The effect of temperature on the production of carbon dioxide over time in *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae is a strain of yeast that can grow under both aerobic and anaerobic conditions, and it is widely used in the food and bio-fuel industry. The purpose of our experiment was to investigate the effect of three different temperatures (31°C, 35°C, and 39°C) on the volume of CO_2 gas produced by wild-type *S. cerevisiae* under aerobic conditions. The volume of CO_2 produced by four replicates and a control of *S. cerevisiae* (3.8×10^7 cells/mL) at each temperature was recorded in five-minute intervals for 80 minutes. Cell concentration of each replicate was measured at the end, and the volume of CO_2 (mL) produced per cell was calculated. The results gave final volumes of $9.69 \times 10^{-9} \pm 1.63 \times 10^{-9}$ (mL CO_2/cell) for 31°C, $5.78 \times 10^{-9} \pm 2.66 \times 10^{-10}$ (mL CO_2/cell) for 35°C, and $4.79 \times 10^{-9} \pm 5.2 \times 10^{-10}$ (mL CO_2/cell) for 39°C. Our two-way ANOVA generated *p*-values of 0.00 for the effect of temperature, 0.00 for the effect of time, and 4.13×10^{-6} for the combined effect of temperature and time on the rate of CO_2 production. The data indicates that the optimal rate of CO_2 production was at 31°C at all times. The effect of temperature may be explained by enzyme kinetics, while the effect of time may be explained by growth phase curves. Not only are both factors significant on their own but increasing temperature also has an effect on CO_2 production rate at different times.

Introduction:

Yeast describes a wide range of unicellular fungi (Moyad 2008). *Saccharomyces cerevisiae* is one of the most commonly used and researched species of yeast and is widely known as baker's or brewer's yeast. According to Herskowitz (1988), the reproduction of *S. cerevisiae* is mostly asexual, via budding, and their cell cycle is about 1.5 hours. Notable characteristics of *S. cerevisiae* include their ability to grow and undergo metabolic processes in both aerobic and anaerobic conditions (Ter Linde *et al*. 1999). Under aerobic conditions, *S. cerevisiae* performs cellular respiration through oxidative phosphorylation of pyruvate, producing CO_2 (Figure 1). The metabolic processes of *S. cerevisiae* are catalyzed by many enzymes (Torija *et al*. 2003). Eisenthal *et al*. (2006) concluded that increasing the temperature would increase the activity of

enzymes and the rate of the reactions they catalyze. They showed that most enzymes have an optimum temperature at which they perform the best. The objective of our experiment was to find the temperature and time at which *S. cerevisiae* has the highest rate of CO_2 production under aerobic conditions. Based on results by Torija *et al*. (2003), Phisalaphong *et al*. (2006), Liu and Shen (2008), and Slaa *et al*. (2009), we predict that the optimum temperature for CO_2 production of *S. cerevisiae* would be approximately 35°C. Liu and Shen (2008) and Slaa *et al*. (2009) predict that the optimal temperature of fermentation enzymes is 37°C. Although these experiments mostly focus on the fermentation of *S. cerevisiae*, we speculated that the same theory would likely apply to enzymes involved in cellular respiration, the metabolic process we are investigating. Our first null hypothesis (H_{o1}) is that increasing temperature has no effect on the CO_2 production rate of *S. cerevisiae* under aerobic conditions with the alternate hypothesis (H_{a1}) stating that increasing temperature has an effect on the CO_2 production rate of *S. cerevisiae* under aerobic conditions. The second hypothesis (H_{o2}) states that increasing time has no effect on the CO_2 production rate of *S. cerevisiae* under aerobic conditions, with the alternate hypothesis (H_{a2}) stating that increasing time has an effect on the CO_2 production rate of *S. cerevisiae* under aerobic conditions. Finally, our third null hypothesis (H_{o3}) states that increasing temperature has no effect on the CO_2 production rate of *S. cerevisiae* at different times under aerobic conditions. The third alternate hypothesis (H_{a3}) states that increasing temperature has an effect on CO_2 production rate of *S. cerevisiae* at different times under aerobic conditions.

Finding the optimal conditions for the metabolic processes of *S. cerevisiae* will be beneficial to society. For example, knowing the ideal temperature of fermentation for *S. cerevisiae* can help with the mass production of products in the alcohol industry in terms of both wine and beer production along with the fuel industry (Phisalaphong 2006).
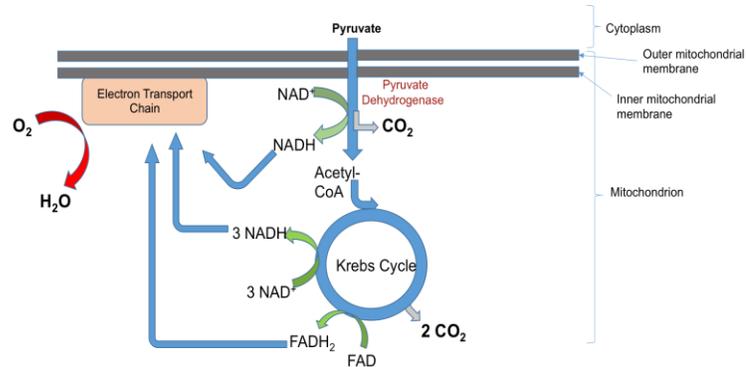
Figure 1. In the presence of oxygen, pyruvate dehydrogenase converts pyruvate to Acetyl Co-A which enters the Krebs cycle. Electrons are then transferred to various electron carrier molecules with oxygen as the final electron acceptor. CO_2 is the byproduct of these reactions.

Methods

We prepared approximately 3.6 liters of *Saccharomyces cerevisiae* wild-type cells in three large flasks at room temperature (approximately 25°C), each covered with aluminum foil to prevent contamination. The medium for growth of *S. cerevisiae* was yeast-extract peptone-dextrose (YPD). In this experiment, we tested and compared the rate of CO_2 production of wild-type *S. cerevisiae* at 31°C, 35°C, and 39°C. We prepared four replicates and a negative control (only the YPD medium, no *S. cerevisiae* cells) for each temperature. The initial cell concentration used for our experiment was 3.8×10^7 cells/mL.

After the culture of *S. cerevisiae* was prepared, we loaded the respirometer tubes for the experimental procedure. The respirometer setup consisted of a 15 mL test tube containing an inverted 4 mL test tube. We added markings on the 4 mL tube in 0.5 mL increments. We loaded the *S. cerevisiae* solution into each respirometer such that the small test tube was completely filled with sample. The control respirometers were filled with YPD growth medium containing no cell culture. We then placed four replicates and a control into each water bath at 31°C, 35°C, and 39°C.

We observed the production of O_2 in the respirometers taking measurements every 5 minutes for 80 minutes. To measure CO_2 production, we read the amount of CO_2 gas formed on top of the *S. cerevisiae* solution with reference to the markings on the test tube (Figure 2). We stopped taking measurements when the small test tube was completely filled with gas or 80 minutes expired. We took a sample from each finished respirometer and added fixative to a sample of cell culture in order to count the final number of cells in each replicate under a microscope.
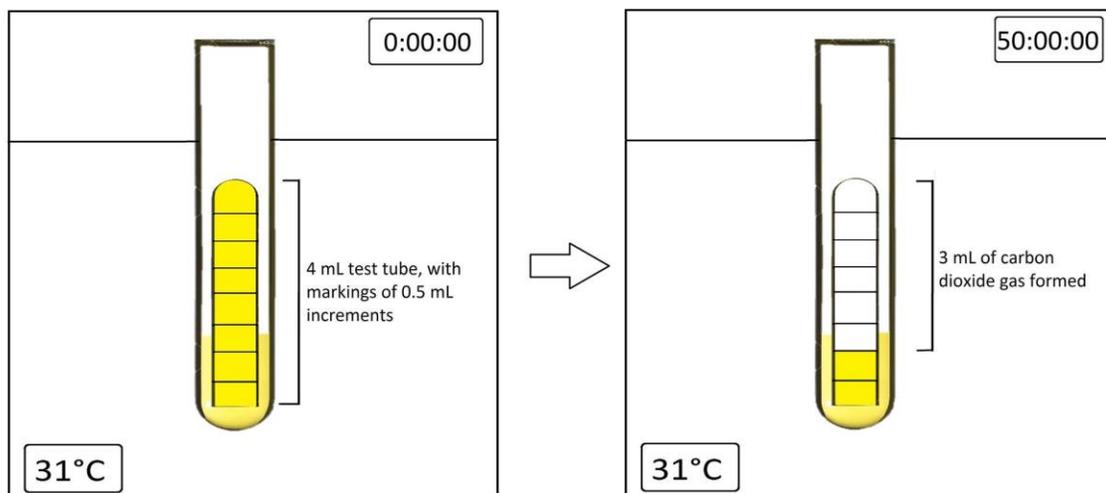
Figure 2. The respirometer set up. The 4 mL test tube is marked with 0.5 mL increments. At the initial time (0 minutes) the test tube is filled with *S. cerevisiae* cells. At 50 minutes, 3 mL of CO_2 gas formed on top of the *S. cerevisiae* cells.

Since acidity affects the rate of cellular respiration and CO_2 production (Petrucci *et al.* 2013), we measured the final pH of each sample to ensure conditions were consistent. The controls at each temperature were constantly observed to ensure that there was no gas production in the absence of *S. cerevisiae* cells.

For each replicate, we divided the final volume of CO_2 produced by the corresponding final cell density in each respirometer to obtain the average CO_2 production per cell. We plotted this data over time, and obtained three temperature curves for ml of CO_2 production per cell over time (Figure 3). Lastly, we performed a two-way ANOVA test, where the independent variables were temperature and time and the dependant variable was the amount of CO_2 gas produced per cell.

Results

A two-way ANOVA was performed to determine whether there was a significant difference in CO_2 production over time and at varying temperatures. In testing the effects of temperature on the rate of CO_2 production a *p*-value of 0.00 was obtained. In testing whether time had an effect on the rate of CO_2 production a *p*-value of 0.00 was obtained. In testing the combined effect of time and temperature we obtained a *p*-value of 4.13 x 10^{-6}.

After 55 minutes we did not have a consistent number of replicates under experimental conditions because at this point the first respirometer reached 4 mL of gas. Due to the change in the number of replicates we decided to perform our statistical analysis with the experiment ending at 55 minutes rather than 80 minutes for all replicates.

Certain trends can be seen in the graphed data; firstly, *S. cerevisiae* cells at 31^oC produced a greater level of CO_2 throughout the experiment than cells under higher temperature conditions. Larger 95% confidence intervals are seen around the points at 31^oC than at 35^oC and 39^oC, graphically shown with error bars (Figure 3). The results for 35^oC and 39^oC are very close values for most of the experiment; the largest difference is at 20 and 25 minutes. Figure 3 shows that between 20 and 40 minutes CO_2 production per cell increased for all temperature conditions. All temperature treatments show a lag in CO_2 production in the first 20 minutes and a plateau in CO_2 production after 40 minutes.
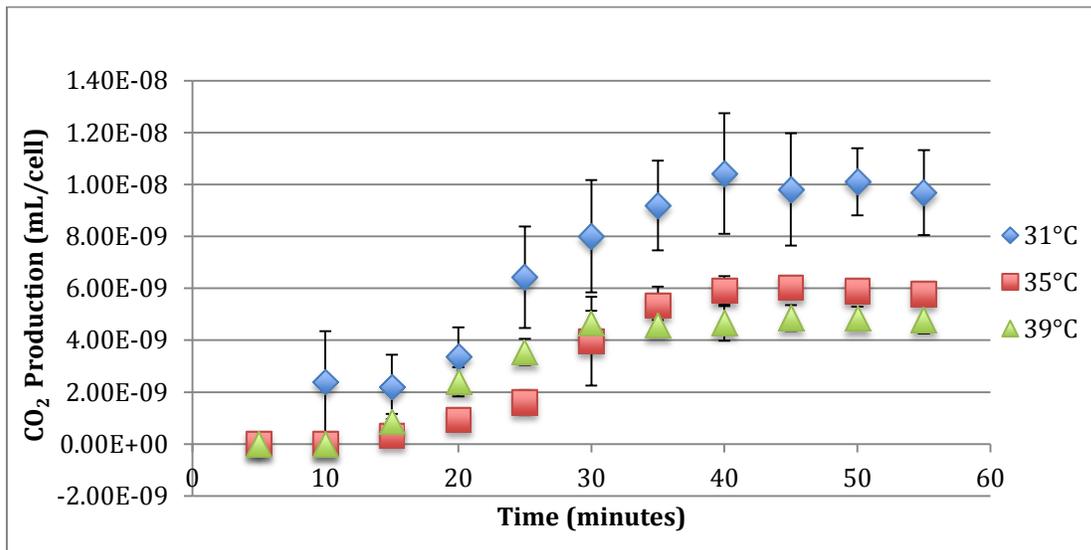
Figure 3. Average production of CO_2 per cell of *Saccharomyces cerevisiae* over 55 minutes at three different temperatures (31^oC, 35^oC and 39^oC) with an n = 4 and 95% confidence intervals.

Discussion

Considering that the *p*-value for the first hypothesis is less than 0.05, we reject the corresponding null hypothesis (Ho_1) and support the alternate hypothesis (Ha_1). Temperature has a statistically significant effect on CO_2 production. Evaluating temperature independently, it is evident that the CO_2 production was overall the highest at $31°C$, and was lower for $35°C$ and $39°C$ (Figure 3). Enzyme kinetics could provide a

possible explanation for this. Under the aerobic conditions of this experiment, cellular respiration is the main reaction responsible for producing CO_2 (Barnett 2003, Otterstedt *et al*. 2004, Uchino *et al.* 2004). *S. cerevisiae* cells contain many enzymes that accelerate this reaction by firmly binding the substrates into the enzyme's active sites forming enzyme-substrate complexes (Segel 1975). The acceleration of the reaction results in higher CO_2 production. At 31°C, potentially the optimal temperature, this complex may have been most effectively formed with the enzyme at its highest level of activity (Segel 1975). This could explain the greater amount of CO_2 production exhibited at 31°C.

A decrease in CO_2 production could be explained by higher temperatures, which can cause enzymes to denature and fill less of the substrate's active sites (Scopes 2002). Even if only one of the enzymes involved in *S. cerevisiae* cellular respiration denatures at 35°C and 39°C, this could explain the observed decrease in CO_2 production for those temperature treatments. While temperatures of 37°C-40°C have been shown to denature certain proteins, (Barton 1979, Nielsen 2001, Mensonides *et al*. 2014) it is important to note that many enzymes do not denature at temperatures less than 40°C and therefore this might not be the best explanation for the observed decrease in this experiment (Wintrode and Arnold 2000).

The macromolecular rate theory may explain the effect of temperature on aerobic metabolism more effectively. This theory explains that when the heat capacity of an enzyme is large, the free activation that must be overcome in order to catalyze the reaction is temperature dependent (LiCata and Liu 2011). Mathematically, the thermal performance curve for the enzyme shows that it may become less active as temperature increases regardless of whether or not it denatures (Schulte 2015). Therefore, as different

proteins have different heat capacities and corresponding curves, this could explain the lower CO_2 production rate observed at 35°C and 39°C (Schulte 2015).

Considering that the *p*-value for the second hypothesis is less than 0.05, we reject the corresponding null hypothesis (Ho_2) and support the alternate hypothesis (Ha_2). Evaluating only time, CO_2 production lagged in the first 20 minutes then underwent an acceleration phase from roughly 20 to 40 minutes before plateauing at approximately 40 minutes (Figure 3).

Growth curves could explain the pattern of CO_2 production over time as more cells lead to greater amounts of CO_2 being produced (Richards 1928, Zwietering *et al*. 1990, Bennett *et al*. 1999, Asaduzzaman 2007, Held 2010). When *S. cerevisiae* is introduced to growth medium they initially experience a lag phase where the cell concentration stays relatively constant (Richards 1928, Zwietering *et al*. 1990, Bennett *et al*. 1999, Asaduzzaman 2007, Held 2010). Following this, cells rapidly divide and enter into an exponential phase of growth (Richards 1928, Zwietering *et al*. 1990, Bennett *et al*. 1999, Asaduzzaman 2007, Held 2010). Eventually, due to high cell density, rapid growth no longer occurs, a stationary phase is reached and metabolic processes (including cellular respiration) slow down (Asaduzzaman 2007 and Held 2010). High cell densities around 40 minutes could have resulted in a nutrient limitation, a decline of one of the reactants required for cellular respiration, such as dextrose in the YPD medium. As a result, the rate of a reaction decreases due to the decline in concentration of the reactants (Ramsden 2000, Collison and McDonald 2001, Schaschke 2014), which may explain the plateau in CO_2 production rate observed after 40 minutes in our experiment.

Finally, considering that the p-value for the third hypothesis is less than 0.05, we reject the corresponding null hypothesis (Ho_3) and support the alternate hypothesis (Ha_3). Not only are both factors significant on their own, but there is also a statistically significant interaction between the two, as increasing temperature has an effect on CO_2 production at different times. One limitation of the two-way ANOVA is that we do not know which temperature treatments are responsible for the statistically significant interaction. The interaction can be observed in Figure 3, exhibited by a shape similar to a growth curve dependent on time, however this trend varies with different temperatures. The shape of the curve at 39°C appears to deviate considerably from that at 35°C between 20 and 30 minutes. The higher rate observed from 20 to 30 minutes at 39°C compared to 35°C could be explained knowing that reaction rates generally increase with temperature (Scopes 2002). At higher temperatures particles collide with one another more frequently and thus it takes less time to produce more CO_2 (Scopes 2002).

It is important to note that the pH was found to be 5.5 in all replicates before and after the respiration process and so can be ruled out as a factor potentially affecting CO_2 production per cell (Heard and Fleet 1988, Slaa 2009, Eed 2013). Although these results are not consistent with our prediction that CO_2 production of *S. cerevisiae* would be optimal at a temperature of 35°C, maximal rates of fermentation, another metabolic process, have been observed at temperatures of both 30°C and 35°C (Nagodawithana *et al.* 1974, Phisalaphong 2006, Torija *et al.* 2003, Slaa 2009). The optimal temperature for cellular respiration enzymes could be slightly different (closer to 30°C) than that of enzymes involved in fermentation.

A limitation of this study was the method of measuring CO_2 production. We used a micropipette to add water in the 4 mL test tube as a guideline to mark the 0.5 mL increments. As this was done by hand, it was difficult to draw the lines completely level, and also too time-consuming to draw lines at 0.1 mL increments. Therefore, the accuracy of measuring CO_2 production was only correct to 0.5 mL. We can avoid this problem in future experiments by using test tubes that are already calibrated, or using more sensitive respirometers such as those with U-tubes and calibrated scales (Scholander *et al.*1951). We assumed that the temperature in each water-bath was kept constant at its corresponding temperature throughout the entire respiration process. However, we had to open the lid of each water-bath almost every minute to check the amount of CO_2 produced for each replicate. The lid may not have been closed firmly every time, or remained removed for varying lengths of time with each measurement. The temperature in each water-bath may have fluctuated throughout the experiment, potentially causing the inconsistencies in the results.

An important source of uncertainty was the cell number counts. As *S. cerevisiae* are only 5-10 μm in diameter (Feldmann 2010), they easily overlap in concentrated samples. When viewed under the microscope, we tried to minimize variation by only counting the cells touching two of the four sides of the grid on the haemocytometer slide. In some cases, the *S. cerevisiae* cells were clumped together making counting more difficult on an individual grid-square, and thus increasing counting uncertainties.

The final source of uncertainty was the large volume of *S. cerevisiae* cells used in our study. We centrifuged the initial *S. cerevisiae* culture in batches and repeated the process eight times to concentrate the cells. As this process took two hours, cells might

have been more concentrated at the bottom of the container. We swirled it to mix the *S. cerevisiae* solution before transferring to each respirometer. However, with large volume of solution, the cell density in each respirometer could still vary significantly.

Conclusion

In conclusion, we are able to reject all three of our null hypotheses (Ho_1, Ho_2, Ho_3) and provide support for our alternate hypotheses (Ha_1, Ha_2, Ha_3). Time and temperature have both an individual and combined effect on the production of CO_2 in respiring *S. cerevisiae*.

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