Effect of caffeine concentration and incubation time on the cell concentration of wild-type *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae is a eukaryotic organism that has been studied intensively. Its genome has been completely sequenced, and many of its pathways and genes have homologues to those in humans. We conducted an experiment to determine the effect of caffeine concentration and incubation time on the cell concentration of wild-type *S. cerevisiae*. We had treatments of 0mM, 10mM, 20mM, and 30mM of caffeine. We counted the number of cells every two hours, from t = 0 to t = 8 hours, using haemocytometers. After collecting the data, we used a two-way ANOVA and found significant differences in cell concentration at varying caffeine concentrations and time intervals. Moreover, the interaction between caffeine and incubation time had a significant effect on cell concentration. Although cell concentration increased as time passed, caffeine had an overall inhibitory effect on the growth of *S. cerevisiae*.

Introduction

Saccharomyces cerevisiae, commonly known as baker's yeast, was the first eukaryotic organism to have its genome completely sequenced (Ostergaard et al. 2000). Yeast cells have a doubling time of 90 minutes and grow best at 30°C (Bergman 2001; Salvadó et al. 2011). Yeast cells share many similarities with mammalian cells, with 31% of protein-encoding yeast genes having mammalian homologs (Botstein et al. 1997). Due to the similarities between yeast and human cells, research on yeast can provide valuable information about humans.

Caffeine is the most widely used drug in the world and it is important to understand the effects caffeine can have on humans (Daly 1998). Testing the effect of caffeine on yeast cells may provide valuable information on how caffeine interacts with human cells. Caffeine is a kinase inhibitor that shows affinity for the TOR1 kinase, which is a part of the TORC1 subunit complex (Kapitzky et al. 2010; Homann et al. 2009). This subunit controls cell growth and division; therefore, inhibition of the TORC1 subunit can trick the cell into believing that it does

not have sufficient resources for cell division (Homann et al. 2009). Caffeine also inhibits cyclic adenosine monophosphate (cAMP), which controls protein kinase A (PKA), an enzyme that controls the amount of available energy in a cell (Kuranda et al. 2006). Inhibition of either the TORC1 subunit or PKA will result in decreased cell division and growth.

Caffeine can inhibit cell growth, reduce cell fitness, arrest the cell cycle, cause DNA damage, and even alter the structure of the cell wall (Kuranda et al. 2006, see Figure 1). For example, when TOR1 kinase is inhibited by caffeine, the Pkc1p-Mpk1p kinase is activated in yeast cells. The Pkc1p-Mpk1p kinase is activated when cell wall integrity is threatened, and the activation of this pathway could lead to dysfunctions in the yeast cell (Kuranda et al. 2006). Due to the widespread consumption of caffeine and the fact that caffeine shares a similar pathway to oxidative stress, heat sensitivity, and acid pH resistance, a study on the effects of caffeine on *S. cerevisiae* could help further research in many fields (Calvo et al. 2009).

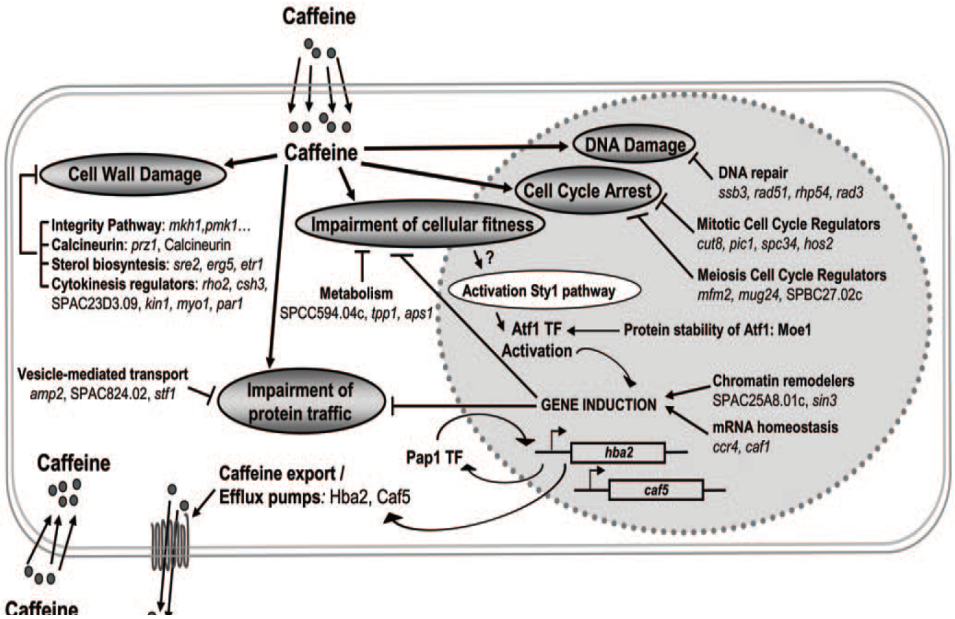

Figure 1: The various effects of caffeine can have on the yeast cell. (Image: Calvo et al. 2009)

Our hypotheses were:

H_{O1}: Caffeine has no effect on the cell concentration of *Saccharomyces cerevisiae*.

H_{A1}: Caffeine has an effect on the cell concentration of *Saccharomyces cerevisiae*.

H_{O2}: Incubation time has no effect on the cell concentration of *Saccharomyces cerevisiae*.

H_{A2}: Incubation time has an effect on the cell concentration of *Saccharomyces cerevisiae*.

H_{O3}: The interaction of caffeine and incubation time has no effect on cell concentration of *Saccharomyces cerevisiae*.

H_{A3}: The interaction caffeine and incubation time has an effect on cell concentration of *Saccharomyces cerevisiae*.

We predict that the rate at which cell concentration increases (growth rate) will decrease in the presence of caffeine due to TOR1 kinase and PKA inhibition (Kuranda et al. 2006, Homann et al. 2009). Our second prediction is that incubation time will increase the cell concentration of *S. cerevisiae b*ecause yeast cells have a doubling time of 90 minutes (Bergman caffeine will affect how quickly yeast cells move from lag phase to exponential phase and to stationary phase. For both our first and third predictions, higher levels of caffeine will result in a greater decrease in the growth rate and will further delay the transition between growth phases.

Methods

For our experiment, we started with a BY4741A wild-type stock solution, 60mM caffeine medium, and Yeast Extract Peptone Dextrose (YPD) medium. Throughout the experiment, we used sterile technique and vortexed our stock solution for 15 seconds for an even distribution of cells. We diluted the stock culture as noted in Figure 2.

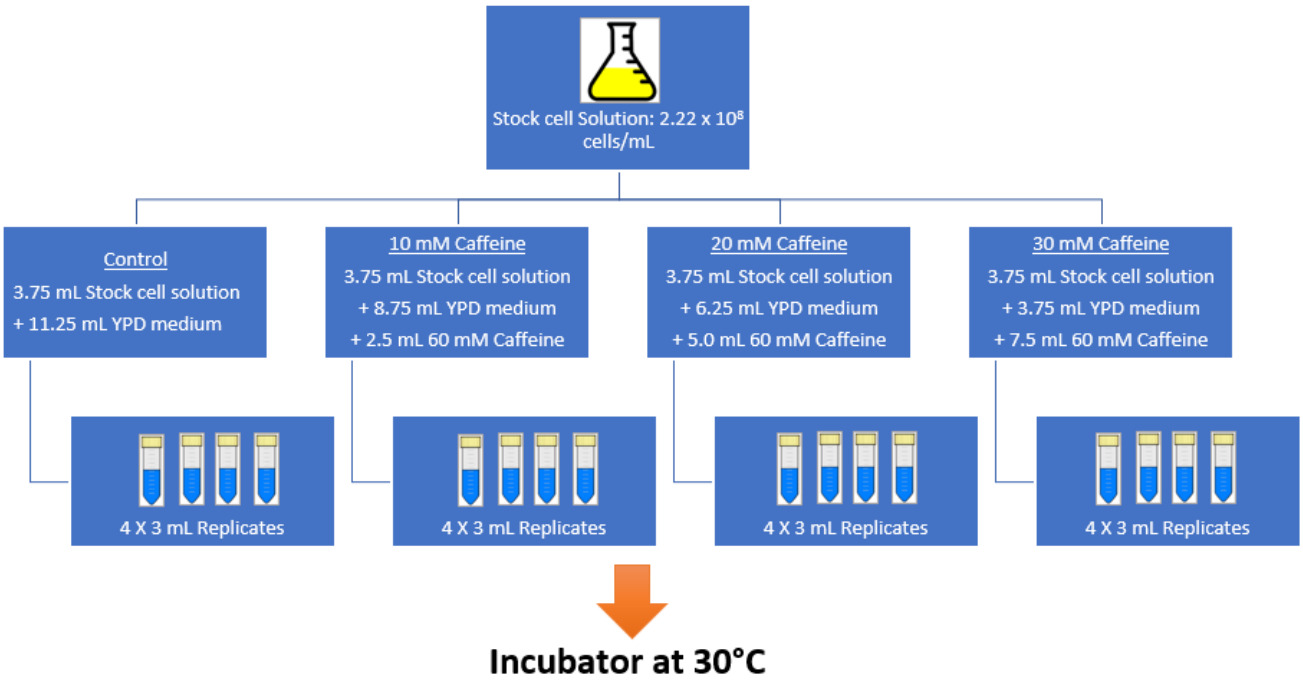

Figure 2. Dilution procedure for control (0mM), 10mM, 20mM, and 30mM caffeine treatments.

Since the focus of our experiment was the effect of caffeine on the cell concentration of *S. cerevisiae*, we used different amounts of caffeine, 0mM, 10mM, 20mM, and 30mM, for our research. Research by Bard et al. (1980) has shown that 50% of caffeine cells will die in the presence of 20mM caffeine, therefore we decided to test the yeast at caffeine concentrations slightly above and below what was suggested in the literature. We placed 3.75 mL of wild-type stock into each of the four treatments. As seen in Figure 2, we pipetted corresponding amounts of YPD medium and 60mM caffeine into these test tubes. After dilution, we started the sampling procedure shown in Figure 3. To inhibit cell growth, we added 5 µL of fixative into our samples. Once we prepared all 16 replicates, we began counting the samples ($t = 0$) following the haemocytometer instructions. We took pictures of each count, a sample of which can be seen in Figure 4.

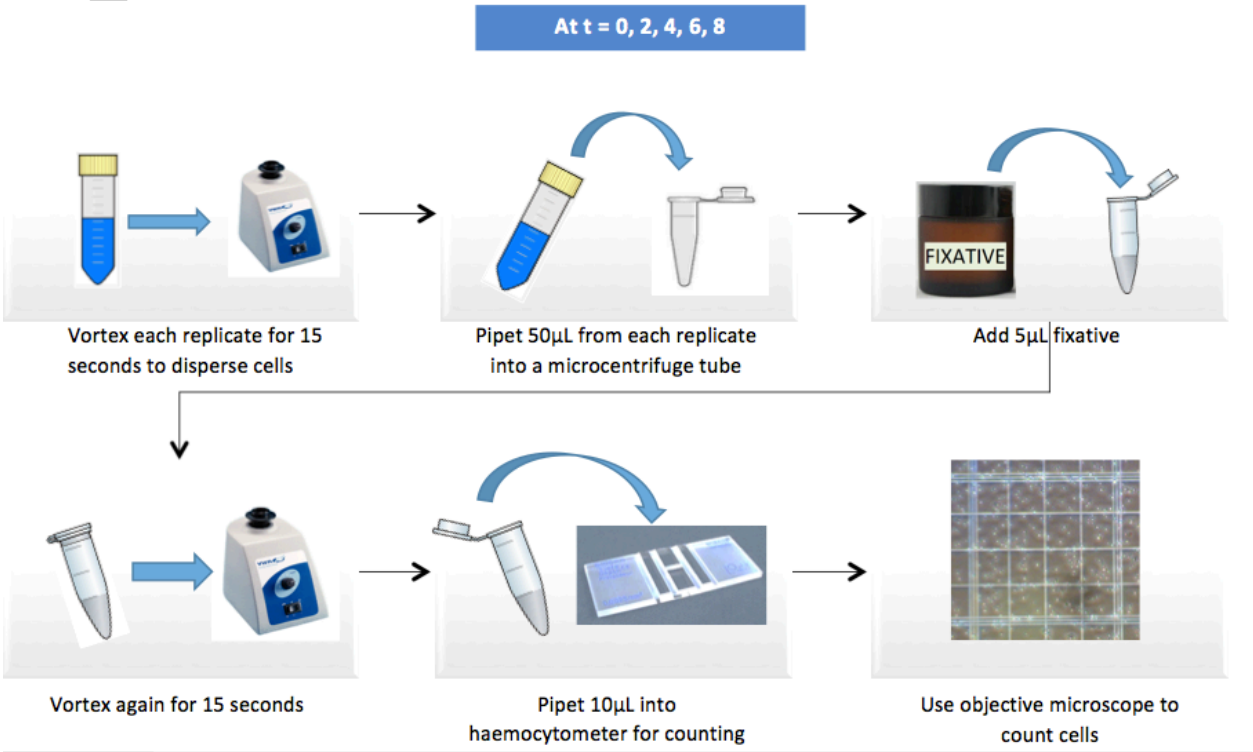

Figure 3. Sampling procedure at t = 0, 2, 4, 6, 8 hours for 0mM (control), 10mM, 20mM, and 30mM caffeine treatments.

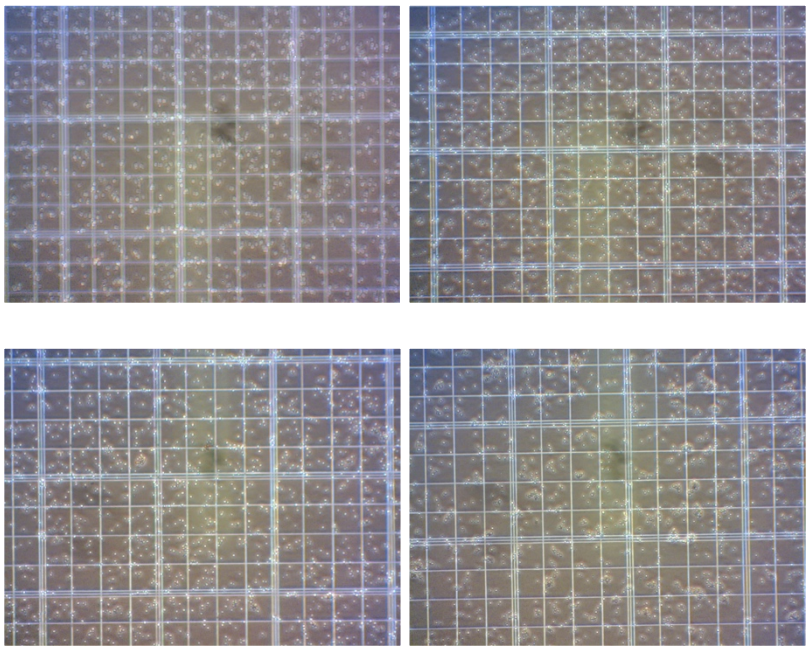

Figure 4. Yeast cells at various levels of caffeine at t = 2. (Top left = control (0mM), top right = 10mM, bottom left = 20mM, bottom right = 30mM). Pictures taken using Dinoxcope at 100x magnification on Axio microscopes under phase contrast. Each small box is 0.05 mm by 0.05 mm in size.

We continued to record cell concentrations for 8 hours (t = 0, 2, 4, 6, 8). Throughout this experiment, we maintained our yeast in an incubator at 30°C, which is the optimal growth temperature for *S. cerevisiae* (Salvadó et al. 2011). We used a two-way ANOVA to test our data and calculated the 95% confidence intervals for each treatment at the various sampling times.

Results

As seen in Figure 5, the initial cell concentration for the control, 10mM, 20mM and 30mM ranged from 2.99 x 10^7 to 4.23 x 10^7 cells/mL. Using a two-way ANOVA test with a 0.05 significance level, we found significant cell concentration differences at different caffeine concentrations (p = 1.03 x 10^{-36}) and at different times intervals (p = 1.4 x 10^{-31}). As well, there was a significant interaction between caffeine concentration and time that affects cell concentration (p = 2.02 x 10^{-38}).

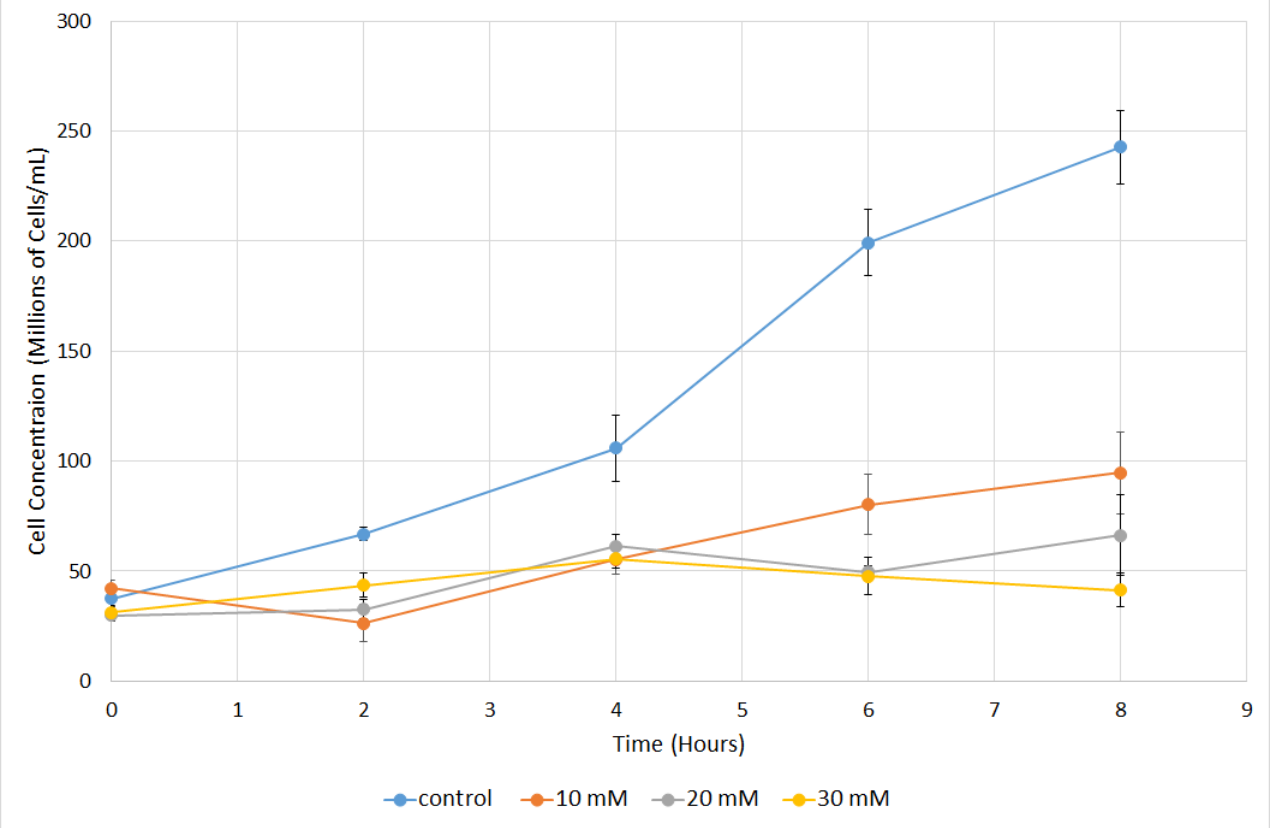

Figure 5. Average cell counts from t = 0 to t = 8 for wild type *S. cerevisiae* with different concentrations of caffeine. Error bars represent 95% confidence intervals.

The cell concentration of the control was significantly higher than all caffeine treatments at times 2, 4, 6, and 8 hours. For the caffeine treatments, the 10mM treatment produced cell concentrations that were significantly higher than the 20mM treatment at 6 hours. As well, the 10mM treatment had significantly higher values than the 30mM treatment at 6 and 8 hours, but significantly lower values at 2 hours. Additionally, the cell concentration of the 20mM treatment was significantly lower than 30mM treatment at the two-hour mark.

There is a positive trend between cell concentration and time in the control and 10mM caffeine treatment. However, this trend does not appear at the higher caffeine concentrations of 20mM and 30mM. After four-hours, there is a trend of higher cell concentration with lower caffeine concentrations. In addition, the mean cell concentration of the 30mM treatment peaked at 4 hours at 55, 672, 222 cells/mL, and decreased after that.

The yeast cells in all treatments appeared round and transparent under 100x magnification using Axio microscopes. In Figure 4, the cells have a light-yellow hue under phase contrast of the microscope. The cells were also observed to cluster around each other. Every two hours, when the replicates were removed from the incubator for sampling, the bottom of every test tube was observed to contain thick light yellow pellets visible to the unaided eye. As time went on, there was an observable increase in the size of the pellet of the control replicates.

Discussion

We are able to reject H_{O1} and provide support for H_{A1} as $p = 1.03 \times 10^{-36}$, as caffeine was observed to have an effect on the growth rate of *S. cerevisiae*. There are significant differences between the cell concentration of the control and caffeine treatment because the p-value is less than 0.05. From t = 2h onwards, the control sample grew to higher cell concentrations than all caffeine treatments. After t = 6, the treatments with higher caffeine concentrations were observed

to have lower numbers of cells. This supports our predictions as the caffeine appears to decrease cell growth. Kuranda et al. (2006) state that the presence of caffeine can lead to the disruption of the TOR pathway and inhibition of cyclic adenosine monophosphate in yeast cells, causing DNA damage, altered cell wall structures, cell cycle arrest, and an overall decrease in cell growth. In addition, Bard et al. (1980) suggested that a growth medium with 20mM caffeine would cause 50% of the yeast cells to die. This study also found that higher caffeine concentrations correlated to higher cell death, which consistent with our findings after $t = 6$.

The p-value for our second hypothesis was 2.02×10^{-38}, allowing us to reject H_{O2} and provide support for H_{A2} which states that time has an effect on the concentration of *S. cerevisiae,* supporting our predictions. As time increased, cell concentrations fluctuated. In the control, the cell concentration increased as time went on. The 10mM caffeine treatment also exhibited this trend after $t = 4$. Bergman (2001) suggested that the doubling time of wild-type yeast is about 90 minutes at 30°C in normal YPD medium during the exponential phase. The quickest doubling time from the samples in our experiment was 2 hours, recorded from the control between $t = 4$ and $t = 6$ hours, in which cell density grew from 1.06×10^8 to 1.99×10^8 cells/mL. However, in Figure 5, the caffeine treatments exhibited varying cell concentrations with time, as shown by the 95% CI, but did not follow the same growth curve of the control. This will be further discussed in the following analysis of hypothesis three.

We are able to reject H_{O3} and provide support for H_{A3} as $p = 2.02 \times 10^{-38}$. This supports our prediction that there is an interaction between the two variables, caffeine and time, that affects cell concentration and growth. Yeast cells grow at different rates depending on the time they have to acclimatize to a new environment and on the concentration of nutrients or waste in the environment. In normal YPD medium, yeast cells exhibit a low growth rate in the lag phase, the fastest growth rate in the exponential phase, and decreased growth in the stationary phase,

where resources become limited at around 5×10^7 and 2×10^8 cells/mL (Bergman 2001). From our data, the cell concentration of the control treatment did not reach its maximum doubling time until $t = 4$ hours. Before this time, the cells may have been in the lag phase; growing slower as they adjusted from the stock medium they were in previously to the diluted medium of the control treatment. As for the caffeine treatments, both 10mM and 20mM caffeine treatments had a slight decrease in cell concentration from $t = 0$ to $t = 2$ and then a an increase once both treatments reached $t = 4$ hours. Kuranda et al. (2006) has stated that caffeine is detrimental to cell health and causes decreased growth. This may have caused the cells to require a longer time to acclimatize to the caffeine environment. Furthermore, although the cell concentration for 30mM treatment increased from $t = 0$ to $t = 4$ hours, it then decreased from $t = 4$ hours to the end of the experiment, indicating that the yeast cells may not tolerate caffeine over 20mM for long periods of time. Overall, both 20mM and 30mM caffeine treatments did not exhibit a standard growth curve and the 10mM caffeine treatment appeared to have a prolonged lag phase as compared to the control in Figure 5.

For future studies, experiments could be designed to test caffeine concentrations with longer incubation times to try to identify any growth patterns. This would allow us to better establish a relationship between growth time and caffeine concentration. In addition, caffeine concentrations of 10mM, 20mM, and 30mM all appeared to decrease cell growth compared to the control sample, thus future studies that look at lower concentrations of caffeine may be beneficial to establish a caffeine concentration where the growth of *S. cerevisiae* may not be as adversely affected.

Sources of error

There were several potential sources of error in our experiment. First, because some of the cells were clustered together or in the process of budding, this could have affected our counting.

Next, there could be an error in pipetting due to uneven cell distribution. In addition, when removing the samples for counting, we first had to remove all the samples from the incubator, which was located in another room. This could have exposed the samples to different temperatures. Finally, when adding fixative, we could not fix all the cells at the same time possibly allowing cells in the other samples to grow more than the others.

Conclusion

Caffeine had an inhibitory effect on the growth rate of the yeast cells, with higher levels of caffeine having lower cell concentrations at $t = 8$ hours. As predicted, incubation time had a positive effect on the cell concentration of *S. cerevisiae*, with cell concentrations increasing over time. The interaction between caffeine and incubation time had an effect on the cell concentration and growth rate of *S. cerevisiae*. This experiment demonstrates that caffeine has an effect on yeast cells and hopefully future studies can focus on how human cells react to prolonged exposure to caffeine.

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