The Effect of Temperature on the Cell Density of *Saccharomyces cerevisiae.*

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Abstract:
We investigated how temperature affects the growth rate of *Saccharomyces cerevisiae*. We hypothesized that change in temperature would increase or decrease the abundance of *S. cerevisiae* over time. To conduct our study, we tested a total of 5 different temperatures; 17°C, 30°C, 35°C, 40°C, 45°C. On day one we used both incubators and water baths. We used 30°C as our control, found to be the optimal growing temperature in previous studies, and ran tests at 30°C in both the incubator and hot water bath to account for any differences in the two methods. On both days, we had 4 replicates at each temperature, growing for three hours, taking samples every hour. Our results showed that at 30°C, the cell density of *S. cerevisiae* cells was significantly higher than tubes incubated at the other temperatures, thus supporting our hypothesis. The reasoning we found to explain these results is that when the temperature is lower than 30°C, the kinetic processes within the cell slow down. In temperatures higher than 30°C, the functionality of the cell also decreases, due to increased ethanol accumulation.

Introduction:

The organism used to perform this experiment is *Saccharomyces cerevisiae*, commonly known as yeast. *S. cerevisiae* is a single-celled organism that has the ability to reproduce rapidly on suitable medium (Hartwell 1974). *S. cerevisiae* reproduce by the process of budding, *S. cerevisiae* bud asymmetrically resulting in a larger mother cell and a smaller daughter cell (Sinclair *et al.* 1998). Each mother cell can produce a fixed amount of daughter cells. As the mother cell ages, the cell cycle slows down and mutation occurs at a higher rate (Sinclair *et al*, 1998). In this experiment, we are testing the effect of change in temperature on the abundance of *S. cerevisiae*. The relative age of *S. cerevisiae* might affect cell growth which in turn affects the abundance of the cell, which is measured in this experiment. The

doubling time for wild-type *S. cerevisiae* is around 100 minutes in optimal conditions which differs from mutant strains of *S. cerevisiae* (Hartwell, 1974).

Temperature is an important abiotic factor affecting *S. cerevisiae* growth. The optimal temperature for growth is around 30°C (Arthur and Watson, 1976). *S. cerevisiae* does not die at lower temperatures (1-10°C), but it does not grow either. At higher temperature, *S. cerevisiae* become stressed and their cellular content becomes damaged. Growth rate decreases and finally at 50°C, *S. cerevisiae* dies (Arthur and Watson, 1976). For our experiment, we based our treatment temperatures on these conditions. The treatment temperatures are 17°C, 30°C, 35°C, 40°C, and 45°C, with 30°C as our treatment control. *S. cerevisiae* is not well known for causing diseases in humans (Munoz *et al.* 2005). Additionally, *S. cerevisiae* fungemia is becoming recognized as associated with infectious disease in humans in recent years (Munoz *et al.* 2005). By exposing *S. cerevisiae* to different temperatures, we could try to inhibit or stop the growth of *S. cerevisiae* that cause damage inside the human body, thus reducing the effect of infection on human body. Since *S. cerevisiae* is particularly important in baking and brewing industries, by changing the temperature *S. cerevisiae* is exposed to we could produce food at a faster rate or produce better quality goods. Overall temperature affects *S. cerevisiae* growth, which in turn affects many aspects of disease control and food production in our daily lives.

Our null hypothesis is that change in temperature away from the optimal temperature does not affect or increases the abundance of *Saccharomyces cerevisiae*, and our alternative hypothesis is that change in temperature will decrease the

abundance of *Saccharomyces cerevisiae*. Our choice of alternative hypothesis is supported by the literature that suggest that *S. cerevisiae* growth is slowed as we move away from its optimal growth temperature (Ratkowsky *et al.* 1982), and that at 50°C, *S. cerevisiae* dies (Arthur and Watson, 1976).

Methods:

First we set up Axio compound microscope using Kohler illumination with the 10X objective lens. This was used to count *Saccharomyces cerevisiae*. Sterile technique was used throughout this experiment. Then we transfered 50 μL of the original *S. cerevisiae* wild-type culture into the haemocytometer. Then we counted the number of *S. cerevisiae* cells in the square that contain number of cells between 40 to 300 cells. Then the calculation for the number of cells per mL of the sample is done.

For this experiment, the starting concentration of our *S. cerevisiae* culture, we were aiming for 1.0×10^{7}cells/mL. Dilution was required for both weeks. For week one of our experiment, we added 0.05mL of *Saccharomyces* solution to 4.95mL of the growth medium to achieve this starting concentration. For week two of our experiment, we added 1.18mL of *Saccharomyces* solution and 3.82mL of growth medium to achieve the starting concentration.

Then all test tubes were labeled with its corresponding temperature treatment and the number of the replicate for each treatment. We pipetted 5mL of diluted *Saccharomyces* culture with starting concentration of 1.0×10^{7}cells/mL into each test tube. Then we placed the 4 test tubes with same treatment temperature

onto the a test tube rack and place in incubator or water bath with the corresponding treatment temperature and take the initial temperature reading of the water. Timer was then set to one hour since cell count is performed every hour for the three hours.

We fixed 100 100μL samples with 10μL of fixative and counted the number of cells using a haemocytometer. For this experiment we also recorded temperature of the water bath or incubator every hour to ensure its consistency throughout the experiment.

Using the cell count we calculated the concentration of our *Saccharomyces* replicates at the end of the hour and recorded the number. Using these end concentrations, first we calculated the average cell concentration of each treatment temperature at the end of one hour, two hour and three hour, and then 95% confidence interval for each treatment at different hour is calculated and compared.

Figure 1: 30°C water-bath with test-tube tray set in water. Temperature is adjusted using knobs on bottom right, and lid is closed during incubation.

Results:

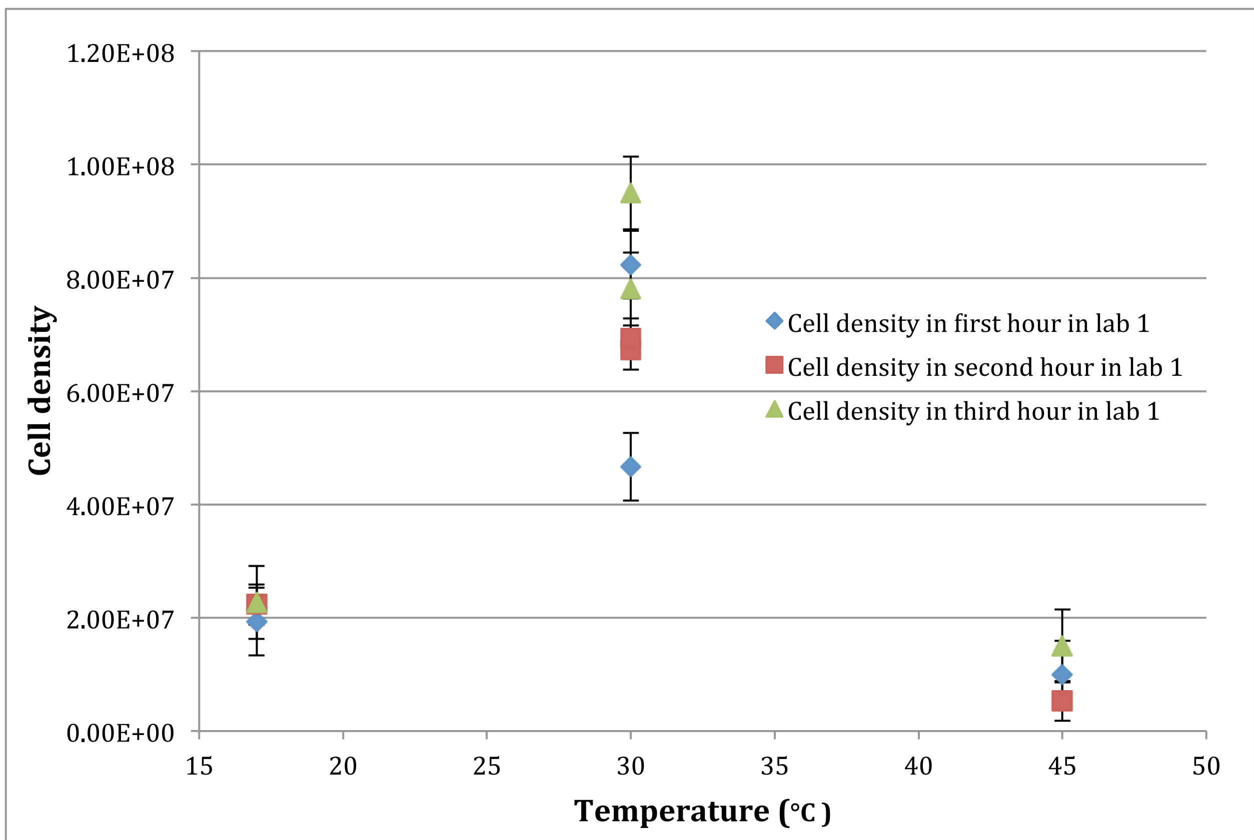

Figure 2: Cell density counts from lab one with temperatures of 30, 45 and 17°C with respect to time.

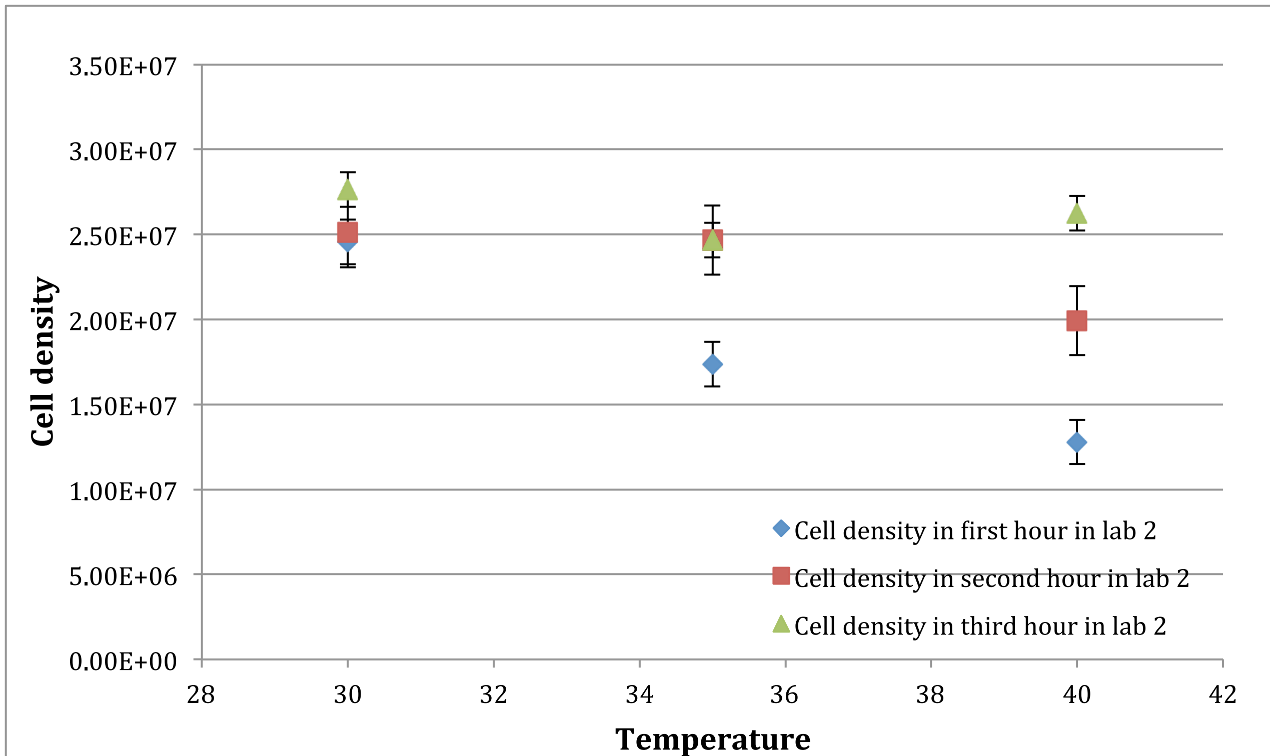

Figure 3: Cell density counts from lab two with temperatures of 30, 40 and 35°C with respect to time

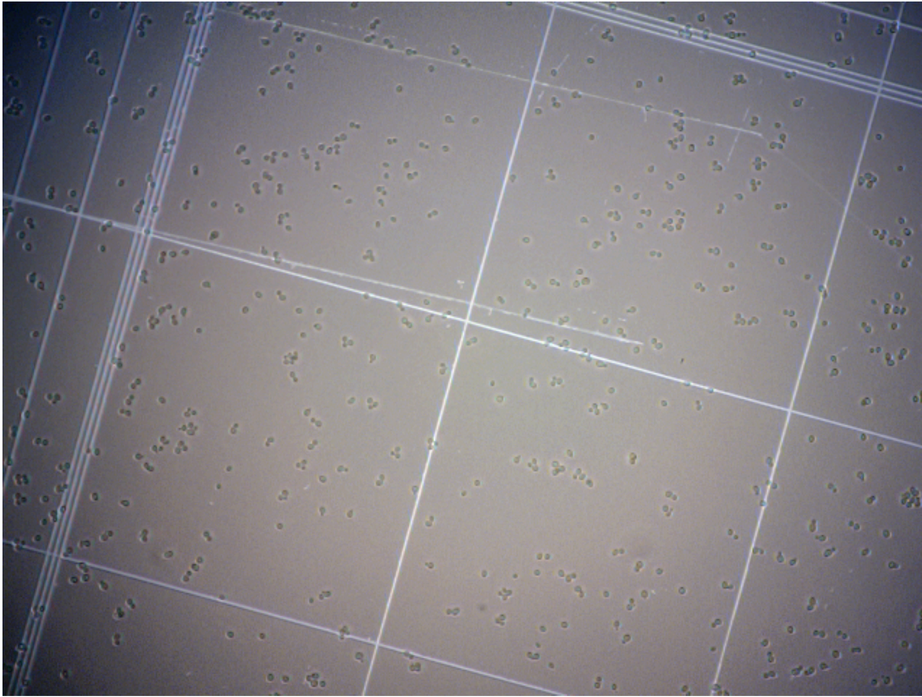

Figure 4: *Saccharomyces cerevisiae* at 30°C after two hours. .

Figure 5: *Saccharomyces cerevisisae* at 45°C after two hours. The large particle in the upper right was not included in our calculations.

Sample Calculation of 30°C of third hour from lab 2:
Average cell count/ml= (118+125+109)/3=117.33 cell/4.00E-06 ml = 2.93E+07 cell/mL
Average cell count of 4 replicate= (2.93E+07 + 2.85E+07 + 2.52E+07 + 2.76E+07)/4= 2.76E+07 cell count/mL
Using excel to calculate the standard deviation to be 1800687.9 and confidence interval (CI) to be 1764674.1

Figure 2 and 3 shows the relationship between cell density and temperature. In Figure 2, we can see a clear trend that the highest cell density occurs at temperature of 30°C and the lowest at temperature of 45°C. The peak cell density at 30°C (9.50 E +07) is very distinct from other temperatures. Similarly, in Figure 3, the highest cell density occurs at 30°C (2.76 E +07 measured in the third hour) and a relatively lower density at 40°C. Moreover, the 95% confidence intervals (CI) for all the data points are quite high. For example in figure 2, the highest CI value occurs at the

second hour at 30°C, which is 14955484. The highest CI for figure 3 is 4446523.1 that occur at the first hour at 35°C.

Discussion:

The results support our alternate hypothesis, and thus cause us to reject our null hypothesis. In the results recorded in lab one, there is a marked difference between the cell density of *Saccharomyces cerevisiae* in the water bath and incubator at 30°C, and the *S. cerevisiae* at 45 and 17°C . The cell densities at 17 and 45°C are significantly lower at all time intervals (of one, two, and three hours) than the cell density of the two groups growing at 30°C, and the 95% confidence intervals do not overlap with the groups at 30°C. When cell density is plotted against temperature, there is a clear trend of a higher cell density at 30°C than at all other temperature intervals. Similarly, in lab two, there is a significant difference between the cell density of *Saccharomyces cerevisiae* incubated at 30°C and the *S. cerevisiae* incubated at 40°C. The cell densities at 40°C are significantly lower at all time intervals (of one, two, and three hours) than the cell density of the group growing at 30°C. The cells growing at 35°C yielded similar results to the cells growing at 30°C, and their 95% confidence intervals overlapped, showing that they are not significantly different. When cell density is plotted against temperature, the cells grown at 40°C are significantly lower than the cells grown at 30 and 35°C at all time intervals except for the third hour interval.

Our results are very similar to the results found in other research, where the optimal growth temperature for *S .cerevisiae* was found to be from 25 to 35°C

(Watson, 1987). Accordingly, in our experiment the greatest amount of growth was observed in cells incubated at 30 and 35°C. In the lower temperature of 17°C, the decreased temperature slows down enzyme kinetics within the cells, which therefore slows down cellular processes (thus affecting growth) (Tai *et al.* 2007a). Additionally, in a previous study, when incubated at 12°C, the amount of glycolytic enzymes present in *Saccharomyces cerevisiae* were 7.5 times lower than the amount at optimal temperatures (Tai *et al*. 2007b). Following this, the cells stored at 17°C were significantly lower than the cells grown at 30 and 35°C. When yeast is exposed to higher temperatures, there is a higher accumulation of ethanol within the cell (Nagdawithina *et al*. 1974). This higher concentration of ethanol causes cell toxicity, which alters the cell membrane of *S. cerevisiae* and thus decreases its functionality (Lucero *et al.* 2000). Accordingly, our strains grown at 40 and 45°C had a significantly lower cell density than the strains grown at 30 and 35°C.

Though our results were supported by previous research, there was still some variation during our experiment. Our two controls of cells stored at 30°C should have yielded approximately the same cell count in lab one as lab two. Though this was the case with the two hour time interval, at the one and three hour intervals the incubator cell density was significantly lower than the water bath cell density (95% confidence intervals did not overlap). This could be attributed to inadequate mixing of the cells with the pipet prior to placing them on the haemocytometer. Additionally, some of the incubator test tubes were mistakenly placed into the centrifuge rather than the vortex, causing the cells to potentially be less concentrated at the point of pipetting which lead to a lower measured cell density.

Another variation in our results was that in the third hour time interval, the cell density of the cells stored at 45°C was not significantly lower than the cells stored at 35°C. This could be attributed to inconsistent temperature in the water bath. There were some inconsistencies in the water bath even when the temperature was adjusted, so the temperature may not have been accurate, and it follows that there could have been increased cell density for cells stored at 45°C. There were definitely some limitations to our study. This experiment would have been more meaningful had we let the yeast grow for longer at the varying temperatures. Allowing them to grow for three hours was enough to measure small differences, however if we'd been able to let them grow for six or more hours, the differences may have been more drastic and meaningful. It may have been beneficial to the study if we had more variance in our temperatures. Since the differences in growth rate weren't all that vast, it may have been useful to have done a few more temperatures further from the optimal temperature.

Conclusion:

Overall, we found that temperature did have an effect on the growth of *Saccharomyces cerevisiae*, and that the cell density decreased at temperatures outside of the optimal growth temperature range (25-35°C).

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