yeast count differed during propagation rounds of the first yeast batch. Even though measurements could not be taken every day one can see that each round yielded different amounts of yeast over time. The first round yielded the highest biomass after three days compared to all other rounds from the first yeast batch. The second round, however, yielded one of the lowest yeast counts on the fourth day of propagation. The results in table 4.1 actually indicated on an "every other" pattern for higher and lower yield of biomass for each round.

4.1.1.2 Propagation time for first yeast batch

As seen from the results above in table 4.1 the propagation times differed from each round. Round 9 had a propagation time of only two days whereas rounds 1, 2 and 5 were standing for

four days. Round 3 was propagated for as long as 7 days and had the highest yeast biomass on its last propagation day.

The time taken for propagation influences largely on the number of yeast cells and what phase the yeast is in. Yeast grows according to a growth curve which means that the growth behavior changes with time due to depletion of nutrients. In the beginning of propagation, the yeast undergoes a brief lag phase. During the lag phase yeast gets prepared for growth by synthesizing essential constituents. Since the wort contains all the nutrients needed and is the same for each round, the lag phase is not very long during propagation. After the lag phase the yeast enters the exponential phase where growth takes place. Yeast growth continues until nutrients are depleted and a phase called stationary phase is reached where the net growth is zero (Madigan and Martinko, 2006). Evidently, time taken for propagation at the brewery will influence the phase in which the yeast is in. If propagation time is very long the sugars in the wort will be fully consumed and no more growth can take place.

The uneven propagation time leads to an uneven pitching rate from round to round. That is the yeast biomass by each round of propagation was different as seen in table 4.1.
The reason behind the propagation time behavior at the brewery is that in process, propagation time is decided by the occurrence of empty fermentation tanks. The recommended propagation time is about two days but as seen from the results, the propagation times at Spendrups are more often three to four days long.

4.1.2 PROPAGATION AT INCREASED TEMPERATURE: SECOND YEAST BATCH

The yeast biomass from propagation rounds of the second yeast batch was shown in table 4.2. The measurements were performed in the same way as for the first yeast batch.

Due to needed alterations for the brewing process at Spendrups some deviations of the propagation parameters were made. The rounds of propagation were held at 20°C, however, due to an in process alteration, the 1st round was held at 11.5°C instead of 20°C. Further deviation during the propagation rounds for the second yeast batch were found in round 2 where the aeration was off the two first days and in round 7 the initial extract was much higher than for the rest of the rounds. Thirteen rounds were propagated with the second yeast batch but only rounds 1, 2, 3, 4, 7, 9, 10, 11, 12 and 13 were followed during the study.

Table 4.2 Yeast biomass recorded during each round of the second yeast batch at 20°C

day	F2:21	F2:22	F2:23	F2:24	F2:27	F2:29	F2:210	F2:211	F2:212	F2:213
1	1.1E+08	2.3E+07	7.2E+07	8.2E+07			5.6E+07	4.4E+07	7.6E+07	7.0E+07
2		3.8E+07		1.5E+08	2.2E+08				2.0E+08	1.2E+08
3		9.0E+07	-	2.4E+08	3.0E+08		2.1E+08			
4	1.1E+09	3.9E+08		2.4E+08	2.9E+08	2.4E+08				
5										
6									2.3E+08	

*Temperature was held at 11.5°C; *No aeration on first two days; *Initial extract was 18°P; *Temperature was held at 11°C

4.1.2.1 Yeast count from propagation rounds of second yeast batch

The growth pattern from propagation round of the second yeast batch is similar to the propagation rounds of the first yeast batch. The 1st round yielded the highest yeast count even though the temperature was held lower than the rest of the rounds of the second yeast batch. Round 2 had a lower initial yeast count and only on the third day the growth really started to increase. Most probably the lack of aeration during the two first days was responsible for the slow growth of round 2. Once the aeration was switched on again the growth increased rapidly, but the final count was much lower compared to the first round. However, compared to the second round of the first yeast batch, the growth was more than double after four days. Round 3 had almost twice the initial yeast count of round 2 but unfortunately the yeast count for the consecutive days was not measured and could therefore not be determined. Round four yielded a lower biomass after four days compared to round 1 and 2. The pattern seen from the propagation rounds of the first yeast batch are slightly detectable from the rounds of the second yeast batch.

Round 13 was on purpose held at 11°C in order to see if a lower temperature could yield a higher yeast growth as in round 1 of the second yeast batch. From the result it was evident that a lower temperature lowers the growth rate. Therefore the high yeast biomass obtained from the 1st round of second yeast batch cannot be a result from low temperature.

4.1.2.2 Propagation time for second yeast batch

The time for propagation rounds of the second yeast batch seemed a bit more uniform compared to the times for the rounds of the first yeast batch. Rounds 1 to 4 were held between 3 and four days.

The lower time differences during propagation rounds from second yeast batch could perhaps help explain the growth pattern showed in table 4.2. If the propagation time did influence the growth, as discussed for first yeast batch, the more uniform growth obtained by rounds from the second yeast batch could be explained due to the fact that the yeast was in similar phase every time a new round was initiated.

4.1.3 YEAST GROWTH AND EXTRACT DROP

The yeast count and extract for the first four rounds of propagation from both yeast batches were shown below in figures 4.1 to 4.8. The results showed how the extract dropped as a result of sugar consumption by yeast for growth. When no more sugars were available for the yeast in the wort the growth ceased.

4.1.3.1 First yeast batch: growth and extract

In figure 4.1 the yeast growth and extract of the first four rounds of propagation from first yeast batch was presented. The yeast was only first measured on the third day and on the fourth day the biomass seemed to have decreased. The biomass did probably not decrease but the measurement could have been done incorrectly. The sample for measurement had to be collected when aeration was switched on. There is a possibility that the measurement on day three was done on a sample taken from yeast while no air pumping was ongoing. That would result in a higher yeast density due to settlement at the bottom when aeration was switched off.

From the graph it could be deduced that the yeast growth was at stationary phase as the extract minima had been reached indicating no available nutrients for more growth.

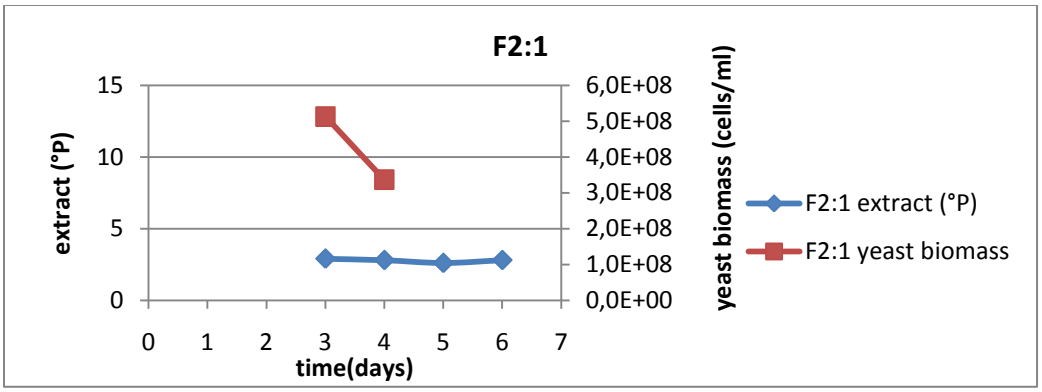


Figure 4.1 Yeast biomass and extract during 1st round of propagation from first yeast batch.

In the 2nd round of propagation from the first yeast batch (figure 4.2) the extract drop was recorded from day one but the yeast biomass could only be recorded on day four. The extract measurements indicate rather well the opposite behavior of yeast growth. The extract dropped slowly the first day and on the second days the extract dropped at a higher rate until day four which was the final day of propagation for this round. By the look of the drop of extract one could assume that the yeast started in a lag phase on the first day and went into exponential phase on day two. One could almost assume that the yeast was in exponential phase when pitched to a fermentation tank after round 2.

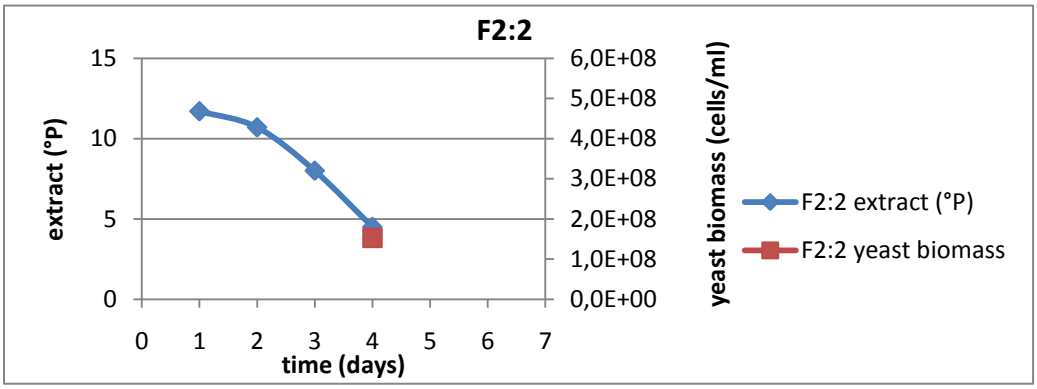


Figure 4.2 Yeast biomass and extract during 2nd round of propagation from first yeast batch.

In figure 4.3 the behavior of yeast growth and extract drop were shown very clearly. From day one to three the yeast growth was of exponential type and stationary phase followed on day four. After day six a second small increase in growth was detected perhaps as a result of switched nutrient (Madigan and Matinko, 2006). The behavior of the yeast growth could be certified by the behavior of extract drop. The two curves follow each other like mirror images.

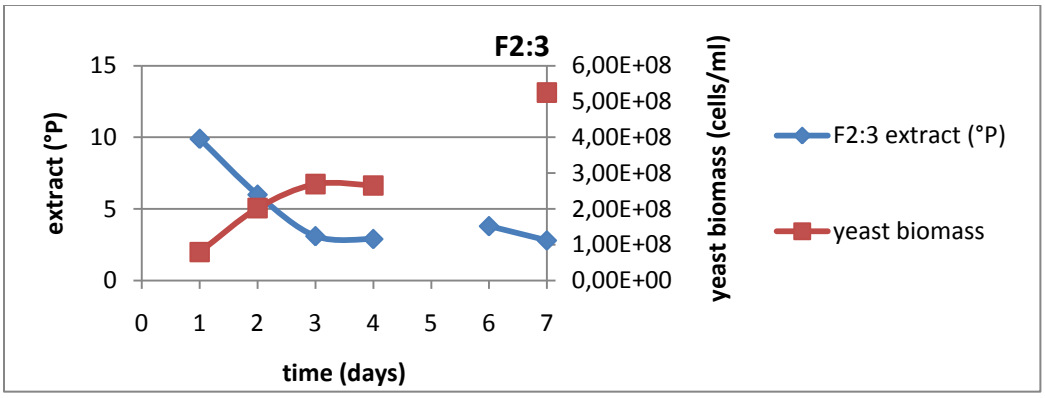


Figure 4.3 Yeast biomass and extract during 3rd round of propagation from first yeast batch.

The 4th round was shown in figure 4.4 and the yeast biomass and extract were once again shown to correlate as in the previous rounds. It looked like the yeast entered the exponential phase and a stationary phase was never initiated.

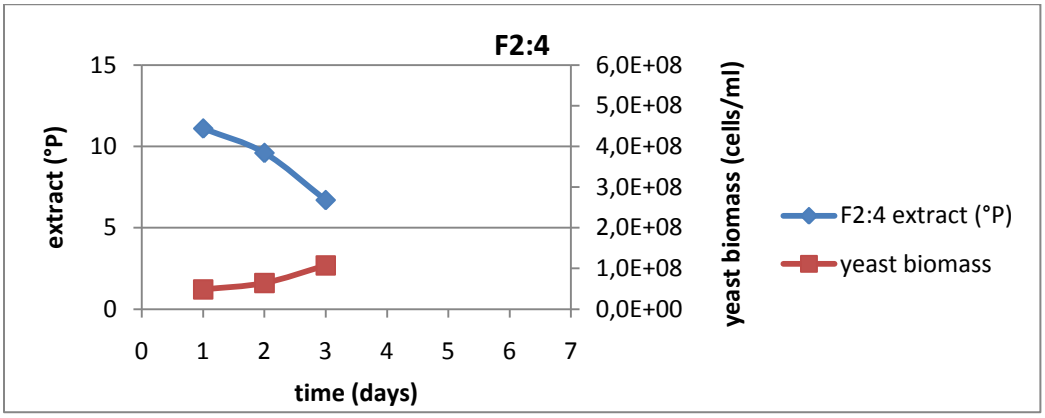


Figure 4.4 Yeast count and extract during 4th round of propagation in first yeast batch.

In figures 4.1 to 4.4 the "every other round" pattern was seen clearly and a possibility could be that the phase which the yeast was in the previous round played a role for the next round's yeast growth. In addition, one could argue that the yeast viability seemed to be higher in around if previous round was terminated while in exponential phase. Since the yeast during exponential phase was the healthiest, the yeast was probably more viable when coming from an exponential phase (Madigan and Martinko, 2006). In addition to high viability, the yeast should be of high vitality in order to successfully and efficiently carry out fermentation reactions (Guido et al., 2004). Even though the exponential phase contains yeast with high viability in its healthiest state it could be assumed that the yeast also were vital during that phase. Therefore yeast in the exponential phase seemed to benefit forthcoming propagations and most importantly the fermentations for which the yeast was ultimately grown.

4.1.3.2 Second yeast batch: growth and extract

The four first rounds of propagation from the second yeast batch were shown in figures 4.5 to 4.8. In these figures the temperature during propagation was included in addition to the yeast biomass and extract measurements. The temperature was included due to the fact that even though the temperature of the propagation was set to 20°C, the incoming wort had a temperature of 9°C thus the initial propagation temperature was always lower than 20°C.

The temperature in the 1st round (figure 2.5) was set to 11.5°C due to the knowledge that it had to stand for over a week and the goal was to actually try and keep the yeast growth low. However the yeast biomass turned out to be the highest throughout the study. The yeast growth seemed to have entered stationary phase before pitching which could be seen from the extract measurement on the fifth day.

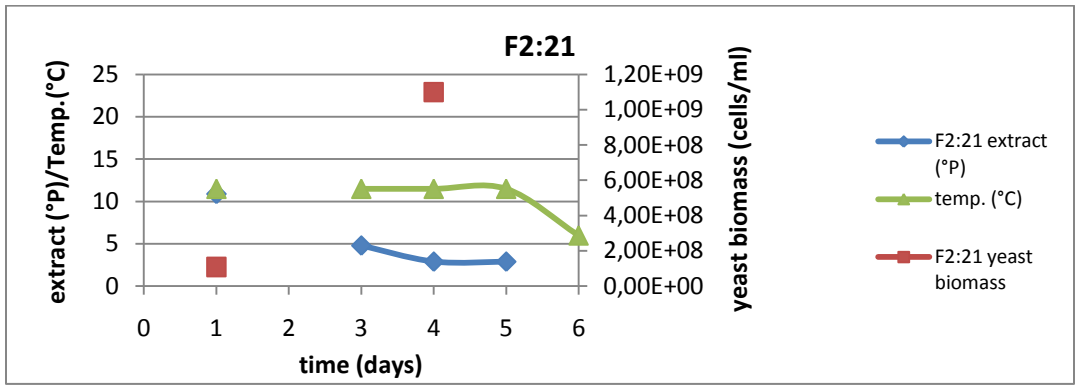


Figure 4.5 Yeast count, extract and temperature during 1st round of propagation in second yeast batch.

The 2nd round of the second yeast batch (figure 4.6) had an unusual slow growth during the first two days which was most probably due to the lack of aeration that occurred (Walker, 2000). Once the aeration was switched on, the growth resumed and increased more than a fourfold on day four. This incidence served to indicate the importance of oxygen for yeast growth during propagation. The high number of cells obtained from this round could be explained by the increase in temperature which actually reached 20°C on day four. The yeast growth seemed to have been in exponential phase upon pitching.

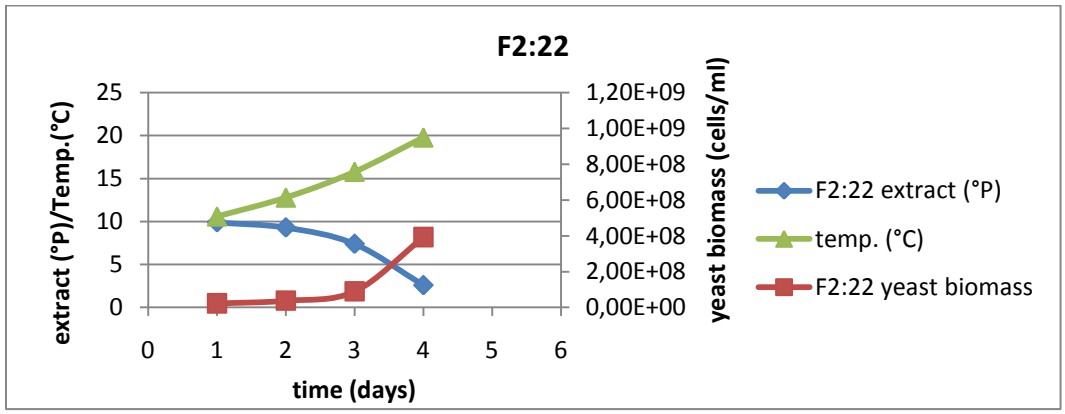


Figure 4.6 Yeast count, extract and temperature during 2nd round of propagation in second yeast batch.

In the 3rd round (figure 4.7) the measurements did not help to indicate in what phase the yeast was in upon pitching. However, the propagation time was only three days and perhaps short enough for still being in exponential phase. Unfortunately only one biomass measurement was performed during the 3rd round which made it difficult to draw any conclusions.

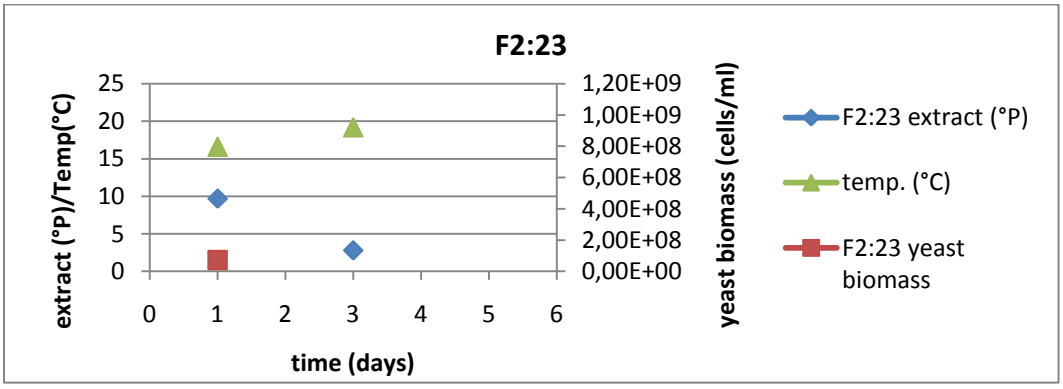


Figure 4.7 Yeast count, extract and temperature during 3rd round of propagation in second yeast batch.

The 4th round (figure 4.8) showed clearly how the yeast entered an exponential phase and stayed in it until the third day of propagation where it entered stationary phase. The yeast biomass was almost only half of the biomass obtained in the 2nd round from the second yeast but once again the yeast biomass was much higher in the 4th round of the second yeast batch compared to the 4th round of the first yeast batch.

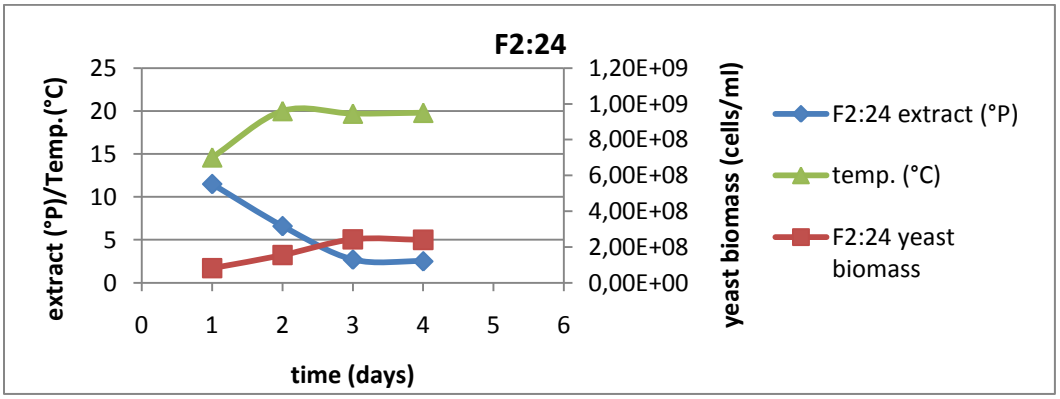


Figure 4.8 Yeast count, extract and temperature during 4th round of propagation in second yeast batch.

Overall in the second batch, the rounds showed higher yeast biomass compared to propagation in first batch. From this, one can deduce that the higher temperature indeed increased the yeast growth seeing that when a higher temperature was employed the extract decreased and the yeast growth increased much faster compared to a lower temperature. However, the first rounds in

both batches seemed to have much stronger growth capacity and as seen in second batch, the first round had lower temperature but still gave the highest yeast count.

From the result presented above an extra test was performed in order to see if yeast growth at lower temperatures would be beneficial. The temperature was changed to 11°C (figure 4.9). As assumed, the yeast growth was much slower and not only was the low yeast count an indication of that but also the slow decrease in extract. The lower temperature was tested on round 13 in the second batch and perhaps the yeast had become weaker after so many cycles.

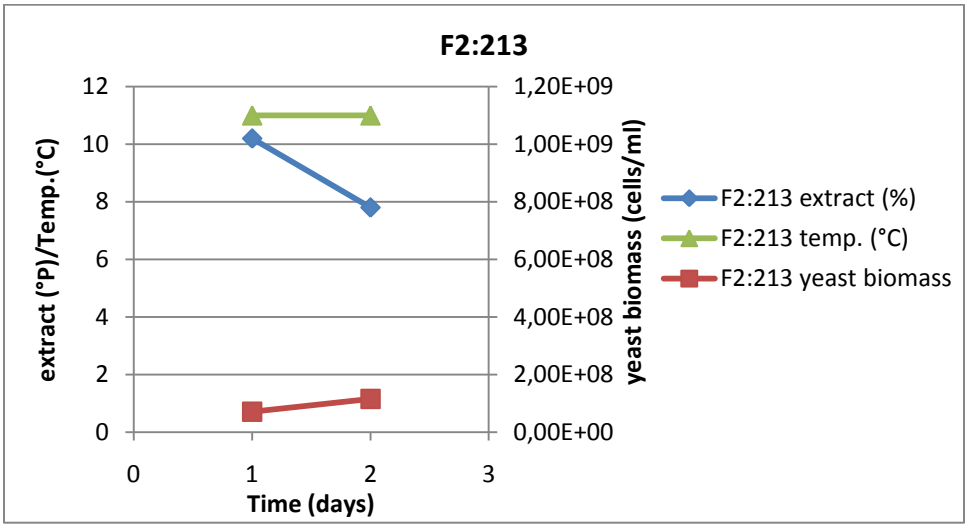


Figure 4.9 Yeast growth, extract and temperature during 13th round of propagation from the second yeast batch at 11°C.

4.2 Fermentation

Each round of propagated yeast was transferred into a small fermenting tank where a volume of approximately 360hl resulted from addition of wort to 30hl propagated yeast.

During the fermentation the yeast entered an anaerobic environment and the yeast behavior changed. The temperature for fermentation was 14°C but the temperature of the added wort was around 9°C and therefore 14°C was only reached after some time. The reason for the low temperature of wort was that the yeast, if started at higher temperatures, could impart unwanted flavors to the beer by release of large quantities of esters and other flavor compounds.

During fermentation the main goal for the yeast was no longer to increase in biomass but to produce alcohol and carbon dioxide. The yeast mass increased, but at a lower rate, in the

beginning as the wort was aerated and some oxygen was available for yeast growth (Walker, 1998).

In this experiment focus was put on the diacetyl production and breakdown due to the fact that it was the time limiting factor for fermentation. The diacetyl must not be present at higher amounts than 100 ppb for desirable lager beer, see section 2.4 for diacetyl formation and reduction.
In figures 4.10 and 4.11 the time taken for diacetyl reduction below 100 ppb were shown for all the fermentations performed during this work.

4.2.1 DIACETYL REDUCTION TIME BY FIRST YEAST BATCH

The diacetyl reduction times during fermentations by the propagated yeast from the first yeast batch were shown in figure 4.10. These reduction times were representing what was the current system and therefore supposedly typical reduction times for Spendrups brewery. The diacetyl was reduced to below 100ppb after about six and seven days of fermentation and did not seem to alter that much from each round of propagated yeast. The uneven pitched yeast which was discussed earlier did not seem to influence the diacetyl largely.

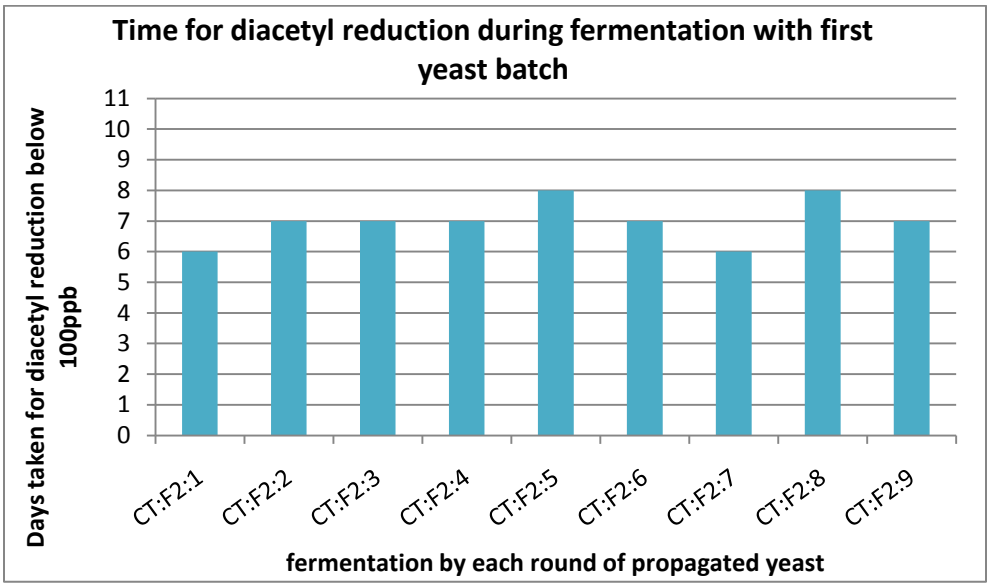


Figure 4.10 Time taken for diacetyl reduction below 100ppb by rounds of propagation of the first yeast batch.

4.2.2 DIACETYL REDUCTION TIME BY SECOND YEAST BATCH

The diacetyl reduction times by the yeast from the second batch (figure 4.11) seemed to fluctuate somewhat more compared to the reduction times in fermentations by the first yeast batch. The reduction times were lower by yeast propagated in round 2 and 4 from the second yeast batch. Whether the results were indicative of the state of the yeast or simply a coincidence cannot be determined.

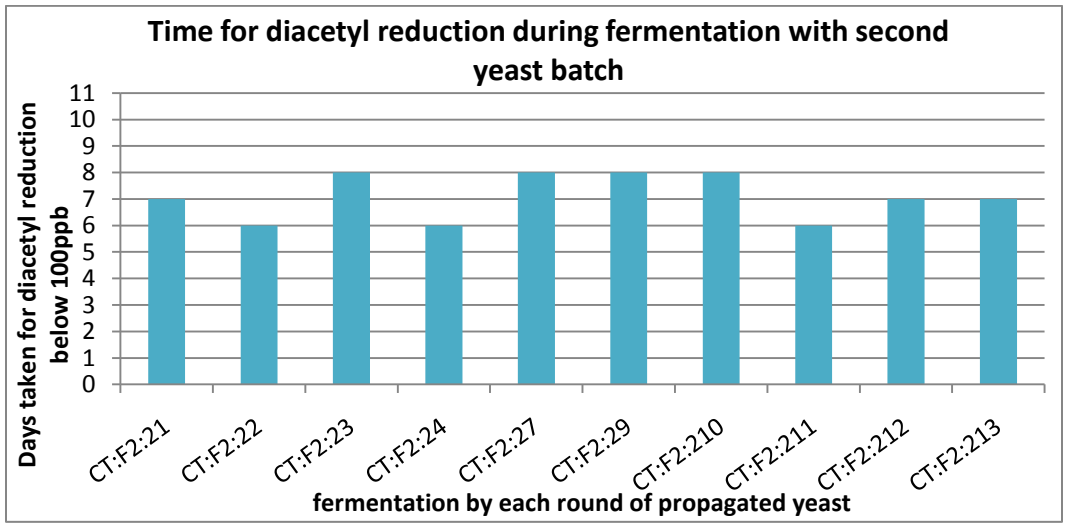


Figure 4.11 Time taken for diacetyl reduction below 100ppb by rounds of propagation of the first yeast batch.

4.2.3 DIACETYL REDUCTION BEHAVIOR

During the study of diacetyl reduction in each of the fermentation corresponding to a round of propagation the diacetyl could not always be recorded on a daily basis. Therefore the first four rounds were used for comparison between the two batches of yeast and their corresponding diacetyl reduction performance.

4.2.3.1 Behavior by first yeast batch

In figure 4.12 it was shown that the reduction of diacetyl seemed to be faster with yeast from the first round at 13°C. That yeast had high biomass when pitched, see table 4.1 and figure 4.1, which could play a role but also just be a coincidence due to other unknowns not studied in this work.

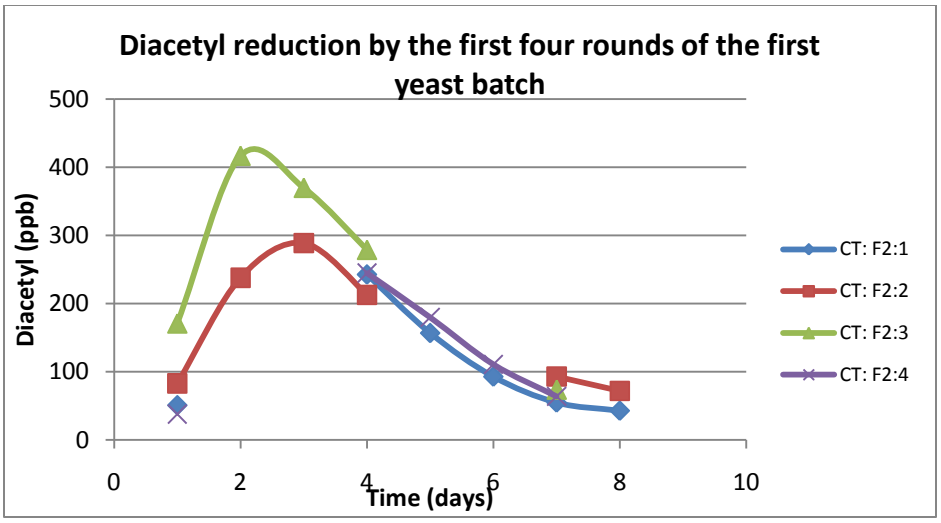


Figure 4.12 Diacetyl reductions during fermentations by the first batch of propagated yeast.

When comparing the four first rounds of propagation in each batch the diacetyl reductions were very similar in both batches. The only difference one could spot was the fact that the diacetyl peaks were higher in the fermentations with the yeast from the second batch shown in table 4.13. Also the highest peaks were reached during fermentation with the yeast that had the highest biomass after propagation. This could be explained by the fact that more yeast will produce more α-acetolactate which will form diacetyl once it has leaked out the yeast cell. Also, the reduction seemed faster and could perhaps be a result of more yeast cells to assimilate diacetyl and enzymatically reduce it into acetoin.

4.2.3.2 Behavior by second yeast batch

In figure 4.13 it was shown how the diacetyl was reduced to below 100ppb after only six days when yeast from second and fourth round of second batch were used. Perhaps there could be a correlation with the metabolic phase the yeast was in when pitched and the capacity of reducing diacetyl. If diacetyl reduction was an action carried out better by faster growing yeast then the amount of yeast does not play as big of a role as what the activity of the yeast cells does.

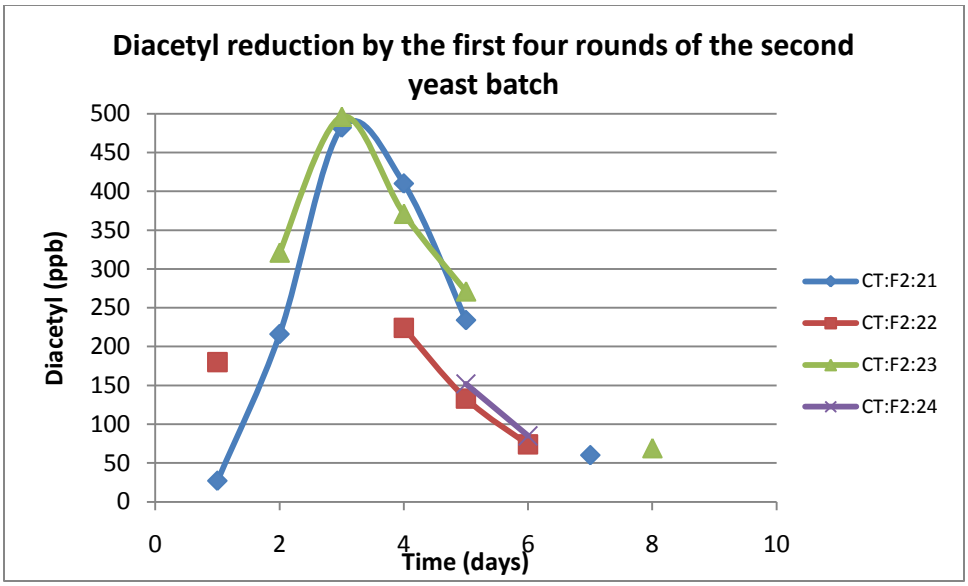


Figure 4.13 Diacetyl reductions during fermentations by the second batch of propagated yeast.

4.3 FINAL DISCUSSION

The time taken for diacetyl reduction below 100ppb did not show to be much shorter due to higher propagation temperature as very similar times were recorded for fermentations by yeast from both batches (figures 4.10 and 4.11). However, one cannot state that the number of cells or the metabolic state of the pitched yeast does not influence the diacetyl reduction at all since the time for diacetyl reduction during fermentation at Spendrups never was the same.

After testing the effect of temperature on propagation of yeast at Spendrups it became clear that the yeast growth increased with increased temperature. It could also be concluded that the first round of propagation by both yeast batches gave higher yeast biomass compared to subsequent rounds. The number of propagations by yeast could play a significant role for the yeast growth during propagation. After all, each round of propagation adds a generation to the propagated yeast which could, in turn, lead to lower metabolic activity.

The increased yeast growth during propagation did not significantly affect the time taken for diacetyl reduction to reach below 100ppb. Thus a high amount of pitched yeast did not automatically decrease the diacetyl reduction times during fermentation. However, from the diacetyl reduction behavior during fermentation with the second yeast batch, the reduction rate seemed to be higher and the diacetyl peak was higher. Compared to the first yeast batch, perhaps

the second yeast batch which yielded a higher pitching rate allowed for stronger reduction capacity even though not seen in time.

The results were based on single tests performed on an in-process production which naturally cannot serve as a ground for determination of whether changed propagation parameters of yeast actually can change the diacetyl reduction time. As a matter of fact it seemed like the propagation system together with the fermentation system at Spendrups brewery exerted a buffering effect due to the large scale incorporated.

For future studies, a broader knowledge of the diacetyl reduction mechanism in yeast cells could lead to huge time savings at the brewery. By studying the possible correlation between the metabolic activity of yeast and the capacity to reduce diacetyl the impact of propagation could perhaps be established. Also, a more careful investigation of whether or not yeast in exponential phase has higher fermentation capability could be of interest. Instead of testing a different propagation temperature rather focus on the behavior of yeast growth from round to round. If a propagation system could be held for the same amount of time each round, the metabolic phase of the propagated yeast could perhaps be more uniform upon pitching.

5 CONCLUSION

With regards to aims of this study, a faster diacetyl reduction could not be confirmed by a propagation system at a higher temperature. However, what can be said is that an increase in propagation temperature increased the yeast growth without negatively affecting the diacetyl reduction time. That means that the brewery could incorporate a higher propagation temperature in order to receive more viable yeast in shorter time without risking a decrease in fermentation capacity by the yeast. Furthermore, a more even propagation time would perhaps decrease fluctuations of the phases in which the yeast exist upon pitching and if the growth phase of the yeast does play a role to its fermentative activity, the fermentations would become more uniform.

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