Investigation of cell density of BY4741A wild-type and YLR044C mutant strain *Saccharomyces cerevisiae* in response to varying dextrose conditions

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**Abstract**

We compared the effect of varying dextrose concentrations on BY4741A wild-type and YLR044C mutant *Saccharomyces cerevisiae* cell densities at late log phase. We had two null hypotheses: 1) The wild-type strain of *S. cerevisiae* will have the same or lower cell density than the PDC1 mutant strain of *S. cerevisiae* at each dextrose concentration at 29$^o$C at late log phase and 2) At higher dextrose concentration treatment levels, the difference between the wild-type and mutant *S. cerevisiae* cell density at late log phase will be the same or decrease, in the sense that the difference will be smaller. We tested three dextrose conditions (25.34g/L, 46.66g/L and 89.34g/L) for each of wild-type and mutant *S. cerevisiae*, with three replicates for each treatment level under the near-optimal temperature condition of 29°C. We took absorbance measurements and converted to cell density by constructing a standard curve through haemocytometer readings of cell density. From our analysis, we reject our first null hypothesis since we observed that the wild-type strain had a significantly higher cell density at late log phase, defined as hour 24 in this experiment, than the PDC1 mutant at 25.34g/L and 46.66g/L dextrose concentrations. Though we did not find a significantly higher cell density at the 89.34g/L dextrose concentration level for wild-type, the wild-type cell density was still higher than mutant. These results are likely occurring because the mutant strain has decreased fermentative growth, which may affect its growth and reproduction. We failed to reject our second null hypothesis since we found that the difference between wild-type and mutant cell density at 89.34g/L was smaller than both the differences in cell density at 25.34g/L and 46.66g/L dextrose medium. Reasons for these results are discussed.

**Introduction**

*Saccharomyces cerevisiae*, also known as bakers' yeast, is a highly researched microorganism. It was the first eukaryote to have its genome completely sequenced (Sherman 2002). It is easily manipulated, can grow in many types of media, and grows optimally at 30°C (Sherman 2002). *S. cerevisiae* can undergo both sexual and asexual reproduction, although the latter is more common, especially in the form of budding (Pronk *et al.* 1996). To produce energy, yeast can follow the aerobic and/or the anaerobic pathway, depending on oxygen conditions (Pronk *et al.* 1996, see Figure 1). During these energy transformation processes, *S. cerevisiae* convert sugars into ethanol and carbon dioxide, using its enzymes, one of which is pyruvate decarboxylase. The aerobic pathway relies on oxidative phosphorylation to generate energy in the form of adenosine triphosphate, ATP, making it more energetically favourable than the anaerobic pathway where ATP production occurs via substrate-level phosphorylation. Oxidative-level phosphorylation produces more ATP (Pronk *et al.* 1996). Fermentative growth is desired for industrial purposes including wine fermentation and production of baked goods (Braconi *et al.* 2011).
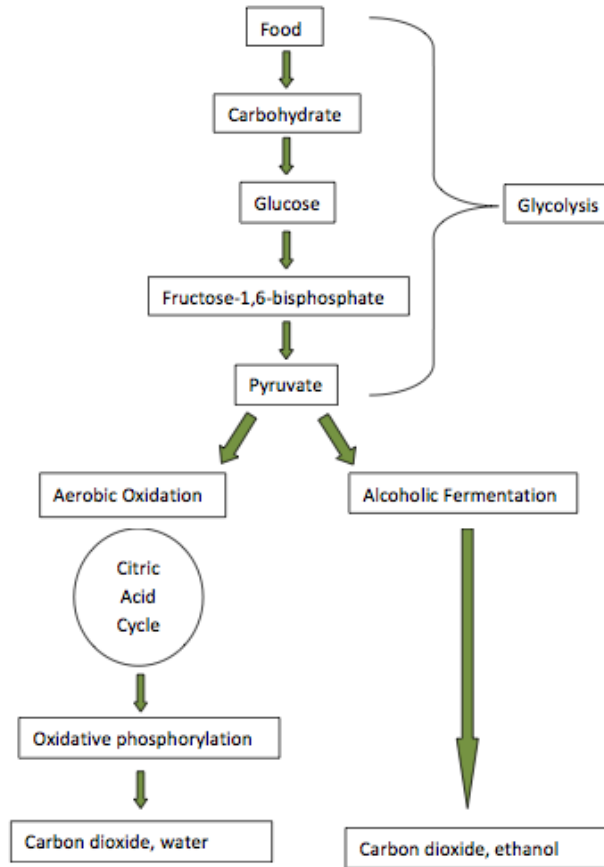
Food

Carbohydrate

Glucose — Glycolysis

Fructose-1,6-bisphosphate

Pyruvate

Aerobic Oxidation | Alcoholic Fermentation

Citric Acid Cycle

Oxidative phosphorylation

Carbon dioxide, water | Carbon dioxide, ethanol

**Figure 1.** The aerobic and anaerobic metabolic pathways involved in pyruvate breakdown in yeast (Kabolizadeh *et al.* 2012)

Yeast comes in many thoroughly researched mutant strains due to its DNA being so easily manipulated. For our research purposes, we focused on two *S. cerevisiae* strains; the wild-type strain, BY4741A, and the PDC1 mutant ,YLR044C. An important characteristic of the PDC1 mutant is reduced rate of vegetative growth in terms of budding and reduced rate of fermentative growth (Eberhardt *et al.* 1999). The PDC1 gene codes for an enzyme called pyruvate decarboxylase that catalyzes the breakdown of pyruvate into acetaldehyde and carbon dioxide (Eberhardt *et al.* 1999). Pyruvate decarboxylase is the key enzyme in the production of alcohol during fermentation. Such mutants tend to show a reduced growth rate in media that is supplemented with dextrose (Steensmati *et al.* 1996). This means that at optimal growth conditions, the wild-type strain should grow more quickly than the mutant. We decided to test the effects of varying sugar concentrations on the growth rate of our wild-type *S. cerevisiae* in comparison to the PDC1 mutant strain under near optimal temperature (29°C). Yeast cells reproduce every 90 minutes in rich medium following a logarithmic growth curve (Bergman 2001). Varying sugar concentrations were

used to see if and how large a role dextrose level plays in the growth rate of our yeast strains. Our results could indicate whether wild-type or PDC1 mutant yeast is more suitable for industrial purposes and which dextrose levels are more conducive to aerobic or anaerobic growth.

Our first null hypothesis was: the wild-type strain of *S. cerevisiae* will have the same or lower cell density than the PDC1 mutant strain of *S. cerevisiae* at each dextrose concentration at 29°C at late log phase. In contrast, our first alternate hypothesis was: the wild-type strain of *S. cerevisiae* will have a higher cell density than the PDC1 mutant strain of *S. cerevisiae* at each dextrose concentration at 29°C at late log phase (Steensmati *et al.* 1996). We created three different conditions of regular growth medium with varying dextrose concentrations. We observed different growth curves for the two different strains. One of the reasons we wanted to test the varying levels of sugar was to not only examine if increasing dextrose concentrations would increase growth rates for both wild-type and mutant, but also to determine whether the difference in growth rates would differ at higher dextrose concentrations. Since the mutant has decreased rates of vegetative and fermentative growth, our second null hypothesis was: at higher dextrose concentrations, the difference between the wild-type and mutant *S. cerevisiae* cell density at late log phase will be the same or decrease, in the sense that the difference will be smaller. Our second alternate hypothesis was: with increasing dextrose concentrations, the difference in the cell densities at late log phase of wild-type as compared to mutant will increase (Michaillet and Mayer 2013).

**Methods:**

*Design:*

The experiment was carried out over a 24-hour period. The seven sampling points were at: 0, 2, 4, 6, 8, 9 and 24 hours after the start of the experiment.

In this experiment, we determined the cell density of both wild-type and PDC1 mutant *S. cerevisiae* were determined over a period of 24 hours at three different treatment levels (25.34g/L, 46.66g/L and 89.34g/L dextrose) using absorbance measurements from a spectrophotometer. Bergman (2001) and Sherman (2002) used 20g/L dextrose as a standard yeast rich medium (YPD). 100g/L is the sugar saturation point at which *S. cerevisiae* reaches highest growth rate (Petrovska *et al.* 1999); therefore, we decided to use 53.33g/L and 106.67g/L as the latter roughly doubles the optimum level. However, due to the original medium (20g/L dextrose) diluting the 53.33g/L and 106.67g/L dextrose media, our final concentrations were 46.66g/L and 89.34g/L dextrose.. We had three replicates

per treatment level for both of wild-type and mutant, giving us a total of eighteen samples. For first null hypothesis, the control was the wild-type at each dextrose level. For the second, the controls were the wild-type and mutant at 25.34g/L.

*Procedure:*

We determined initial wild-type and mutant sample concentrations by haemocytometer count. Cells were fixed with Prefer™ (1:10 prefer-to-sample ratio); we observed the cells using a Zeiss Axio compound microscope at 10x magnification).

**Equation 1.** Cell concentration calculation from haemocytometer cell count

$$Cell\ density\ \left(\frac{cells}{mL}\right) = \frac{cell\ count}{grid\ volume} = \frac{cell\ count}{4.0 * 10^{-6}}$$

Since the populations of cells were very dense, both samples were diluted 10x using the standard growth medium in order to facilitate cell counting. After initial determination of wild-type and mutant sample concentrations, dilution to the same initial concentration of $2.5 \times 10^7$ cells/mL was achieved through the addition of standard growth medium.

For each of the three treatment levels, we further diluted the solution through addition of the appropriate amount of dextrose concentration media until we obtained 50mL of each of wild-type and mutant sample at $5.0 \times 10^6$ cells/mL concentration. We chose this concentration as it was reported to be the "start-point" of logarithmic growth (Bergman 2001). We obtained three replicates for each treatment level by pipetting 15mL of the appropriate sample into a 50mL Erlenmeyer flask. We then placed all samples in an incubator set at 29°C and took the samples out only briefly when measurements were made.

At each of the seven sampling points, we recorded absorbance measurements at an optical density of 600nm (Bergman 2001) for all eighteen samples, using 20g/L growth medium as the blank for the spectrophotometer. Additionally, we withdrew and fixed six 25.34g/L dextrose wild-type and mutant samples for haemocytometer counting at each sampling point. From this, we established a standard curve of absorbance versus cell density so we could extrapolate from absorbance measurements to obtain cell density counts. For the last two sample points (hour 9 and 24), we fixed and counted cells in all eighteen samples, as the absorbance measurements were all over 1.0, which is the point at which spectrophotometer readings become unreliable (Bergman 2001). For all samples counted in the haemocytometer , we made qualitative descriptions of cell size and appearance.

*Data Analysis:*

Firstly, we established a standard curve plotting absorbance at 600 nm (OD600) against cell density in order to elucidate the relationship between absorbance and cell density. Using this relationship, we obtained cell density counts for all seven sampling points for the eighteen samples. For each treatment level, we obtained an average cell density at each sampling time by averaging the data of the three replicates. We then plotted this against time to obtain a growth curve for each dextrose treatment level of both wild-type and mutant.

We compared wild-type and mutant cell density counts throughout the yeast population growth cycle at different dextrose concentrations by finding 95% confidence intervals (CI) at all sampling times for each treatment level. Because we started with different starting cell densities for mutant and wild-type samples, we conducted fold change analysis, which looks at the change between sampling points rather than absolute values. Fold change is calculated as a ratio of a final value to an initial value. We analyzed fold change between each sequential sampling points (e.g., hour 0-2, hour 2-4 etc.).

## Results

We plotted cell density of the six wild-type and mutant samples treated with 25.34g/L dextrose at all sampling points against their corresponding absorbance measurement at OD600 (optical density or absorbance measured at a wavelength of 600 nm) (Figure 2). We found that the relationship between cell density and absorbance fit an exponential model, with a best-fit equation of:

**Equation 2:** Best-fit equation of a standard curve of cell density vs. absorbance
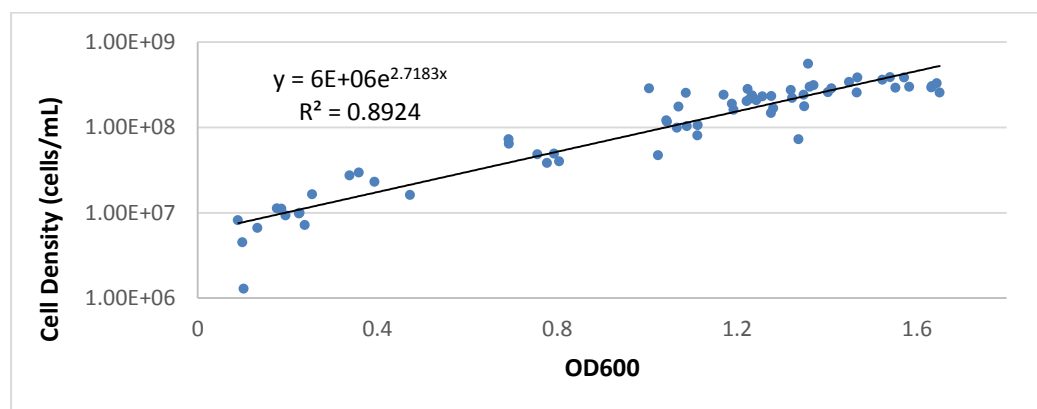
$$Cell\ density = 6 * 10^6 e^{2.7183 * absorbance}$$



**Figure 2.** The relationship between OD600 and cell density (cells/mL) of wild-type and mutant *S. cerevisiae* treated with 25.34g/L dextrose. The best fit curve has an equation of y = 6E+06e$^{2.7183x}$ with an R² value = 0.8924.

Equation 2 was used to convert measured OD600 to cell density. For example, an OD600 of 0.103 would correspond to a cell density of $6E+06e^{2.7183\times0.103} = 7.94 \times 10^6$ cells/mL. After converting all the measured OD600 to cell densities, the mean and 95% confidence intervals of the 3 replicates were calculated for each wild-type and mutant sample.

For example, at time 0, the cell densities of the 3 replicates (n=3) of the wild-type sample treated with 25.34g/L dextrose were $7.94\times10^6$, $7.87\times10^6$, and $7.66\times10^6$ cells/mL:

$$\text{Average, } \overline{X} = \frac{(7.94\times10^6 + 7.87\times10^6 + 7.66\times10^6)}{3} = 7.83 \times 10^6 \text{ cells/mL}$$

$$\text{Variance, } s^2 = \frac{\Sigma(x_i-X)^2}{n-1} = \frac{(7.94\times10^6 - 7.83\times10^6)^2 + (7.87\times10^6 - 7.83\times10^6)^2 + (7.66\times10^6 - 7.83\times10^6)^2}{2} = 2.0787 \times 10^{10}$$

$$\text{Standard Error, } \sigma = \frac{s}{\sqrt{n}} = \frac{1.44176\times10^5}{\sqrt{3}} = 8.3240 \times 10^4 \text{ cells/mL}$$

$$\text{95\% confidence interval} = X \pm 1.96\frac{\sigma}{\sqrt{n}} = 7.83 \times 10^6 \pm 1.96 \times 8.3240 \times 10^4 = 7.83 \times 10^6 \pm 1.63 \times 10^5 \text{ cells/mL}$$

The average of the cell density of all 3 replicates for each sample against time was plotted in Figure 3, with error bars indicating 95% confidence intervals.
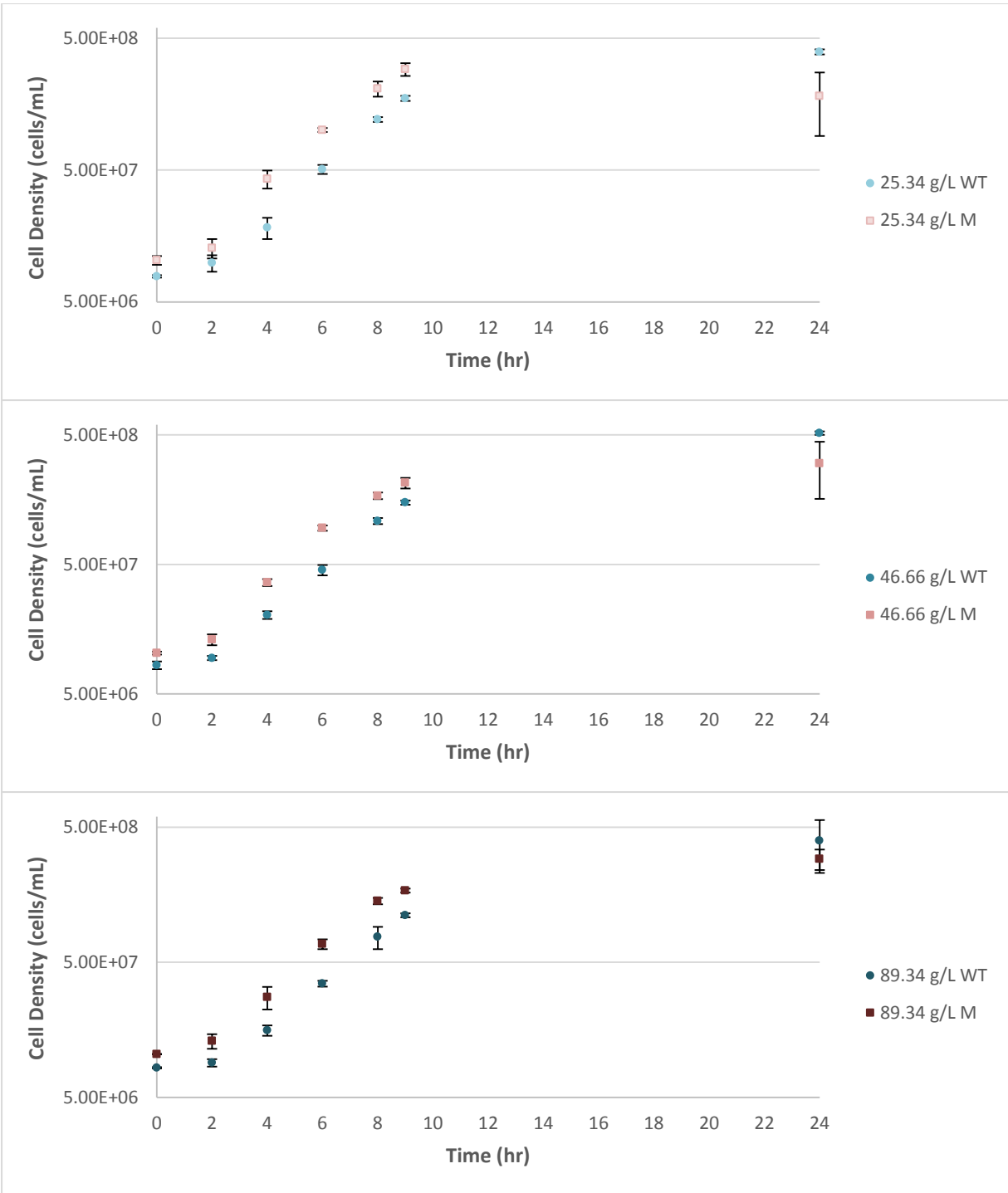
**Figure 3.** The growth curve of wild-type (WT) and mutant (M) *S. cerevisiae* at each dextrose treatment level in a 24-hour period in logarithmic scale. Error bars indicate 95% confidence intervals.

Figure 3 shows that the starting cell densities of our wild-type samples and mutant samples under each treatment level were significantly different from each other. As time went by, the cell densities of wild-type and mutant samples treated with the same concentration of dextrose were also significantly different from each other

with the exception of two points: the cell densities of the wild-type and mutant samples treated with 25.34g/L dextrose at hour 2 and the final cell densities of the wild-type and mutant treated with 89.34g/L dextrose at hour 24. Moreover, at hour 24, the cell densities of the wild-type samples treated with 25.34g/L dextrose and 46.66g/L dextrose were significantly higher than the corresponding mutant samples even though the starting cell densities of the wild-type samples were lower than the mutant samples. There was no significant difference in the cell densities of the wild-type and mutant samples treated with 89.34g/L dextrose at hour 24, but wild-type cell density was still higher. Additionally, although the cell densities of most samples were constantly increasing, the cell density of the mutant treated with 25.34g/L dextrose decreased from hour 9 to 24.
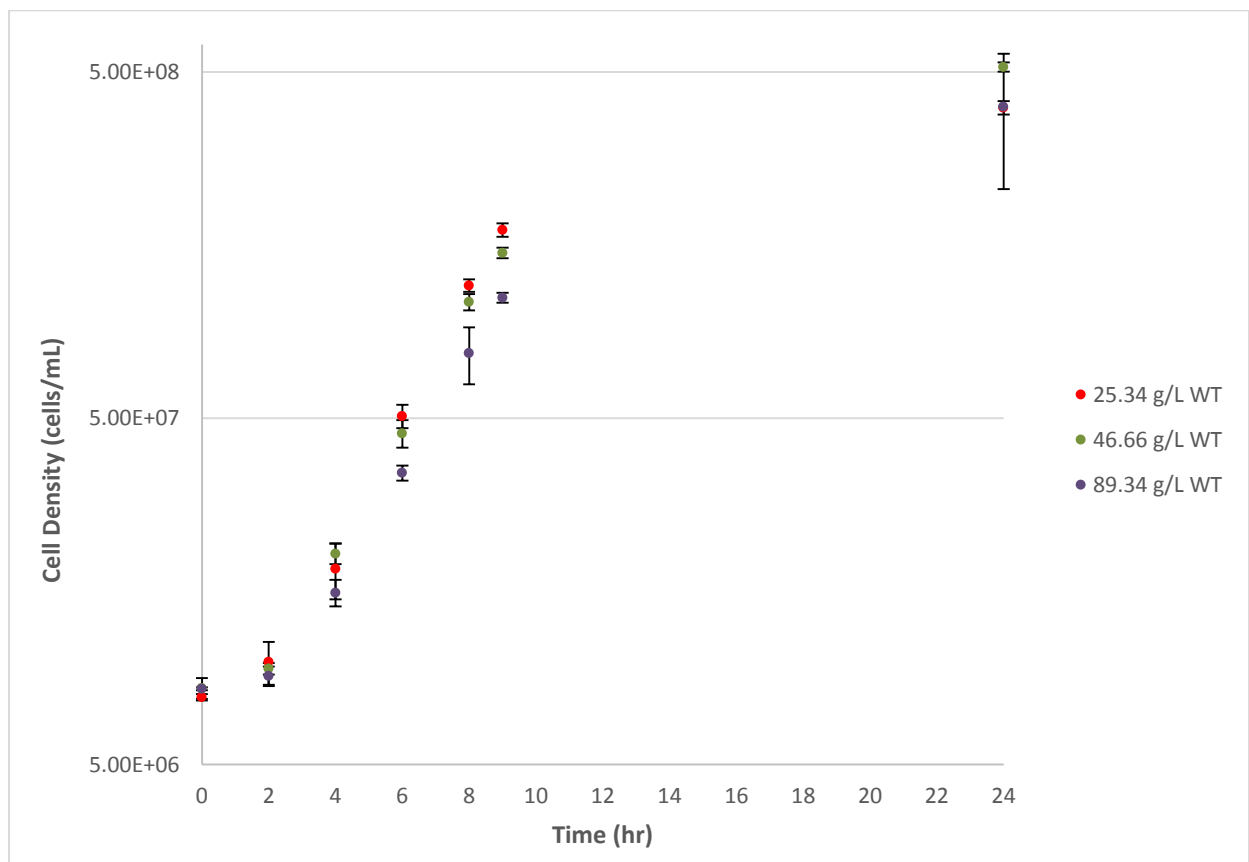


**Figure 4a.** The growth curve of wild-type (WT) *S. cerevisiae* under different concentrations of dextrose in a 24-hour period in logarithmic scale. Error bars indicate 95% confidence intervals.

Figure 4a compares the growth curve of the wild-type samples containing different concentrations of dextrose. At hour 8 and hour 9, the cell densities of all three samples were significantly different from each other as their error bars are not overlapping. The cell density of the wild-type sample with 89.34g/L dextrose was also

significantly different from the cell densities of the other two samples at hour 6 while there was no significant difference in the cell densities between those two samples with 25.34g/L dextrose and 46.66g/L dextrose. Moreover, the samples with lower concentrations of dextrose seemed to grow faster (steeper slope) than the samples with higher concentrations of dextrose at the sampling points where there were significant differences in cell density.
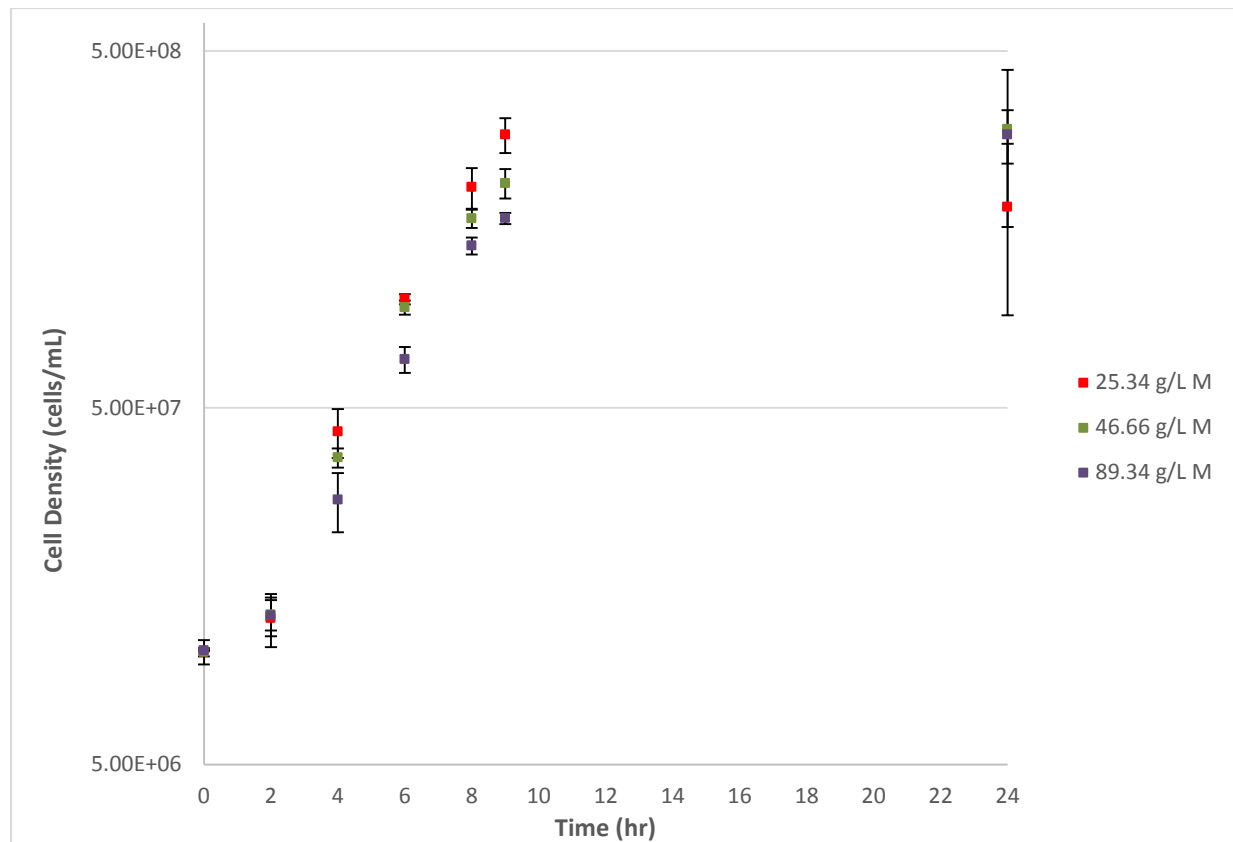


**Figure 4b.** The growth curve of mutant (M) *S. cerevisiae* under different concentrations of dextrose in a 24-hour period in logarithmic scale. Error bars indicate 95% confidence intervals.

Similar to Figure 4a, Figure 4b compares the growth curve of the mutant samples containing different concentrations of dextrose. At hour 8 and hour 9, the cell densities of all three samples were significantly different from each other as their error bars were not overlapping. The lowest dextrose treatment level had the highest cell density and the highest dextrose treatment level had the lowest cell density at both sampling points. The cell density of the mutant sample with 89.34g/L dextrose was also significantly different from the cell densities of the other two samples at hour 4 and 6 while there was no significant difference in the cell densities between those two samples with 25.34g/L dextrose and 46.66g/L dextrose. Generally, the samples with lower concentrations of dextrose seemed

to grow faster than the samples treated with higher concentrations of dextrose at the sampling points where there were significant differences in cell density.

**Table 1.** The slopes of the best-fit lines of the growth curves of wild-type and mutant *S. cerevisiae* during the early log phase (hour 2-9).

| Treatment Level (g/L dextrose) | Slope | |
|---|---|---|
| | **Wild-type** | **Mutant** |
| **25.34** | 0.4241 | 0.4377 |
| **46.66** | 0.4008 | 0.3997 |
| **89.34** | 0.3672 | 0.3795 |

Table 1 compares the slopes of the growth curve of each wild-type and mutant pair with the same concentration of dextrose. The slopes for each pair are quite similar and thus indicate there was not much of a difference in the growth rate of the wild-type and mutant *S. cerevisiae* in each treatment level during the early log phase (hour 2-9). In addition, the decreasing slope value of both wild-type and mutant *S. cerevisiae* with increasing concentration of dextrose also indicates that the samples treated with lower concentrations of dextrose grew faster than the samples treated with Changes in OD600 (Table 2) and changes in fold-change of cell density (Table 3) were also calculated to examine the magnitude of changes between adjacent sampling points as we started with different cell densities between the wild-type and mutant samples.

The change in OD600 for a replicate of one sample in a particular time period can be calculated as: OD600 at $t_{a+1}$ - OD600 at $t_a$. Then the average of the change in of all the three replicates was used as the change of the sample. For example, the change in for the wild-type sample treated with 25.34g/L dextrose from the starting hour 0 to hour 2 is calculated as:

Change in OD600 of replicate #1 = OD600 of replicate #1 at hour 2 – OD600 of replicate #1 at hour 0

$$= 0.133 - 0.103$$

$$= 0.030$$

Change in OD600 of replicate #2 = 0.126

Change in OD600 of replicate #3 = 0.097

Change in OD600 of the wild-type sample treated with 25.34g/L dextrose = Average of the 3 replicates

$$= (0.030 + 0.126 + 0.097)/3$$

$$= 0.084$$

**Table 2.** Change in OD600 of wild-type and mutant *S. cerevisiae* cells between adjacent sampling points.

| | WILD-TYPE | | | MUTANT | | |
|---|---|---|---|---|---|---|
| | 25.34g/L dextrose | 46.66g/L dextrose | 89.34g/L dextrose | 25.34g/L dextrose | 46.66g/L dextrose | 89.34g/L dextrose |
| **0hr - 2hr** | 0.084 | 0.048 | 0.031 | 0.077 | 0.088 | 0.081 |
| **2hr - 4hr** | 0.227 | 0.281 | 0.204 | 0.444 | 0.373 | 0.273 |
| **4hr - 6hr** | 0.376 | 0.294 | 0.293 | 0.317 | 0.357 | 0.336 |
| **6hr - 8hr** | 0.320 | 0.322 | 0.290 | 0.265 | 0.211 | 0.270 |
| **8hr - 9hr** | 0.136 | 0.120 | 0.139 | 0.125 | 0.083 | 0.065 |
| **9hr - 24hr** | 0.299 | 0.455 | 0.447 | -0.201 | 0.110 | 0.196 |

The fold-change of cell density for a replicate of one sample in a particular time period can be calculated as:

Cell Density at $t_{a+1}$ / Cell Density at $t_a$. Then, the average of the fold-changes of all the three replicates was used as the fold-change of the sample. For example, the fold-change of cell density for the wild-type sample treated with 25.34g/L dextrose from the starting hour 0 to hour 2 is calculated as:

Fold change of replicate #1 = Cell Density of replicate #1 at hour 2 / Cell Density of replicated #1 at hour 0

$$= \frac{8.61 \times 10^6}{7.94 \times 10^6}$$

$$= 1.085$$

Fold change of replicate #2 = 1.408

Fold change of replicate #3 = 1.302

Fold change of the wild-type sample treated with 25.34g/L dextrose = Average of the 3 replicates

$$= \frac{(1.085 + 1.408 + 1.302)}{3}$$

$$= 1.265$$

**Table 3.** Fold-Change of the cell density of wild-type and mutant *S. cerevisiae* between adjacent sampling points.

| | WILD-TYPE | | | MUTANT | | |
|---|---|---|---|---|---|---|
| | 25.34g/L dextrose | 46.66g/L dextrose | 89.34g/L dextrose | 25.34g/L dextrose | 46.66g/L dextrose | 89.34g/L dextrose |
| **0hr - 2hr** | 1.265 | 1.141 | 1.088 | 1.237 | 1.277 | 1.252 |
| **2hr - 4hr** | 1.881 | 2.148 | 1.743 | 3.341 | 2.775 | 2.138 |
| **4hr - 6hr** | 2.798 | 2.225 | 2.221 | 2.376 | 2.641 | 2.545 |
| **6hr - 8hr** | 2.388 | 2.400 | 2.210 | 2.060 | 1.775 | 2.087 |
| **8hr - 9hr** | 1.447 | 1.387 | 1.471 | 1.405 | 1.255 | 1.197 |
| **9hr - 24hr** | 2.258 | 3.446 | 3.586 | 0.647 | 1.398 | 1.722 |

As we can see from both Table 2 and 3, the changes of OD600 and the fold-changes of cell density were greater in the wild-type samples than in the mutant samples beginning from hour 6, indicating that the wild-type samples grew faster than the mutant samples from that time on.

**Table 4.** The difference in cell density between wild-type and mutant *Saccharomyces cerevisiae* samples under different concentrations of dextrose at hour 24.

| Treatment Level (g/L of dextrose) | Cell Density (cells/mL) |
|---|---|
| 25.34 | $2.11 \times 10^8$ |
| 46.66 | $2.15 \times 10^8$ |
| 89.34 | $1.05 \times 10^8$ |

Table 4 shows that at hour 24, the difference in cell density between wild-type and mutant samples treated with 25.34g/L dextrose and 46.66g/L dextrose were similar to each other and the difference at 89.34g/L dextrose was smaller.

**Discussion**

Following analysis of our results, we were able to reject our first null hypothesis and support our first alternate hypothesis. We found significantly higher cell density for wild-type as compared to mutant at the dextrose levels 25.34g/L and 46.66g/L, and a non-significantly higher cell density for wild-type as compared to mutant at the dextrose level 89.34g/L. We failed to reject our second null hypothesis. We find that at the highest dextrose level (89.34g/L), the difference in cell density between wild-type and mutant samples were the smallest.

We observed that at all 3 dextrose levels at hour 24, wild-type samples had higher cell density than mutant samples; for the two levels 25.34g/L and 46.66g/L, cell density was significantly higher (Fig 3). This may be explained by the fact that the YLR044C mutant strain has abnormal vacuole morphology where vacuoles do not fragment easily as compared to the wild-type strain of *S. cerevisiae* (Michaillat and Mayer 2013). Vacuoles are important for the cells because they function in pH and ion homeostasis, osmoregulation and the storage of amino acids and ions (Michaillat and Mayer 2013). However, our mutant lacks numerous fragmentation factors that facilitate membrane curvature change and promote vacuole fragmentation (Michaillat and Mayer 2013). This decreases vacuole functionality in the mutant, as vacuolar fragmentation is needed to increase the surface area to volume ratio in order to accommodate the intake of nutrients. When nutrients are limited, vacuoles coalesce into a single organelle and expand their volume. During the growth phase in the wild-type, *S. cerevisiae* typically have two to five vacuoles, and as steady-state growth phase is reached, vacuoles generally begin to coalesce into one big organelle. In the mutant, the vacuoles begin to coalesce early on in the growth phase in comparison to the wild-type,

which may affect its population growth (Michaillat and Mayer 2013). These observations are consistent with our results as we see a higher population growth in the form of higher cell density for wild-type as compared to mutant by late log phase (hour 24).

According to Flikweert *et al.* (1999)*,* if *S. cerevisiae* is reported to have a mutation in PDC then they are unable to sustain prolonged continuous growth on dextrose media. This may be because prolonged growth means that the oxygen in the medium will be consumed, so *S. cerevisiae* will be forced to resort to anaerobic respiration. The decreased fermentative activity in the mutant may affect its energy production, which affects reproduction and death rates (Eberhardt *et al.* 1999).  This may explain our observation that the cell density of the 25.34g/L treatment level of mutant sample decreased from hour 9 to hour 24 (Figure 4b). Although 24 hours may not be considered a "prolonged" time period for growth, we did not place the cultures in a shaker so the oxygen in the immediate vicinity of the yeast may have been used up, forcing the yeast to undergo anaerobic respiration.

From hour 2-9 (early log and log phase), wild-type and mutant *S. cerevisiae* exhibited very similar growth rates at each dextrose treatment level when growth rate is calculated by slope of the growth curve (Table 1). This similar growth rate early on may be accounted by the fact that both wild-type and mutant samples are mainly utilizing aerobic respiration at this point as oxygen is still abundant in the medium; the YLR044C mutant strain has no defect affecting aerobic respiration, only anaerobic respiration so growth rate should be similar.

In contrast to our earlier observation of similar growth rates between wild-type and mutant at early log and log phase (hour 2-9), we observed that from hour 6 (log and late log phase), growth rate as quantified by changes in OD600 and fold-changes of cell density were greater in the wild-type samples than the mutant samples (Table 2 and 3). As stated earlier, this may be due to the oxygen consumption over time. By log and late log phase, much of the oxygen near the yeast may have been consumed, forcing anaerobic respiration to occur. The YLR044C mutant strain of *S. cerevisiae* has a well-documented disadvantage in anaerobic respiration, leading to lower energy production, which affects reproduction (Eberhardt *et al.* 1999). The observation that mutant growth rate drops off as time passes while wild-type growth rate does not is well explained by the fact that the mutant samples have decreased fermentation ability (Eberhardt *et al.* 1999).

Our observations at hour 8 and 9 for both our wild-type and mutant samples reveal that the lowest dextrose treatment levels have the highest cell density, and vice versa, with significantly different cell densities among all treatment levels (Figure 4a and 4b). In addition, we observed a decreasing growth rate for both wild-type and mutant samples with increasing concentration of dextrose from hour 2-9. This may be explained by the Crabtree effect. This effect is well documented in the literature and shows that under high glucose conditions, *S. cerevisiae* will produce ethanol through fermentation even in aerobic conditions (Beck and Meyenburg 1968, Pronk *et al.* 1996, Steensmati *et al*. 1996, Lei *et al.* 2001). So at the higher dextrose levels of 46.66g/L and 89.34g/L, the Crabtree effect may be causing fermentation to occur. As the process of fermentation yields much less ATP than the process of aerobic respiration, less energy is produced, which may lead to a lower growth and reproduction rate (Eberhardt *et al.* 1999). This may explain why we observed lower growth rate (Table 1) and lower cell density (Table 4) at hour 24 for both wild-type and mutant samples at higher dextrose concentrations.

From hour 0 to 9, the 25.34g/L dextrose concentration level had the highest cell densities for both wild-type and mutant samples; however by hour 24, wild-type and mutant at 25.34g/L had the lowest cell density. This drastic change may be explained by the fact that at hour 0 to 9, the 46.66g/L and 89.34g/L dextrose concentrations had repressed aerobic respiration due to excess dextrose as discussed above. By hour 24, this may no longer be the case since the dextrose had continuously been consumed, leaving less than the required amount of dextrose for the 20g/L cultures to sustain its high growth rate. In fact, the dextrose may have been almost entirely consumed, leading to significantly decreased cell densities for the 25.34g/L dextrose level for both wild-type and mutant from hour 9 to hour 24. In contrast, the dextrose levels in the 46.66g/L and 89.34g/L level may have been brought down so that repression of aerobic respiration is no longer a major factor, leading to a sustainable steady-state growth rate. This matches our observation that the cell densities for the 46.66g/L and 89.34g/L dextrose levels remain steady at hour 24.

At hour 24, the difference in cell density between wild-type and mutant samples treated with 25.34g/L dextrose and 46.66g/L dextrose were very similar to each other and the difference at 89.34g/L dextrose was smaller (Table 4). This smaller difference for the 89.34g/L dextrose concentration level may due to the fact that for wild-type sample at hour 24 for 89.34g/L level, we had an outlier for replicate 2 where the absorbance measurement dropped significantly from hour 9 to 24. This meant that the corresponding calculated cell density dropped

significantly, making the average cell density for wild-type lower and closer to the calculated average mutant cell density.

For our experiment, there were several possible sources of error. Firstly, we dropped our pipette tip into our mutant sample, which may have been a source of contamination, ultimately affecting our mutant cell count as the outside of the pipette was also in contact with the original solution. Secondly, there could have been variability and human error when doing the cell count from the haemocytometer reading. We tried to minimize inter-rater variability by all doing three haemocytometer counts on the same squares initially and by agreeing on standards for counting. Counting is a vital data collection procedure for our experiment, because the cell density count highly depends on the initial cell count of each sample due to the dilution factors. For instance, a change in cell count of ten in a haemocytometer reading could amount to a difference of 110 cells when taking into account an 11x dilution. This error, along with the pipette contamination error above may explain why the starting cell densities for our wild-type and mutant samples were very different although we initially counted on the haemocytometer then diluted to the same concentration. The pipette contamination may have increased the cell density in the mutant sample as cells may have stuck on the outside of the pipette when it was originally used to withdraw solution. Variability in counting may have drastically affected either our initial count before making the sample, skewing our dilutions, or it may have affected our calculation for our starting cell density. Lastly, although our yeast cultures were well maintained in a 29°C incubator, while we withdrew samples for the haemocytometer and absorbance readings, the cultures were exposed to lower temperatures which may have affected the cell growth of our wild-type and mutant strains.

**Conclusion**

We rejected our first null hypothesis stating that under the near-optimal temperature condition (29°C), the wild-type strain of *S. cerevisiae* will have the same or a lower cell density at late log phase (hour 24) than the PDC1 mutant strain of *S. cerevisiae* at each dextrose concentration and support our alternate hypothesis. We observed a higher cell density for wild-type than mutant at late log phase for each dextrose level. We failed to reject our second null hypothesis which states that at higher dextrose levels, the difference in cell density at hour 24 between wild-type and mutant will be the same or lower than the difference in cell density at hour 24 between wild-type and mutant at lower dextrose level. We observed no significant differences when comparing cell density differences between wild-

type and mutant at late log phase between the 25.34g/L and 46.66g/L dextrose levels, and a smaller cell density

difference at the 89.34g/L level than 25.34g/L and 46.66g/L.


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**Literature Cited**

Beck, C., and Meyenburg H.K. 1968. Enzyme Pattern and Aerobic growth of *Saccharomyces cerevisae* under
   various degrees of glucose limitation. Journal of Bacteriology, **96** (2): 479-486.

Bergman, L.W. 2001. Growth and maintenance of yeast. pp 9-14. In: Two-Hybrid Systems: Methods and Protocols,
   Humana Press Inc., Totowa, NJ.

Braconi, D., Amato, L., Bernardini, G., Arena, S., Orlandini, M., Scaloni, A., and Santuccia, A. 2011. Surfome
   analysis of a wild-type wine *Saccharomyces cerevisiae* strain. Food Microbiology, **28** (6): 1220-1230.

Eberhardt, I., Cederberg, H., Li, H., König, S., Jordan, F., and Hohmann S. 1999. Autoregulation of yeast pyruvate
   decarboxylase gene expression requires the enzyme but not its catalytic activity. European Journal of
   Biochemistry, **262** (1): 191-201.

Flikweert, M.T., Swaaf, M., Dijken, J.P., and Pronk, J.T. 1999. Growth requirements of pyruvate-decarboxylase-
   negative *Saccharomyces cerevisiae*. FEMS Microbiology Letters, **174**(1): 73-79.

Kabolizadeh, M., Parhar, B. K., Pourrahmat, M., and Qaiser, Y. 2012. Carbon dioxide production in wild type and
   PDC1 mutant *Saccharomyces cerevisiae* undergoing the Crabtree effect. The Expedition, **2**: 24.

Lei, F., Rotboll, M. and Jorgensen, S.B. 2001. A biochemically structured model for *Saccharomyces cerevisiae*.
   Journal of Biotechnology, **88**: 205-221.

Michaillat, L., and Mayer, A. 2013. Identification of genes affecting vacuole membrane fragmentation in
   *Saccharomyces cerevisiae*. PLoS ONE, **8** (2): e54160.

Petrovska, B., Winkelhausen, E. and Kuzmanova, S. 1999. Glycerol production by yeasts under osmotic and sulfite
   stress. Canadian Journal of Microbiology, **45**: 695-699.

Pronk, J. T., Steensmays, H. Y., and Van Dijken, J. P. 1996. Pyruvate Metabolism in *Saccharomyces cerevisiae*.
   Yeast, **12**: 1607-1633.

Sherman, F. 2002. Getting Started with Yeast. Methods in Enzymology, **350**: 3-41.

Steensmati, H., Van Dijkent, J.P., and Pronkt, J.T. 1996. Pyruvate decarboxylase: an indispensable enzyme for growth of *Saccharomyces cerevisiae* on glucose. Yeast, **12**: 247-257.