# The Effect of Temperature on the Growth Rate of *Saccharomyces cerevisiae*

Tim Cheung, Jessica Ko, Jae Lee, and Manpreet Takhi

**Abstract:**

We conducted our experiment to determine whether increasing incubation temperature would have an effect on the growth rate of *Saccharomyces cerevisiae*. Our four treatments were incubation at 12°, 17°, 30°, and 35° C, and cell counts were taken every 2 hours for 10 hours and once again after 24 hours. Our data analysis focused on the 6 to 10 hour time period, the interval where the greatest amount of growth occurred. We found the highest growth rate was at 35°, with a mean growth rate $4.98 \times 10^6$ cells/hour, compared to values of $7.59 \times 10^3$, $9.37 \times 10^4$, and $4.45 \times 10^6$ at 12°, 17°, and 30°, respectively. There was a significant effect of temperature on growth rate (analysis of variance, $p = 5.07 \times 10^{-6}$). We suggest that at higher temperatures, phospholipids in the cell become free allowing higher rates of particle transport, thus increasing reproduction.

**Introduction:**

The organism used to conduct this experiment was *Saccharomyces cerevisiae*, commonly known as yeast. *S. cerevisiae* reproduces asymmetrically by budding off new daughter cells that contain genetically identical information to their parent cell (Sinclair *et al*. 1998). The age of *S. cerevisiae* is not determined by the time elapsed from their production, but rather by the number of times it buds (Sinclair *et al.* 1998).

Temperature is one of the important factors affecting the growth of *S. cerevisiae.* Therefore, we investigated the effect of temperature on the growth rate of *S. cerevisiae*. The optimum temperature for growth is around 30°C (Salvado 2011). At low temperatures (1-10°C), the cells are viable but they do not grow well (Arthur and Watson 1976). The growth rate increases at temperatures from 28°C to 37°C and the growth rate does not increase at temperatures from 39°C to 41°C, but the cells are still viable (Mensonides *et al.* 2002). However, the viability of

cells decreases at 42°C and higher temperatures, which in turn, decreases the growth rate of *S. cerevisiae* (Mensonides *et al.* 2002).

The common uses of *S. cerevisiae* are for bread rising, brewing beer, and fermenting wine, which depend on the ability of fermentation of *S. cerevisiae*. According to Babiker *et al.* (2010), the temperature is controlled to grow the yeast at optimum level, since the metabolic pathway of *S. cerevisiae* produces heat. This production of heat could affect the fermentation process. Since this particular strain of yeast is widely used in bread and wine making industries, the effect of temperature can be very useful in producing better quality goods. Moreover, the effect of temperature on *S. cerevisiae* can be used in pathological industry. *S. cerevisiae* is well known as a Generally Recognized As Safe (GRAS) microorganism, however the number of reported mucosal and systemic infection in human population has increased and even fatal infections have occurred in relatively healthy individuals (Muller *et al.* 2011). The expression of genes of *S. cerevisiae* can be altered by heat stress, which induces protein folding that ultimately affects the growth (Helen *et al.* 2001). Thus, the temperature effect on protein folding can be studied further to figure out which temperature inhibits such protein production to prevent *S. cerevisiae* from being a pathogen.

Our null hypothesis was that increasing temperature will decrease or have no effect on the growth rate of *Saccharomyces cerevisiae* and our alternate hypothesis was that increasing temperature will increase the growth rate of *Saccharomyces cerevisiae*. Our alternate hypothesis is supported by literature suggesting that the growth of *S. cerevisiae* increases significantly at temperatures from 28°C to 37°C (Mensonides *et al.* 2002).

## Methods

The treatment temperatures of our experiment were 12°C, 17°C, 30°C and 35°C, with 30°C as our control since it is the approximate optimum temperature at which *S. cerevisiae* grows. We observed their growth every two hours because the doubling time for this organism varies between 60 minutes to 100 minutes (Mortimer and Johnston 1959).

The initial cell density of our yeast sample was calculated by pipetting 10µL of the sample onto a haemocytometer and counting the number of cells by using an Axio compound microscope at 100X magnification.

From the counts we were able to determine that we needed to dilute 312.5µL of yeast sample with 49.69mL of growth medium. We filled 12 test tubes to get three replicates for each of our four treatments. Figure 1 below shows a picture of one of our members preparing the 50mL replicates.

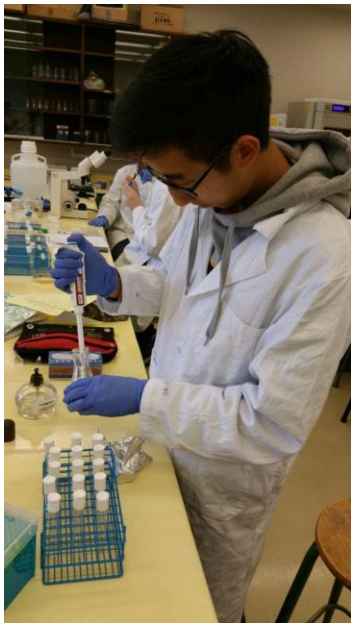

**Figure 1:** One of our group members diluting the yeast sample into a large test tube.

We placed the four treatments into incubators at 12°C, 17°C, 30°C, and 35°C. We noticed that the 35°C incubator did not have a light source compared to the other incubators, so to keep the light intensity constant we placed a black garbage bag over the other treatments. The samples were then left to grow in the incubators.

We took samples every 2 hours for 10 hours and also after 24 hours and measured the cell density. Using sterile technique, we pipetted 100μL of yeast sample with 10μL prefer into a microcentrifuge tube. After putting the samples into microcentrifuge tubes, we put the treatments back into the incubators as quickly as possible so that the time it was out of the incubator was limited. We then waited two hours for the solution to grow, before we repeated the same procedure to count the cell density. In total the cell density for each replicate was recorded at 6 time intervals.

To determine the cell density at different time intervals, we followed the same steps as for the determination of the initial cell density. While counting we also took pictures of each replicate in the microscope, this gave us a reference of the appearance of the cells in addition to the cell count.

From the data obtained we calculated the rate of growth from 6-8 hours and 8-10 hours. The rates were than averaged and the growth rate for each treatment was determined. These rates were then graphed with 95% confidence intervals. One-way Analysis of Variance (ANOVA) test was done to determine the p-value and this value was used to compare means of the treatments.

**Results:**

After creating an initial plot of the growth of our cell cultures over time, we determined that the period of greatest growth was during the 6 to 10 hour interval. Therefore, we focused on analyzing the growth rate during this period for each two- hour period, subtracting the initial cell

count from the final cell count and dividing by the time elapsed. We performed a one-way ANOVA test using growth rates calculated at each of our four treatments, which resulted in a calculated p-value of $5.07 \times 10^{-6}$. This indicates a significant difference among the growth rates at the four temperatures. Figure 2 illustrates the relationship between temperature and growth rate; the cultures incubated at 30°C and 35°C showed a significantly higher growth rate than the 12°C and 17°C treatments, with mean growth rates of $4.45 \times 10^{6}$ cells/hour $\pm$ $5.55 \times 10^{5}$ and $4.98 \times 10^{6}$ cells/hour $\pm$ $1.66 \times 10^{6}$ over every 2 hours from our chosen time interval, compared to $7.59 \times 10^{3}$ and $9.37 \times 10^{4}$ at 12°C and 17°C, respectively. This shows a trend towards 35°C as the optimal growth temperature for *S. cerevisiae*. There was relatively little variation found in the 12°C and 17°C treatments, with standard deviations of $3.20 \times 10^{3}$ and $5.37 \times 10^{3}$, and this is indicated by the lack of error bars in Figure 2.

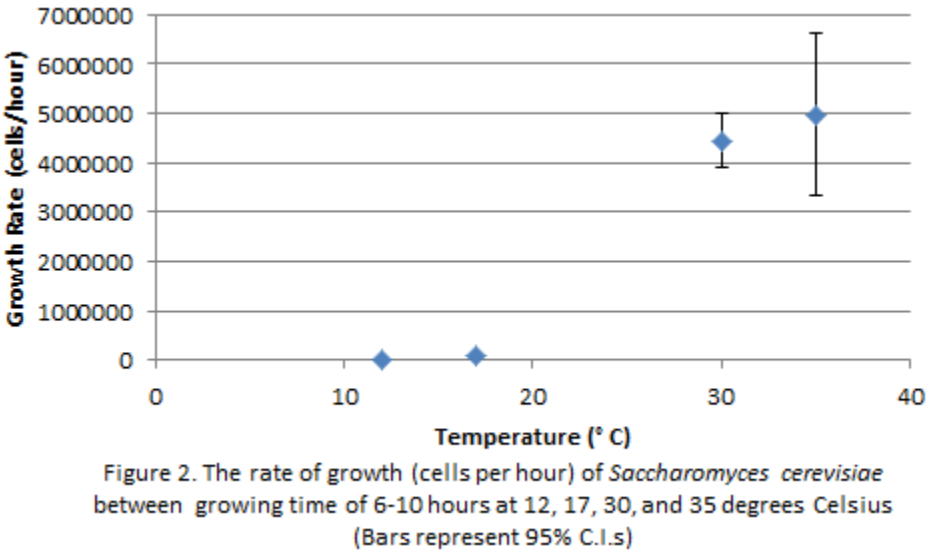


Figure 2. The rate of growth (cells per hour) of *Saccharomyces cerevisiae* between growing time of 6-10 hours at 12, 17, 30, and 35 degrees Celsius (Bars represent 95% C.I.s)

In terms of qualitative observations, we found that the cells were circular and white when viewed under the microscope, as shown in Figures 3 and 4. At higher temperatures and longer intervals, the cells were often found in small groups of 4 or 5 due to budding. By the time we took out our samples at 8 hours, we noticed that when the 30°C and 35°C treatments were mixed

a cloudy solution would appear. The cloudy solution may have appeared because the growth in these treatments was larger compared to the other treatments.

Figure 3. *Saccharomyces cerevisiae* at 30 degrees after 6 hours

Figure 4. *Saccharomyces cerevisiae* at 30 degrees after 10 hours

**Discussion:**

In our experiment, the primary focus was on the growth phase, where yeast tends to bud at an exponential rate. Our p-value of $5.07 \times 10^{-6}$, allowed us to reject the null hypothesis and give support to our alternate hypothesis, where increasing temperature increases the growth rate of *S. cerevisiae*.

Figure 2 shows the growth rate varies with temperature. At 12℃ and 17 ℃, we see that the growth rate is small. While growth rate at 30℃ has increased tremendously. At 12℃ and 17℃ we see small error bars, while there are larger error bars at 30℃ and 35 ℃. This shows that as temperature increases there is more variation among replicates. Furthermore, we see overlap of error bars between 30℃ and 35℃.

Our results were very similar to the ones obtained by other researchers such Salvado *et al.* (2011) in which they determined the optimal temperature for yeast to grow is 32.3° C. But unlike Salvado *et al*.'s (2011) research, which showed that cells continued to divide until 45.4° C, our data showed that the growth rate plateaued at 35℃. In Figure, 2 we had a growth rate of $4.45 \times 10^6$ cells/hour $\pm$ $5.55 \times 10^5$ at 30° C whereas it was $4.98 \times 10^6$ cells/hour $\pm$ $1.66 \times 10^6$ at 35° C. The growth rate does increase beyond optimal temperatures, but it plateaus. Nutrients may become limited, which prevents *S. cerevisiae* from dividing optimally (Tai *et al.* 2007). This is known as the stationary phase. At this phase toxins and wastes accumulate, making a poor environment for *S. cerevisiae (*Lucero 2000). Toxins develop from high concentrations of ethanol being produced by *S. cerevisiae* (Nagodawithana 1974).

Tai *et al.* (2007) discuss how temperature is important for cellular processes such as protein synthesis and substrate transport to enzymes. Lower temperature slows down enzyme kinetics. Temperature is also important in maintaining membrane fluidity and membrane production. Phospholipids also are affected by changing temperature (Tai *et al.*, 2007). As temperature decreases, there is more production of lipids on the membrane making it more constricted (Hunter and Rose, 1972). As temperature increases, diffusion occurs at an increased rate. This is where the growth phase occurs.

Charoenchai *et al*. (1998) conducted an experiment of the effect of temperature on the cell biomass of *S. cerevisiae.* They found very similar results in which the cell biomass increased within the optimal temperature range. However, Charoenchai *et al*.'s (1998) experiment was different in terms of methods. They were incubated at different temperatures which were 10° C, 15 ° C, 20° C, and 25° C. Samples were taken every 12 hours instead of 2 hours, which was our case. They focused on the cell density growth whereas our focus was on growth rate. In the end, however, we both saw similar trends. During the exponential growth, growth rate increased as the cell density increased.

Our experiment had sources of variation and sources of relevant errors. A major source of error was having light intensity affecting our treatments. In three out of the four incubators there was a light source. We tried to decrease the difference by putting a black garbage bag over the ones that were placed in an incubator with a light source. However, we realized after that there was no garbage bag covering the sides of the test tubes. Perhaps we could have done this to minimize light. Another error we made was that when we opened the incubator to obtain our test tubes, we left the door open for a while as we went to go retrieve our sample. As a result, temperatures could have changed, thus altering our results. We should have closed the door immediately. In other articles, samples were taken every 12 hours (Hartwell 1974), but in our case it was every 2 hours.

**Conclusion**

In this experiment, we were able to reject our null hypothesis and provide support for our alternate hypothesis. We found out that the growth rate of *Saccharomyces cerevisiae* increased with increasing temperature.

**Acknowledgement**

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