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Original article

The role of yeast propagation aeration for subsequent primary fermentation with respect to performance and aroma development

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Summary During the primary fermentation of beer, the yeast simultaneously carries out a series of processes such as cell growth, pH shift, and the formation and degradation of essential flavour components. Harvested yeast from a previous fermentation can be used to inoculate the fermentation or fresh cells can be produced through aerobic propagation. This study investigated the influence of different aeration conditions during *Saccharomyces pastorianus* ssp. *carlsbergensis* propagation on the cell count development and the production of secondary metabolites during the subsequent primary beer fermentation. Propagations were conducted by applying six different dissolved oxygen concentrations, and the cells were used as inoculum for the subsequent fermentation. Cell count, pH shift, and the development of key aroma compounds were monitored throughout the primary fermentation to evaluate any difference between the conducted fermentations. The outcomes revealed significant distinctions between fermentations using yeast propagated under elevated oxygen levels and those propagated under reduced oxygen levels. Cells propagated using lower oxygen concentrations showed earlier cell growth with 40% lower final cell counts, resulting in 50% reduced biomass yields. Additionally, lower oxygen concentrations during propagation led to lower pH shifts during primary fermentation with 20% more higher alcohols and elevated formation of acetaldehyde, and esters.

Keywords Fermentation, oxygen, propagation, *Saccharomyces*, yeast.

Introduction

Brewing is one of the oldest and most complex food production processes. The brewing procedure consists of a series of individual steps; the number of ingredients, on the other hand, is typically minimal. Only water, malt, hops, and yeast are commonly used for traditional brewing. Therefore, the optimal physiological condition of yeast is essential for reliably consistent beer quality (Walker & Walker, 1998). To meet the yeast demand in a brewery, the yeast cells can be cropped after fermentation and reused for the following fermentation or produced freshly in an aerobic fermentation. The repitching process of harvested cells can be repeated several times, even though the bottom-fermenting yeast *Saccharomyces pastorianus* is commonly not used for more than 5–10 successive fermentations and top-fermenting yeast up to fifty times and more (Briggs *et al.*, 2004; Manger *et al.*, 2018).

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Two metrics are available in practice to assess the condition of a yeast culture: vitality and viability. The viability indicates the proportion of dead cells in the population. In many cases, however, toxic effects or stress responses do not lead to cell death. These factors can cause morphological, intracellular, or metabolic changes and, in sum, are considered by vitality. So, vitality defines the physiological capabilities of a cell. Vitality determination covers various aspects of the cell's physiology, such as cellular ATP content, mitochondrial membrane potential, and intracellular enzyme activities (Kwolek-Mirek & Zadrag-Tecza, 2014). Repitched yeast often shows deviation from batch-specific fermentation characteristics due to shifting exposure to different stress factors such as osmotic, ethanol, oxidative, and mechanical stress during repeated fermentations.

A more reliable cultivation method for yeast is the propagation process. Propagation is an aerobic fermentation that generates biomass as a starter culture for subsequent beer fermentation. Starting

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fermentations with fresh cultures of selected yeast strains minimises the risk of the accumulation of mutations and low fermentation performances over time and ensures sufficient quantity and the so-called vitality of the yeast. During fermentation, yeast is intended to achieve different goals simultaneously in a balanced manner. At the beginning of the fermentation, rapid yeast growth and a high pH shift should be achieved. In addition, the off-flavour diacetyl should be broken down as quickly as possible during secondary fermentation, and additional flavour components should be built up (Narziß, 2008). Oxygen concentration is already known as a process parameter that significantly influences cell growth during propagation (Beugholt *et al.*, 2023). However, there is still a lack of knowledge concerning the impact of oxygen during propagation on the pitching yeast and the quality of the subsequent fermentation.

One quality determining factor for efficient propagation is adequate aeration of the process to avoid oxygen limitations. Oxygen plays a versatile role in many yeast metabolism pathways, so adequate aeration and minimal oxidative stress must be balanced to optimise the growth conditions during propagation. One of the primary roles of oxygen in yeast cells is the biosynthesis of unsaturated fatty acids and ergosterol, both vital molecules for cell growth and cell membrane formation (Verbelen *et al.*, 2009b). In case of insufficient sterol and unsaturated fatty acid content, the cell will change its membrane structure, and the membrane-bound enzymes will be affected. Thus, the oxygen feed for yeast propagation is crucial due to the versatile function of oxygen in yeast cell metabolism (Verbelen *et al.*, 2009a).

Over-aeration increases the cells' reactive oxygen species (ROS) content and can cause oxidative stress for the yeast cells. ROS are partially reduced forms of molecular oxygen and can react with numerous intracellular components, damaging DNA, lipid structures, and proteins (Jamieson, 1995; Bayir, 2005). Intensive aeration can destabilise the balance between antioxidant potential and ROS generation in *S. pastorianus* and harm the cells up to triggering apoptosis (Lushchak & Gospodaryov, 2005; Perrone *et al.*, 2008; Beugholt *et al.*, 2022). Therefore, the aeration of the propagation has an essential role in manipulating the balance between oxygen depletion and over-aeration.

During fermentation, the main catabolic pathway is the conversion of fermentable sugars into CO_2 and ethanol, but as side products, a broad range of secondary metabolites are produced (Thesseling *et al.*, 2019). Despite their low concentrations, these secondary metabolites are responsible for the unique flavour composition of the final beer. Even small changes in the pitching yeast preparation can result in flavour changes since many environmental factors, like oxidative stress, affect the cell's production of volatile aroma compounds (Procopio *et al.*, 2011; Olaniran *et al.*, 2017). Due to the individual and partially low taste thresholds of the various components in the complex medium beer, insignificant changes in the final concentrations can generate an entirely new flavour profile (Renger *et al.*, 1992). Therefore, this work aims to investigate whether the dissolved oxygen concentration influences not only yeast propagation but also the subsequent primary fermentation of beer and its composition.

Materials and methods

Preculture and propagation

Experiments were conducted using *Saccharomyces pastorianus* ssp. *carlsbergensis* TUM 34/70, a genetic hybrid of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus* that is widely used in industrial brewing. Precultures for the propagation were prepared according to Beugholt *et al.* (2023). In short, precultures for the propagation were inoculated with a spatula tip of dry yeast in shake flasks and cultivated in multiple stages for 144 h at room temperature. Lager cast wort of an industrial brewery was used as medium. It had an original gravity of 11.28 ± 0.1°P and 197.6 ± 3.7 mg L^{-1} free amino nitrogen (FAN), and its pH was 5.14 ± 0.04. The propagation was inoculated with 5×10^6 living cells mL^{-1} from the preculture and was processed in pilot scale propagator (Esau&Hueber, Schrobenhausen, Germany) at a total volume of 60 L and a temperature of 12 °C for 84 h. The design of the propagator is shown in Beugholt *et al.* (2023). The oxygen content was set to a constant value between 2 mg L^{-1} and 8 mg L^{-1} using an airflow controller (MASS-STREAM, Bronkhorst, Germany) and a PID controller with a maximum airflow of 0.025 vvm to generate different conditions in each propagation. To ensure comparable starting conditions, the intracellular pH of the cells was measured after each propagation for cell vitality determination. The measurement of the ICP is based on flow cytometry and was described in detail by Weigert *et al.* (2009) and Imai & Ohno (1995). For the ICP measurement, cells are incubated with a pH-sensitive fluorescent marker. This fluorescent dye can be detected using flow cytometry and provides a direct correlation to the intracellular pH value which is an indirect marker for the yeast's metabolic and vitality state.

Cell count

The cell count was measured manually using a microscope and a haemocytometer according to MEBAK III 10.4.3.1 with methylene blue for viability tests according to MEBAK 10.4.4 (Jacob, 2013).

Fermentations

Fermentations were conducted in triplicates in 2 L reactors at a temperature of 12 °C using the same lager cast wort for the propagation and the preculture. The fermentation's initial living cell count was 15×10^6 cells mL^{-1}. The propagation broth was centrifuged to harvest the cells and to reduce any influence of the propagation medium on the fermentation and the upcoming analyses. The fermentation was performed for 7 days to cover the entire primary fermentation and had an initial dissolved oxygen concentration set to 4 mg L^{-1}.

Alcohol, fermentation attenuation and pH value

The alcohol content, pH, and fermentation attenuation were measured using Alcolyser ME and DMA 5001 (Anton Paar Group AG, Graz, Austria). For this purpose, the samples were degassed, and cell material and other sediments were removed by centrifugation for 10 min at 4000 r.p.m.

Volatile metabolites

The analyses of vicinal diketones, higher alcohols, esters, and aldehydes were performed using gas chromatography. The Hewlett Packard 5890 Series II gas chromatograph was used to analyse vicinal diketones. A headspace method with an electron capture detector (ECD) was used according to MEBAK 2.21.5.1 (Jacob, 2013). The higher alcohols and esters were analysed in the Hewlett Packard HP 6890 Series gas chromatograph with a flame ionisation detector (GC-FID) following MEBAK 2.21.6 (Jacob, 2013). Table 1 summarises the analysed volatile metabolites and adds literature values for their aroma descriptions, and taste thresholds (Hughes, 2009; Schieberle, 1991; Collin *et al.*, 1994; Fritsch & Schieberle, 2005; Kobayashi *et al.*, 2008; Preedy, 2008; Saison *et al.*, 2009).

Results and discussion

Propagation

The dissolved oxygen concentration for each propagation was set to one of six different values to investigate the influence of aeration during propagation on primary fermentation. Figure 1 displays the dissolved oxygen concentrations during the propagations with fixed values between 2 mg L^{-1} and 8 mg L^{-1} generated by pulsed aeration. Since antifoam agents are usually not used in industrial brewing, excessive aeration is no option to further increase the dissolved oxygen concentration in wort due to the resulting foaming of the medium. An oxygen concentration of 8 mg L^{-1} was chosen as the highest concentration in the following experiments to run the experiments in industry-related conditions without needing antifoam agents. The lowest concentration was set to 2 mg L^{-1} to ensure aerobic conditions in the medium. The geometric mean of the inoculum ICP value for the fermentations was 5.94 ± 0.14, which indicates good yeast vitality according to the literature and comparable starting conditions for each fermentation (Weigert *et al.*, 2009).

Cell growth during primary fermentation

One critical indicator of the influence of various aeration conditions during propagation on metabolism is changes in cell growth (Maemura *et al.*, 1998). Figure 2 shows the cell count during the primary fermentation triplicates. The reactors were inoculated with a living cell count of 15 million cells mL^{-1} but showed deviating behaviour during the 7 days of fermentation. To

Table 1 List of volatile metabolites analysed during primary fermentation

Name	Molecular weight (g mol^{-1})	Flavour descriptions	Compound classification	Taste threshold (mg L^{-1})
Acetaldehyde	44.05	Grassy, green apple	Aldehyde	19.8
1-Propanol	60.09	Alcoholic, solvent-like	Alcohol	700
Ethylacetate	88.11	Solvent, fruity, sweet	Ester	28.2
2-Methylpropanol	74.12	Malty, solvent-like	Alcohol	113.3
3-Methylbutanol	88.15	Alcohol, banana, malty	Alcohol	45
2-Methylbutanol	88.15	Malty, alcoholic, vinous, banana	Alcohol	57.5
2-Methylpropylacetate	11.16	Banana, sweet, fruity	Ester	1.05
Ethylbutanoate	116.16	Papaya, butter, sweet	Ester	0.4
3-Methylbutylacetate	130.18	Fruity, banana, pear, solvent	Ester	1.2
2-Methylbutylacetate	130.18	Fruity, banana, apple	Ester	0.8
Ethylhexanoate	144.21	Sour apple, aniseed, fruity, sweet	Ester	0.21
Diacetyl	86.09	Butterscotch, buttermilk	Ketone	0.087

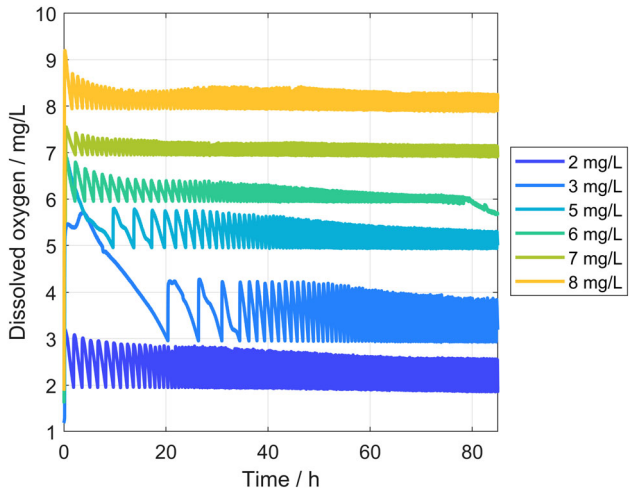

Figure 1 Dissolved oxygen concentration during the conducted propagations colour-coded as target values of the respective propagations.

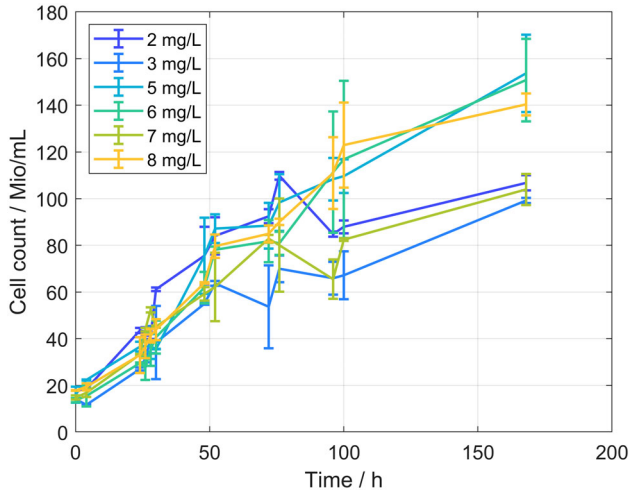

Figure 2 Development of the cell count during primary fermentation, depending on the oxygen concentration during propagation.

ensure comparable starting conditions, the intracellular pH value (ICP) was determined for every yeast culture used for pitching as a measure of yeast vitality. The geometric mean of the ICP value was 5.94 ± 0.14, which indicates excellent yeast vitality according to the literature (Weigert *et al.*, 2009). During the first 24 h of the fermentation, the cell count seems to develop similarly for all tested propagation conditions. After 90 h, two groups separate from each other. One group (P-value < 0.01) consisted of samples with higher oxygen concentrations during propagation (5 mg L^{-1}, 6 mg L^{-1}, and 8 mg L^{-1}). These samples result in higher final cell counts during the subsequent fermentation. The other group (p-value <0.01) consists of the samples with lower oxygen concentrations during the propagation (2 mg L^{-1}, 3 mg L^{-1}) and the 7 mg L^{-1} samples. The results indicate that lower oxygen concentrations during propagation cause lower final cell counts during fermentation. Similar results were demonstrated by Cheong *et al.* (2008) and Maemura *et al.* (1998), where higher aeration rates during propagation led to higher final cell counts during the subsequent fermentation. Cheong *et al.*, 2008 proposed that the observed augmentation in cell counts could primarily be attributed to an enhanced synthesis of sterols and unsaturated fatty acids within the yeast membrane during propagation.

Further mathematical analyses are necessary to calculate continuous curves from the measured cell count data points. This continuous data reveals characteristics of microbial growth, like different growth phases and the maximal growth rate. Microbial growth is assumed to follow a specific growth curve; hence, a logistic curve was adopted to model the development of the yeast cells. The idea of a logistic growth curve is based on the model first calculated by Pearl (1927), which is widely used for population calculations and, therefore, is a reasonable basis for this paper (Vadasz *et al.*, 2001). For a better characterisation of the cell count development, the corresponding fit for the mean cell counts of the fermentations was computed according to eqn (1).

$$y(t) = \frac{y_0 \cdot y_{\text{end}}}{y_0 + (y_{\text{end}} - y_0) \cdot e^{-y_{\text{end}} \bullet k \bullet t}} \tag{1}$$

$y(t)$ = cell count a time t, y_0 = initial cell count, y_{end} = final cell count, k = growth factor, t = time.

The measured cell count during fermentation was linearised using eqn (2) as a first step to finding the ideal fit for cell growth.

$$y_{\text{lin}}(t) = \ln\left(\frac{1}{y(t)} - \frac{1}{y_{\text{end}}}\right) \tag{2}$$

$y_{\text{lin}}(t)$ = linearised cell count a time t, $y(t)$ = cell count a time t, y_{end} = final cell count factor.

The optimal value for y_{end}, which describes the final cell count during the stationary phase, was determined iteratively with the least squares method of the resulting linear regression (Fig. 3a).

Performing calculations with various values for the final cell count enhances the method's robustness against fluctuations in cell quantification and scenarios where the last cell count determination precedes the attainment of the stationary phase. Therefore, different y_{end} values were tested for optimal linear regression, leading to very high determination coefficients. Based on the linear regression aimed at determining the optimal y_{end} value, the growth rate (k) was derived

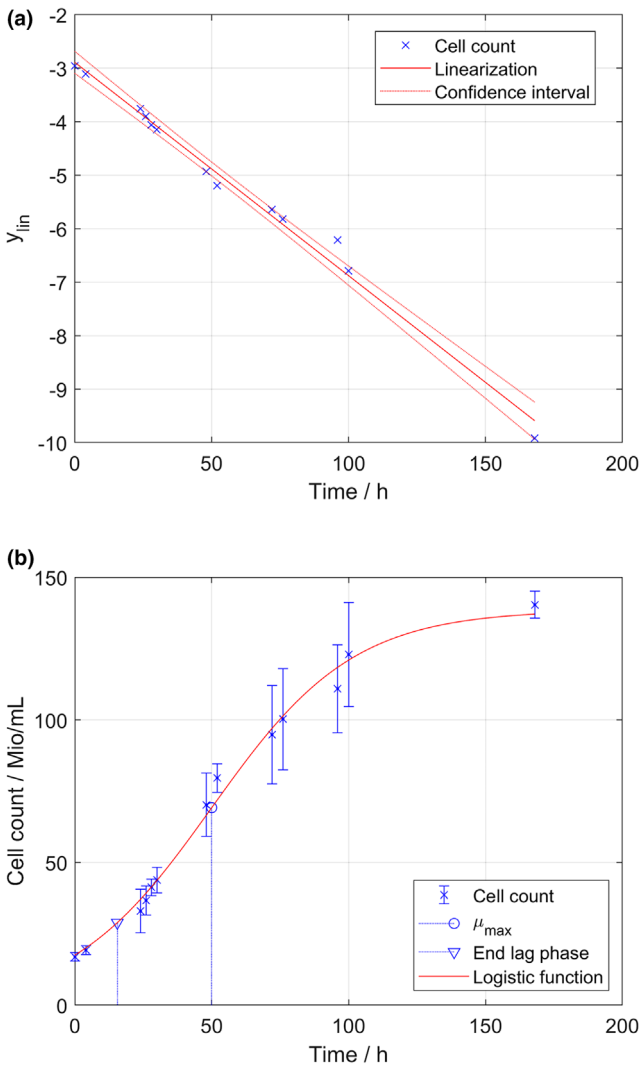

Figure 3 (a) Representative linearisation of the cell count of the fermentation propagated with 8 mg L^{-1}. (b) Logistic fit ($R^2 = 0.99$) of the measured cell count based on the estimated parameters phase from the linearisation. The circle marks the point of the maximum growth rate $\mu_{max,}$ and the triangle marks the end of the lag phase.

according to eqn (3), and the initial value (y_0) was determined following eqn (4), using the slope (m) and the y-axis intercept (y_{int}).

$$k = -\frac{m}{y_{end}} \tag{3}$$

k = growth factor, m = slope of the linear regression, y_{end} = final cell count.

$$y_0 = \frac{y_{end}}{1 + y_{end} \cdot e^{y_{int}}} \tag{4}$$

y_0 = initial cell count, y_{end} = final cell count, y_{int} = the y-axis intercept of the linear regression. With this parameter estimation, an average logistic fit for the cell count of every fermentation triplicate can be calculated, as shown in Fig. 3b. This figure shows a representative logistic fit of the cell count for the fermentations propagated with 8 mg L^{-1}. Calculating the first and second derivatives of the logistic function enables the identification of two significant points: the local maximum of the first derivative defines the point of maximum growth rate μ_{max} displayed in Fig. 3 as a circular marker, and the local maximum of the second derivative specifies the end of the lag phase shown as a triangular marker. The logistic fit was calculated for each fermentation triplicate, representing the mean development of the cell count. The statistical evaluatin of the calculated fits are shown in Table 2.

Figure 4 shows the mean point of μ_{max} values during the fermentations, calculated with the first derivative of the logistic fit in Fig. 3b. The results show the same two groups as for the final cell count in Fig. 2. Besides higher final cell counts, higher oxygen concentrations during propagations lead to a later occurrence of μ_{max} values during the fermentation. The group with higher oxygen concentrations during propagation (5 mg L^{-1}, 6 mg L^{-1}, and 8 mg L^{-1}) not only shows higher final cell counts in the fermentation but also indicates earlier maximum growth rates than the group with lower oxygen concentrations during propagation (2 mg L^{-1}, 3 mg L^{-1}) and the 7 mg L^{-1} samples. These results suggest that lower oxygen concentrations during propagation delay the cell count development during the subsequent fermentation. This behaviour might occur due to different adaptions of the cells to the new medium after inoculation. Yeast has developed adaptive mechanisms to sense and respond to decreasing oxygen concentrations by regulating oxygen homeostasis and coherent gene expression (Trendeleva *et al.*, 2014). Additionally, the growth of *Saccharomyces* yeast in anaerobic conditions depends on aerobic growth factors such as ergosterol, which could be produced in higher amounts during propagations with higher oxygen concentrations. It is known that oxygen

Table 2 Statistical evaluation of the calculated logistic fit for each triplicate

Oxygen concentration	2 mg L^{-1}	3 mg L^{-1}	5 mg L^{-1}	6 mg L^{-1}	7 mg L^{-1}	8 mg L^{-1}
R^2	0.97	0.92	0.96	0.99	0.91	0.99
RMSE	6.2	7.7	8.7	5.8	8.3	4.1

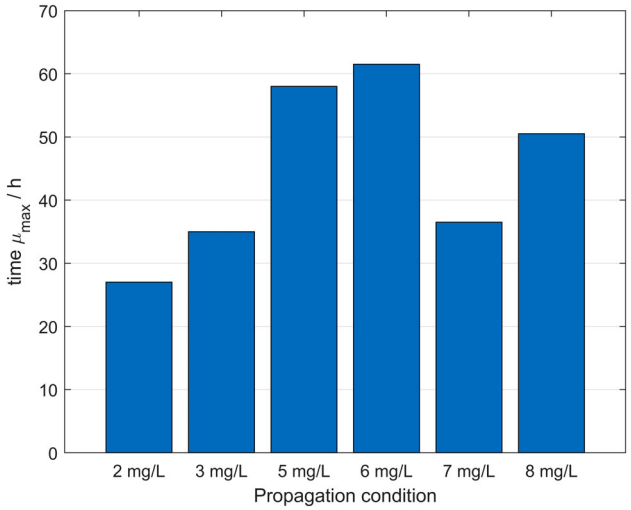

Figure 4 Point of the highest growth rate during fermentation for different oxygen concentrations during propagation. μ_{max} was calculated from the first derivative of the logistic fit of the cell count.

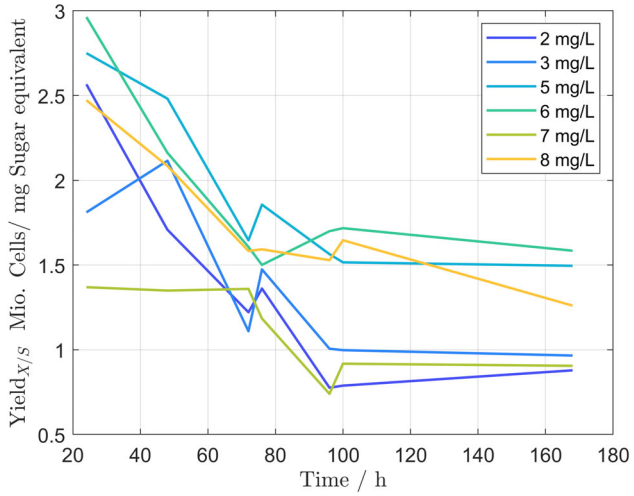

Figure 5 Biomass yield $y_{x/s}$ presented as cells mg^{-1} sugar equivalent produced during primary fermentation, depending on the oxygen concentration during propagation.

positively affects ergosterol production in *Saccharomyces* yeast, although the specific mechanisms involved require further investigation (Blaga *et al.*, 2018; Marbà-Ardébol *et al.*, 2018; Jordá & Puig, 2020).

Biomass yield

One of the objectives during primary fermentation is biomass generation. Fermentable sugars undergo metabolism in the yeast cells to mainly produce biomass, carbon dioxide, and ethanol. In the case of brewing, the complex medium wort is used as a growth medium. It contains a combination of various fermentable sugars, amino acids, proteins, and minerals, summarised by the extract. The extract serves as a carbon source for the yeast cells and is measured in °P, where 1 °P is equivalent to the mass fraction of an aqueous 1% saccharose solution. The sugar uptake is therefore calculated in sugar equivalents with the correlation between the wort extract and the specific density taken from Plato (1900). Effective biomass formation is characterised by a considerable increase in biomass with simultaneous low sugar uptake. This relationship can be expressed through the biomass yield $Y_{X/S}$.

$$Y_{X/S} = \frac{q_x}{q_s} = \frac{dc_x}{dc_s} \tag{5}$$

$Y_{X/S}$ = biomass yield, q_x = specific growth rate, q_s = specific substrate uptake rate, c_x = biomass concentration, c_s = substrate concentration.

Figure 5 shows the biomass yield that is defined as the proportion of carbon intake that is converted into biomass. In this case, the biomass yield is shown as a ratio of the produced cells using the logistic models and the uptake of sugar equivalents from the medium. The unit mio. cells per milligram sugar equivalent was chosen in contrast to the standard unit gram biomass per gram carbon source to avoid the influence of changes in the single cell weight, which can be caused by changing growth conditions (Polakis & Bartley, 1966; Łabędź *et al.*, 2017). The biomass yield starts at high values and decreases until 100 h of fermentation, when it reaches a static behaviour. The absorbed carbon is distributed between the maintenance metabolism and the anabolism, as shown by a fast increase in cell count in Fig. 2. After 100 h, the proportion of anabolism decreases while the proportion of catabolism and maintenance metabolism increases. As a result, the biomass yield decreases throughout the fermentation. Later in the process, the biomass yield reaches a constant value with low biomass increase and low sugar uptake. The data show a correlation between higher oxygen concentrations during propagation and higher biomass yields during primary fermentation.

pH shift during primary fermentation

The pH shift during primary fermentation is essential for the final beer's shelf life and, in addition, is a measure of the yeast cells' metabolic activity. The assimilation of sugars by the yeast cells results in a pH decrease in the course of fermentation. This occurs due to the expulsion of protons and the formation and discharge of organic acids and carbonic acid

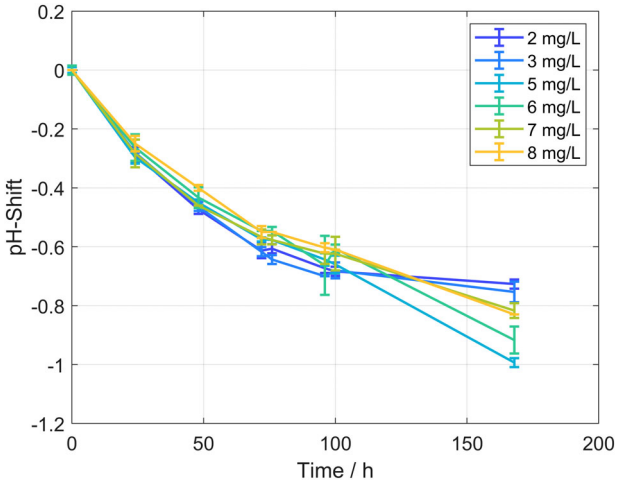

Figure 6 pH shift during primary fermentation. Results are shown as a difference to the starting pH to compensate for small changes in the fermentation medium.

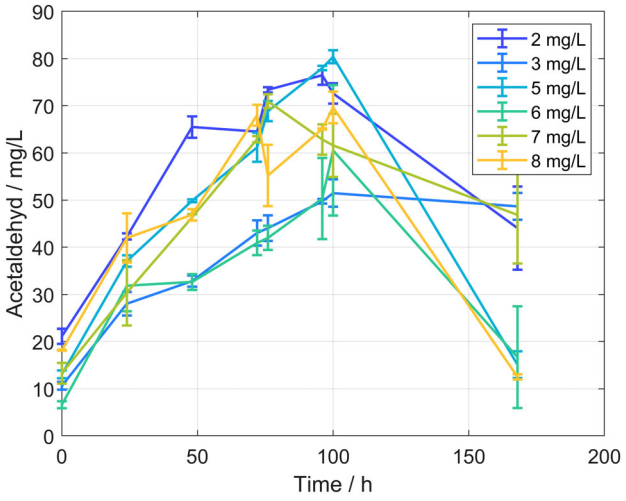

Figure 7 Formation and degradation of acetaldehyde during primary fermentation depending on the oxygen concentration during propagation.

originating from the dissolution of CO_2 in the fermenting wort (Miller *et al*., 2013). Therefore, the pH shift shown in Fig. 6 indicates the yeast's condition after propagation and during fermentation. The pH value is represented as a pH shift to account for minor fluctuations in the initial values, which fall within the range of 5.94 ± 0.14. The slight variability in the initial conditions is attributed to the wort as the fermentation medium. The wort originates from an industrial brewery and is subject to minor variations due to the production process and the natural resources used. The pH profiles shown represent the typical trend observed in industrial-scale fermentations, with substantial pH decreases at the beginning of the process and gradually decreasing pH shifts towards the end of the fermentation. At the beginning of the primary fermentation, the pH shift is very similar for all tested fermentations. After 70 h, the pH values start to separate between the different runs, and after 100 h, the pH shifts stop for the cells propagated with 2 mg L^{-1} and 3 mg L^{-1}. The observations from the final samples at the 168-h mark illustrate that elevated oxygen concentrations result in more pronounced pH shifts. Conversely, lower oxygen concentrations during propagation accelerate the ending of the pH shift. Consequently, fermentations involving cells propagated with reduced oxygen concentrations exhibit elevated final pH levels. The pH shift is caused by the formation of organic acids as by-products and affects the final product's stability, character, and durability (Li & Liu, 2015). In the shown primary fermentations, lower dissolved oxygen concentrations during propagation led to earlier ends of the pH shift and a higher final pH value of the medium. Miller *et al*. (2013) hypothesised that freshly propagated yeast has recently undergone aerobic growth and is likely to have generated substantial amounts of sterols and unsaturated fatty acids before primary fermentation compared with repitched yeast. Consequently, upon pitching, these cells can more effectively use free amino nitrogen (FAN), leading to a more significant reduction in pH. This hypothesis could be extrapolated to the present data, where higher oxygen concentrations may have resulted in increased formation of sterols and unsaturated fatty acids, thus causing a more pronounced decrease in pH.

Formation of volatile metabolites

Gas chromatographic analyses of volatile aroma components were carried out during primary fermentation to assess the influence of propagation on the cells' metabolic activity and the final beer's aroma profile. The focus of these analyses was on the investigation of the prominent taste components of young beer. The formation and degradation of acetaldehyde and diacetyl, as well as the formation of higher alcohols and esters shown in Table 1, are of particular interest.

Figure 7 shows the formation and degradation of acetaldehyde with its typical course during primary fermentation. Acetaldehyde is formed as an intermediate product of yeast metabolism by the decarboxylation of pyruvate, and its green apple flavour is an essential flavour of young beer (Xu *et al*., 2019). In the experiments conducted, the acetaldehyde concentration peaks after about 100 h before degradation begins. Again, two groups can be identified in the samples taken after 168 h. The cohort exposed to elevated

oxygen levels during propagation (5 mg L^{-1}, 6 mg L^{-1}, and 8 mg L^{-1}) exhibited increased acetaldehyde concentrations after 100 h. However, this group also showed more pronounced degradation of the acetaldehyde formed, resulting in lower acetaldehyde levels in the final samples. The other group consists of the samples propagated with lower oxygen concentrations (2 mg L^{-1}, 3 mg L^{-1}) and the 7 mg L^{-1} samples. These fermentations show lower degradation of acetaldehyde after 100 h, resulting in higher concentrations after 168 h. Thus, the final concentration of acetaldehyde in fermentations with higher oxygen concentration during propagation is lower than for fermentations with lower concentrations during propagation. However, it must be noted that the presented measurements of acetaldehyde concentration exhibit strong fluctuations. These fluctuations complicate the interpretation of the data, which is why trends are described here.

Figure 8 shows the formation and degradation of diacetyl with its typical course during primary fermentation. Diacetyl is often used as a key component for monitoring the maturation of the beer (Kruse *et al.*, 2024). In addition to the other mentioned aroma components, diacetyl significantly influences the final product's taste and is one possible control variable of fermentation (Krogerus & Gibson, 2013b). Diacetyl is a metabolic product of yeast formed during fermentation. Depending on the beer style, even in small quantities of diacetyl, with its buttery aroma, can be seen as an off-flavour that can significantly alter the beer taste. The typical course of diacetyl in the wort during primary fermentation is an increase in diacetyl concentration with a subsequent breakdown (Beckett *et al.*, 2003). The results presented show this progression and, in addition, very similar diacetyl concentrations between the different runs in the first 100 h of the primary fermentation. The final measurement after 168 h shows large variations in the measured values without significant differences between fermentations with different propagation conditions. The presented results showed deficient amounts of diacetyl and no significant changes between the tested fermentations. Diacetyl is formed during the degradation of acetolactate, a valine synthesis product that is excreted from the cells (Gjermansen *et al.*, 1988). Acetolactate is degraded outside the cell by spontaneous oxidative decarboxylation, and the cells take up the resulting diacetyl. The insignificant differences in the final diacetyl concentration between the fermentations may be caused by the spontaneous extracellular reaction that is not influenced by the cells (Krogerus & Gibson, 2013a). Thus, propagation conditions do not necessarily affect diacetyl formation since the decarboxylation is the rate-limiting step.

Higher alcohols are mainly formed during the primary fermentation by transamination, decarboxylation, and reduction of various amino acids. This process is also known as the Ehrlich mechanism (Narziß, 2008). As essential bouquet substances, esters are also products of yeast metabolism. They are formed throughout fermentation by enzymatically catalysed reactions and significantly influence beer aroma. Compared with vicinal diketones, higher alcohols, and esters remain in the beer and are only slightly degraded (Tokpohozin *et al.*, 2019). Figure 9 shows

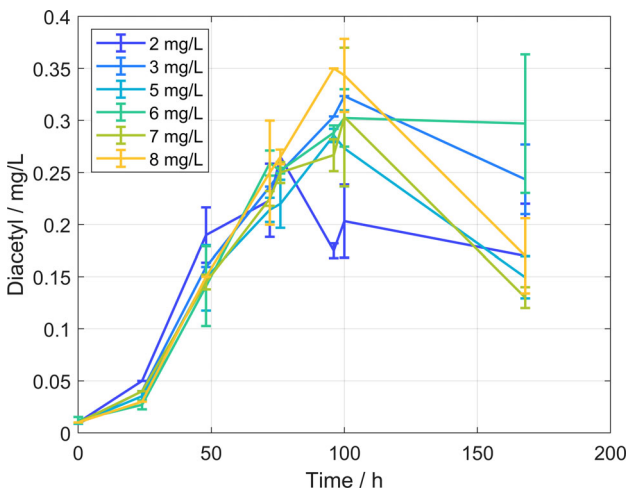

Figure 8 Formation and degradation of diacetyl during primary fermentation depending on the oxygen concentration during propagation.

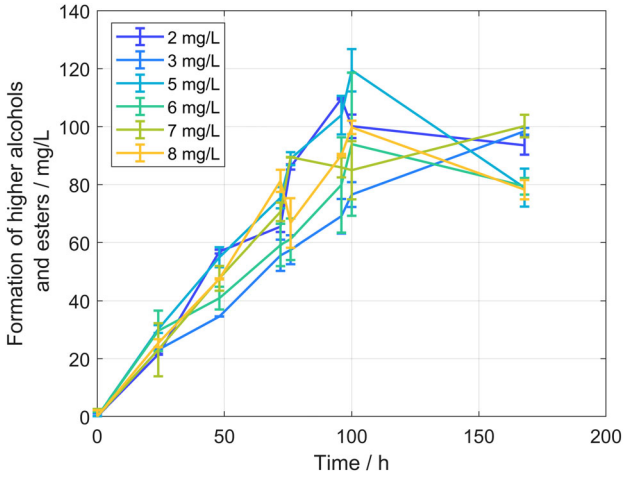

Figure 9 Formation of higher alcohols and esters during primary fermentation depending on the oxygen concentration during propagation. The results are shown as cumulative values of the tested volatile components.

the cumulative amount of the higher alcohols and esters listed in Table 1 during the primary fermentation. Higher oxygen concentrations during propagation led to elevated concentrations of higher alcohols after 100 h of primary fermentation. The group exposed to elevated oxygen levels during propagation (5 mg L^{-1}, 6 mg L^{-1}, and 8 mg L^{-1}) exhibited reduced concentrations of higher alcohols and esters after 168 h. Samples propagated with lower oxygen concentrations (2 mg L^{-1}, 3 mg L^{-1}), and the 7 mg L^{-1} samples showed higher concentrations at the end of the primary fermentation. This trend correlates with findings from Dzialo *et al.* (2017), which showed a correlation between lower cell growth and the elevated formation of higher alcohols. Further research from Visinoni *et al.* (2022) showed that the relationship between cell growth and the production of higher alcohols depends on the cell's physiological state, as higher alcohols are a product of the amino acid metabolism via the Ehrlich pathway. The observed elevated amounts of higher alcohols could result from changes in the amino acid metabolism in connection with the lower growth rate of the cells propagated with lower oxygen concentrations.

In all the results, the cells propagated at 7 mg L^{-1} are considered outliers because they behave differently from the other propagations with higher oxygen concentrations but like those in the lower oxygen concentration group.

It must be clarified whether the varying cell counts can cause the differences in the tested characteristics during fermentation. Svenkrtova *et al.*, 2016 showed a correlation between a higher rate of cell division and increased resistance against oxidative stress. In this study, cells with higher division rates accumulated more trehalose, a carbohydrate connected with stress resistance. These findings could be transferred to the present data and explain the occurrence of two groups with different behaviours due to oxidative stress resistance.

Conclusions

In this study, *Saccharomyces pastorianus* was used to test the effect of different oxygen concentrations during propagation on the subsequent primary beer fermentation. Lower oxygen concentrations during propagation resulted in lower cell counts (Fig. 2) and earlier maximal growth rates (Fig. 4). The biomass yield during primary fermentation was reduced for the cells propagated with lower oxygen concentration. Additionally, lower aeration during propagations led to a lower pH shift. Although the aeration did not affect the concentration of diacetyl, increased acetaldehyde concentrations and higher alcohols and esters were observed for propagations with lower aeration. Based on the findings in the present study, high oxygen concentrations during propagation seem to benefit the subsequent primary fermentation. Higher alcohols contribute significantly to the taste impression of the final beer; however, excessive concentrations can be perceived as off-flavours depending on the beer style. Therefore, excessive concentrations should be avoided. The higher cell counts during primary fermentation enable higher microbial activity, which is beneficial during the maturation of the beer. It should be stated that adequate aeration of the propagation process with high oxygen concentrations in brewing comes with additional challenges. The wort medium tends to excessive foaming, and antifoam agents are either not allowed or are not typical for their impact on the foam stability of the final product. This drawback needs to be compensated through bigger tanks or other process strategies. Nevertheless, the advantages of the presented data prevail and form the foundation for future considerations regarding improved yeast propagation in view of subsequent process steps.

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Author contributions

Alexander Beugholt: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; software; formal analysis; project administration; data curation; writing – review and editing. **Dominik Ulrich Geier:** Funding acquisition; writing – review and editing. **Thomas Becker:** Writing – review and editing; resources; supervision.

Ethical statement

The authors declare no conflict of interest. Neither ethical approval nor informed consent was required for this study.

Peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

References

Bayir, H. (2005). Reactive oxygen species. *Critical Care Medicine*, **33** (12 Suppl), S498–S501.

Beckett, E.M., Abelmann, A. & Maier, M.A. (2003). Diacetyl. In: *Patty's Toxicology* (edited by E. Bingham, B. Cohrssen & C.H. Powell). Pp. 1–19. New york, USA: Wiley.

Beugholt, A., Büchner, K., Geier, D.U. & Becker, T. (2022). Quantification of oxidative stress in saccharomyces pastorianus propagation: gene expression analysis using quantitative reverse transcription polymerase chain reaction and flow cytometry. *Frontiers in Chemical Engineering*, **4**, 1035348.

Beugholt, A., Geier, D.U. & Becker, T. (2023). Improvement of saccharomyces propagation performance through oxygen-enriched air and aeration parameter variation. *Frontiers in Chemical Engineering*, **5**, 1193230.

This source was cited because of its detailed physical description of the fumigation process. Additionally the same propagator was used for the generation of the experimental data.

Blaga, A.C., Ciobanu, C., Caşcaval, D. & Galaction, A.-I. (2018). Enhancement of ergosterol production by *Saccharomyces cerevisiae* in batch and fed-batch fermentation processes using n-dodecane as oxygen-vector. *Biochemical Engineering Journal*, **131**, 70–76.

Briggs, D., Boulton, C., Brookes, P. & Stevens, R. (2004). *Brewing*. Boca Raton, Florida: CRC Press.

Cheong, C., Wackerbauer, K., Si, K.L. & Soon, A.K. (2008). Optimal conditions for propagation in bottom and top brewing yeast strains. *Food Science and Biotechnology*, **17**, 739–744.

This source was cited to appreciate the basic idea of the paper. The authors have followed a similar approach to this paper, but have left room for further development in the crucial details addressed in this paper.

Collin, S., Derdelinckx, G. & Dufour, J.-P. (1994). Relationships between the chemical composition and sensory evaluation of lager beers. *Food Quality and Preference*, **5**, 145–149.

Dzialo, M.C., Park, R., Steensels, J., Lievens, B. & Verstrepen, K.J. (2017). Physiology, ecology and industrial applications of aroma formation in yeast. *FEMS Microbiology Reviews*, **41**(Supp_1), S95–S128.

Fritsch, H.T. & Schieberle, P. (2005). Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian pilsner-type beer. *Journal of Agricultural and Food Chemistry*, **53**, 7544–7551.

Gjermansen, C., Nilsson-Tillgren, T., Petersen, J.G., Kielland-Brandt, M.C., Sigsgaard, P. & Holmberg, S. (1988). Towards diacetyl-less brewers' yeast. Influence of ilv2 and ilv5 mutations. *Journal of Basic Microbiology*, **28**, 175–183.

Hughes, P. (2009). A quality perspective – beer flavor. In: *Beer – A Quality Perspective*. Pp. 61–83. London: Academic Press.

Imai, T. & Ohno, T. (1995). The relationship between viability and intracellular pH in the yeast *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, **61**, 3604–3608.

(edited by Jacob, F.) (2013). *Wort, beer, beer-based beverages*. Mebak: Freising-Weihenstephan.

This source was cited because the basic analytical methods for brewing science are collected in this publication. This work sets a standard for an entire field of research that is recognised in the professional world and is agreed as a common consensus by an expert committee for analytics.

Jamieson, D.J. (1995). The effect of oxidative stress on *Saccharomyces cerevisiae*. *Redox Report: Communications in Free Radical Research*, **1**, 89–95.

Jordá, T. & Puig, S. (2020). Regulation of ergosterol biosynthesis in *Saccharomyces cerevisiae*. *Genes*, **11**, 1–18.

Kobayashi, M., Shimizu, H. & Shioya, S. (2008). Beer volatile compounds and their application to low-malt beer fermentation. *Journal of Bioscience and Bioengineering*, **106**, 317–323.

Krogerus, K. & Gibson, B.R. (2013a). 125th anniversary review: Diacetyl and its control during brewery fermentation. *Journal of the Institute of Brewing*, **119**(3), 86–97.

Krogerus, K. & Gibson, B.R. (2013b). Influence of valine and other amino acids on total diacetyl and 2,3-pentanedione levels during fermentation of brewer's wort. *Applied Microbiology and Biotechnology*, **97**, 6919–6930.

Kruse, J., Wörner, J., Schneider, J., Dörksen, H. & Pein-Hackelbusch, M. (2024). Methods for estimating the detection and quantification limits of key substances in beer maturation with electronic noses. *Sensors (Basel, Switzerland)*, **24**, 3520.

Kwolek-Mirek, M. & Zadrag-Tecza, R. (2014). Comparison of methods used for assessing the viability and vitality of yeast cells. *FEMS Yeast Research*, **14**, 1068–1079.

Łabędź, B., Wańczyk, A. & Rajfur, Z. (2017). Precise mass determination of single cell with cantilever-based microbiosensor system. *PLoS One*, **12**, e0188388.

Li, G. & Liu, F. (2015). Changes in organic acids during beer fermentation. *Journal of the American Society of Brewing Chemists*, **73**, 275–279.

Lushchak, V.I. & Gospodaryov, D.V. (2005). Catalases protect cellular proteins from oxidative modification in *Saccharomyces cerevisiae*. *Cell Biology International*, **29**, 187–192.

Maemura, H., Morimura, S. & Kida, K. (1998). Effects of aeration during the cultivation of pitching yeast on its characteristics during the subsequent fermentation of wort. *Journal of the Institute of Brewing*, **104**, 207–211.

Manger, H.-J., Annemüller, G. & Lietz, P. (2018). The Yeast in the Brewery. In: *Management – Pure Yeast Cultures – Propagation*, 2nd edn. Berlin: VLB Berlin.

Marbà-Ardébol, A.-M., Bockisch, A., Neubauer, P. & Junne, S. (2018). Sterol synthesis and cell size distribution under oscillatory growth conditions in *Saccharomyces cerevisiae* scale-down cultivations. *Yeast*, **35**, 213–223.

Miller, K.J., Box, W.G., Jenkins, D.M., Boulton, C.A., Linforth, R. & Smart, K.A. (2013). Does generation number matter? The impact of repitching on wort utilization. *Journal of the American Society of Brewing Chemists*, **71**, 233–241.

Narziß, L. (2008). *Abriss der Bierbrauerei*, 7th edn. Weinheim: Wiley-VCH.

Olaniran, A.O., Hiralal, L., Mokoena, M.P. & Pillay, B. (2017). Flavour-active volatile compounds in beer: production, regulation and control. *Journal of the Institute of Brewing*, **123**, 13–23.

Pearl, R. (1927). The growth of populations. *The Quarterly Review of Biology*, **2**, 532–548.

Perrone, G.G., Tan, S.-X. & Dawes, I.W. (2008). Reactive oxygen species and yeast apoptosis. *Biochimica et Biophysica Acta*, **1783**, 1354–1368.

Plato, F. (1900). *Die Dichte, Ausdehnung und Kapillarität von Lösungen Reinen Rohrzuckers in Wasser*. Berlin, Heidelberg: Springer.

Polakis, E.S. & Bartley, W. (1966). Changes in dry weight, protein, deoxyribonucleic acid, ribonucleic acid and reserve and structural carbohydrate during the aerobic growth cycle of yeast. *The Biochemical Journal*, **98**, 883–887.

Preedy, V.R. (2008). *Beer in Health and Disease Prevention*. San Diego: Elsevier Science & Technology.

Procopio, S., Qian, F. & Becker, T. (2011). Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. *European Food Research and Technology*, **233**, 721–729.

Renger, R.S., van Hateren, S.H. & Luyben, K.C.A. (1992). The formation of esters and higher alcohols during brewery fermentation; the effect of carbon dioxide pressure. *Journal of the Institute of Brewing*, **98**, 509–513.

Saison, D., de Schutter, D.P., Uyttenhove, B., Delvaux, F. & Delvaux, F.R. (2009). Contribution of staling compounds to the aged

flavour of lager beer by studying their flavour thresholds. *Food Chemistry*, **114**, 1206–1215.
Schieberle, P. (1991). Primary odorants of pale lager beer. *Zeitschrift fr Lebensmittel-Untersuchung und – Forschung*, **193**, 558–565.
Svenkrtova, A., Belicova, L., Volejnikova, A., Sigler, K., Jazwinski, S.M. & Pichova, A. (2016). Stratification of yeast cells during chronological aging by size points to the role of trehalose in cell vitality. *Biogerontology*, **17**, 395–408.
Thesseling, F.A., Bircham, P.W., Mertens, S., Voordeckers, K. & Verstrepen, K.J. (2019). A hands-on guide to brewing and analyzing beer in the laboratory. *Current Protocols in Microbiology*, **54**, e91.
Tokpohozin, S.E., Fischer, S. & Becker, T. (2019). Selection of a new saccharomyces yeast to enhance relevant sorghum beer aroma components, higher alcohols and esters. *Food Microbiology*, **83**, 181–186.
Trendeleva, T.A., Aliverdieva, D.A. & Zvyagilskaya, R.A. (2014). Mechanisms of sensing and adaptive responses to low oxygen conditions in mammals and yeasts. *Biochemistry*, **79**, 750–760.
Vadasz, A.S., Vadasz, P., Abashar, M.E. & Gupthar, A.S. (2001). Recovery of an oscillatory mode of batch yeast growth in water for a pure culture. *International Journal of Food Microbiology*, **71**, 219–234.
Verbelen, P.J., Saerens, S.M.G., van Mulders, S.E., Delvaux, F. & Delvaux, F.R. (2009a). The role of oxygen in yeast metabolism during high cell density brewery fermentations. *Applied Microbiology and Biotechnology*, **82**, 1143–1156.
Verbelen, P.J., Depraetere, S.A., Winderickx, J., Delvaux, F.R. & Delvaux, F. (2009b). The influence of yeast oxygenation prior to brewery fermentation on yeast metabolism and the oxidative stress response. *FEMS Yeast Research*, **9**, 226–239.
This source was cited because the author makes a fundamental contribution to the research area described. Numerous publications have created a fundamental understanding of the influence of yeast in the brewing process.
Visinoni, F., Zhang, P., Hollywood, K.A. *et al.* (2022). Volatile aroma compound production is affected by growth rate in S. Cerevisiae. *Applied and Environmental Microbiology*, **88**, e0150922.
Walker, G. & Walker, G.M. (1998). *Yeast Physiology and Biotechnology*. Chichester/New York: Wiley.
Weigert, C., Steffler, F., Kurz, T., Shellhammer, T.H. & Methner, F.-J. (2009). Application of a short intracellular pH method to flow cytometry for determining *Saccharomyces cerevisiae* vitality. *Applied and Environmental Microbiology*, **75**, 5615–5620.
Xu, X., Niu, C., Liu, C. & Li, Q. (2019). Unraveling the mechanisms for low-level acetaldehyde production during alcoholic fermentation in *Saccharomyces pastorianus* lager yeast. *Journal of Agricultural and Food Chemistry*, **67**, 2020–2027.

