The effect of temperature on respiration rate in *Saccharomyces cerevisiae*

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Abstract

Respiration plays a crucial role in the function of cells and is necessary for the continuation of life. Aerobic respiration is one of two methods in which *Saccharomyces cerevisiae*, also known as yeast, is able to break down glucose. In this study, data were collected to determine whether or not temperature has an effect on the respiration rate of *S. cerevisiae* under aerobic conditions. Using respirometers, yeast culture medium was placed into four experimental water baths set to temperatures of 20°C, 25°C, 30°C, and 40°C, respectively. An additional positive and negative control temperature of 35°C was used with yeast and without yeast respectively. Yeast aggregates obtained through centrifugation were mixed with standard YPD medium and placed into a respirometer in the appropriate water bath. Displacement of fluid in the respirometers was measured at 15-minute intervals for a total of 60 minutes and total height difference as a result of fluid displacement via carbon dioxide pressure was determined. The data suggest that yeast respiring at 30°C rather than the optimal temperature of 35°C (Arroyo-Lopez *et al.* 2011) displace the highest amount of fluid with a value of 1.37 cm. Based upon comparisons of 95% confidence intervals and rates of fluid displacement (RFD) calculated, the null hypothesis that higher temperatures either decrease or have no effect on respiration rates of *S. cerevisiae* could not be rejected.

Introduction

Saccharomyces cerevisiae, also known as yeast, are living organisms important in the food industry. *S. cerevisiae* is responsible for ethanol production in alcoholic drinks as well as the rising of bread, hence the common aliases “brewer’s” yeast and “baker’s” yeast (Bioweb 2002). *S. cerevisiae* produce ethanol and carbon dioxide as by-products of anaerobic fermentation in low oxygen environments. In oxygen-abundant environments, however, *S. cerevisiae* undergoes aerobic respiration, producing carbon dioxide, ATP, and water (Bioweb 2002). As with any living organism, there is a temperature range that is optimal for growth of *S. cerevisiae*, and thus an optimal yield of the abovementioned by-products. The effective use of yeast depends on the knowledge of this characteristic, as the organism may be inadvertently subjected to unfavourable

conditions in its various applications. Numerous studies and experiments test for this optimal temperature range (e.g. experiments of Charoenchai *et al*. 1998 and Salvadó *et al.* 2011).

The basic objective of this study was to observe how *S. cerevisiae* respires in environments where all conditions except temperature are held constant. With a reputation of being one of the most important model organisms in the world, countless aspects of *S. cerevisiae*, certainly temperature, have already been tested (Bioweb 2002). We measured respiration rates of *S. cerevisiae* via carbon dioxide production in an oxic environment (to ensure only aerobic respiration occurs). The results of this study are useful in commercial applications of *S. cerevisiae* (e.g. the food industry), as well as in the study of the organism itself. The respiration rates of *S. cerevisiae* in various temperatures provide important information for experiments that test other characteristics of the organism. The unintentional exposure to either optimal or suboptimal temperatures will effectively skew the outcomes of other studies.

The alternate hypothesis for the study postulates that higher temperatures will increase the respiration rates of *S. cerevisiae*. The corresponding null hypothesis states that higher temperatures will have either no effect or decrease the respiration rates of *S. cerevisiae*. Our alternate hypothesis is based on the fact that *S. cerevisiae* has an optimum growing temperature, thus highest respiration rate, in the 25°C – 35°C range, but has the ability to grow in temperatures up to 45°C (Salvadó *et al.* 2011). It should be noted that "higher temperatures" is a somewhat subjective term, but for the purposes of our study will refer to temperatures higher than that of the optimum range.

Methods

The experiment was conducted over a period of two lab sessions, each three hours in duration. Within the two 3-hour lab periods we tested the effect of temperature on the respiration rates of wild type *S. cerevisiae* (BY741a). We tested a wide range of temperatures: 20˚C, 25˚C, 30˚C, 35˚C, and 40˚C. We used 35˚C as our control temperature since it is closest to the optimum temperature of 34˚C (Arroyo-Lopez *et al.* 2011). For the negative control, standard medium was used, also at a temperature of 35˚C. This negative control was necessary to ensure there was no additional production of carbon dioxide as a result of the experimental conditions.

Preparatory step: Five water baths were set up to ensure the desired temperatures were maintained throughout the lab. We then poured approximately 45mL of wild type medium into the centrifuge tubes. Once four tubes were filled with wild type medium, they were placed into the centrifuge on the highest setting for a total of five minutes (Figure 1). Once centrifugation was complete, the supernatant was poured into a waste flask, leaving the remaining pellet in the centrifuge tube. The pellet from each test tube was removed by mixing with 5mL of standard medium. Once fully mixed, the pellet medium was poured out into another 500mL flask. This step was repeated until there were fourteen complete sets. Next, an extra 5mL of standard medium was directly added to the pellet medium to ensure that there was sufficient amount of pellet medium to carry out the entire experiment. The pellet and medium combination was finger-vortexed before usage to ensure an even distribution.

Figure 1. Set up for the centrifugation step.

Experiment: Yeast cells in the pellet medium were counted using a haemocytometer. Once the cell count was confirmed, we carried out the experiment by loading pellet medium into the respirometer. First, the larger test tube was filled with pellet medium until it was 3/4 full. Then, a smaller inverted test tube was placed inside the larger test tube. For the next step we covered the opening of the larger test tube with our fingers and inverted it slowly until the smaller test tube was at the bottom of the larger test tube, containing the yeast and medium mixture, with no air bubbles present (Figures 2 and 3).

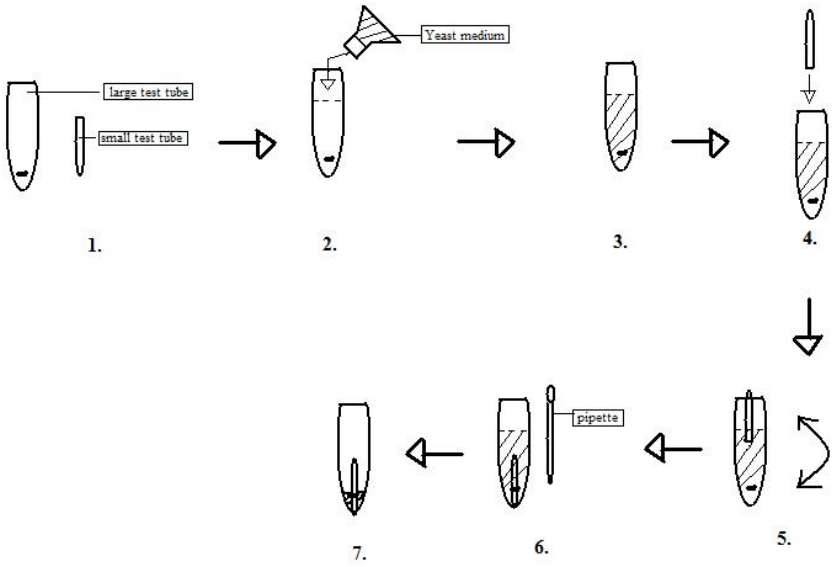

Figure 2. A simple diagram illustrating the steps to using a respirometer.

Figure 3. The inversion of the respirometer.

Figure 4. Photo to show the removal of excess medium from the respirometer

Next, we used a pipette to remove any excess medium back into the pellet medium flask until we reached the black line marked on the large test tube (Figure 4). The black line was approximately 1 cm above the bottom of the test tube. Of the five temperatures, we set 20°C, 25°C, 30°C, and 40°C as our four experimental temperatures. We set both our positive and negative controls at 35°C based on the fact that optimum growing temperatures occurs around this value (Salvadó *et al.* 2011). We placed three respirometers holding yeast samples into each respective water bath and let them sit for 60 minutes. We checked and recorded the displacement of fluid in each sample once every 15 minutes. After the full 60 minutes, we measured the final displacement and used it to calculate the respiration rate using the following equation:

$$\text{Respiration rate} = \frac{\text{Total Displacement}}{\text{Duration}}$$

The collected data were used to calculate 95% confidence intervals to see if there were significantly different respiration rates at different temperatures.

Results

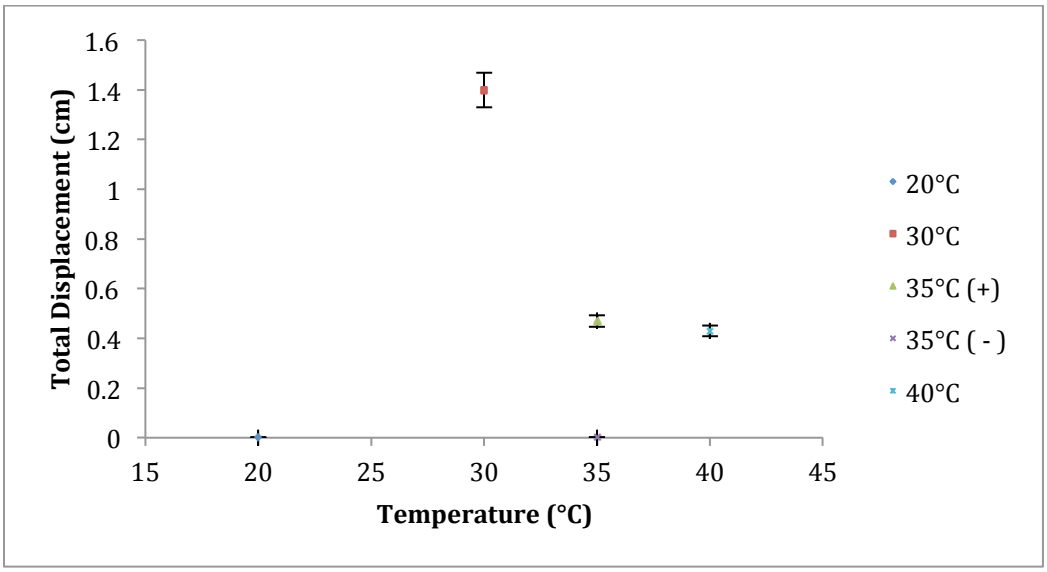

Figure 5. The relationship observed between the average total height of fluid displaced after 60 min. and the temperature of the respirometer that contained *S. cerevisiae* in trial 1 of the experiment. The error bars present on the graph represent 95% confidence intervals.

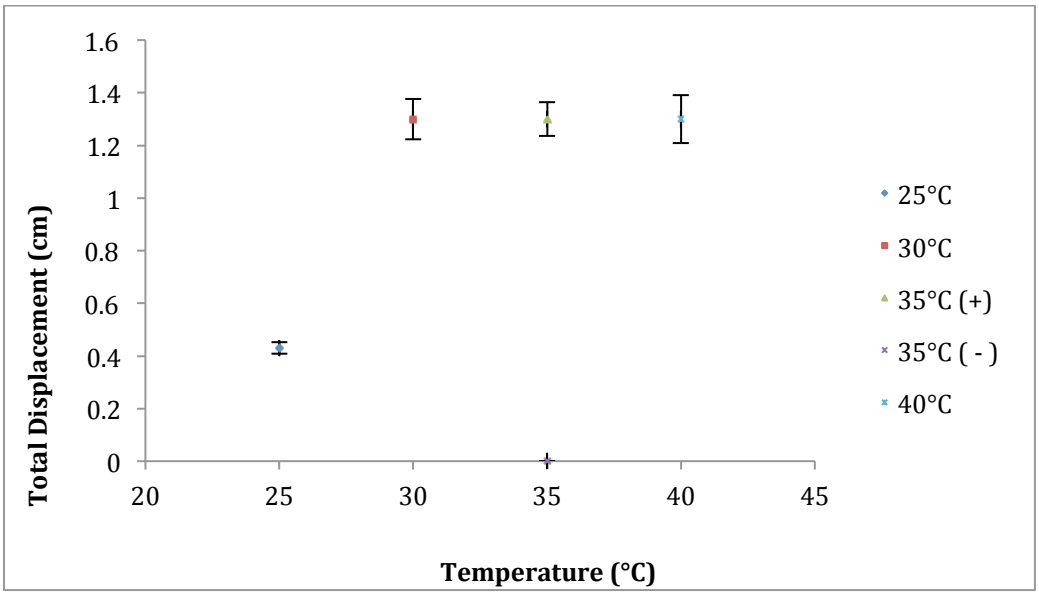

Figure 6. The relationship observed between the average total height of fluid displaced after 60 min. and the temperature of the respirometer that contained *S. cerevisiae* in trial 2 of the experiment. The error bars present on the graph represent 95% confidence intervals.

In trial 1 of the study, at 20°C, the yeast yielded an average total fluid height displacement of 0.0+/-0.0 cm. The yeast subjected to 30°C temperature conditions produced an average total fluid height displacement of 1.4+/-0.1cm. Our positive control, which contained yeast, at 35°C yielded average height difference values of 0.47+/-0.03cm. On the other hand, our only negative control at the 35°C without yeast culture produced no height difference at all. The 40°C treatment provided an average height difference of 0.43+/-0.04cm. The 30°C treatment was determined to have displaced the most fluid via carbon dioxide production when comparing 95% confidence intervals (C.I.). While the positive control and the 40°C treatment possessed no significant difference from each other, there was a statistically significant difference between these two temperatures and the other points (Figure 5). From 95% C.I. comparisons, it was determined that the positive control and 40°C temperature yielded the second highest amount of fluid displaced. The 20°C and negative control both had the least amount of fluid displaced (Figure 5)

In trial 2 of our study, the 25°C treatment, our replicates displayed an average difference in height of 0.43+/-0.04cm. The yeast subjected to 30°C temperature conditions, produced an average height displacement of 1.3+/-0.08cm. Our positive control at 35°C had an average height difference value of 1.3+/-0.07cm. As with our trial 1 measurements, our negative control at the same temperature produced no height difference at all. The 40°C treatment provided an average height difference of 1.3+/-0.09cm.

During the second trial, the heights displaced by the 30°C, 40°C, and the positive control temperature of 35°C, were not statistically significant from one another.

When compared with the 25°C and negative control, however, it was determined that they produced the highest amount of fluid displacement. The 25°C treatment produced the second highest fluid displacement, while the negative control yielded the least fluid displacement (Figure 6).

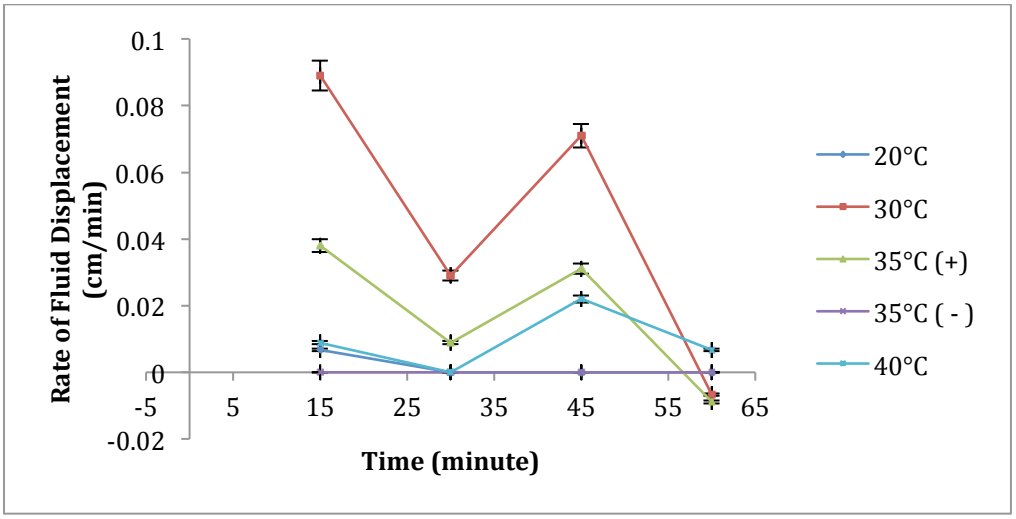

Figure 7. The relationship between the rates of fluid displaced and time in the respirometer that contained *S. cerevisiae* on the first day of the experiment. The error bars present on the graph represent 95% confidence intervals.

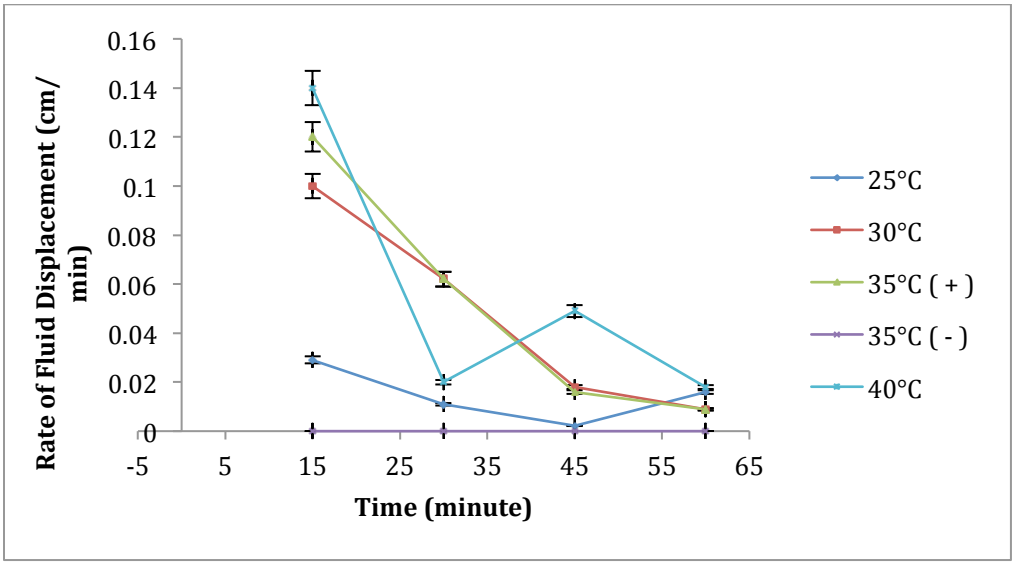

Figure 8. The relationship between the rates of fluid displaced and time in the respirometer that contained *S. cerevisiae* on the second day of the experiment. The error bars present on the graph represent 95% confidence intervals.

During the first trial, the highest rates of respiration measured generally occurred at 15 and 45 minutes. For our 20°C treatment replicates, we observed an average RFD of 0.0067+/-0.0003cm/min in the first 15 minute interval. However, the 45 minute interval yielded an RFD of 0.0+/-0.0cm/min. For our 30°C treatment, the 15 minute mark had an RFD of 0.089+/-0.005cm/min. The RFD at 45 minutes was 0.071+/-0.004cm/min. For our positive control treatment at 35°C, the RFD measured during the first 15 minutes was 0.038+/-0.002 cm/min and 0.031+/-0.002 cm/min at 45 minutes. The negative control at 35°C yielded an RFD of 0 cm/min for every interval we measured at. For the yeast subjected to 40°C temperature, the RFD were measured at 15 minutes was 0.0089+/-0.0005cm/min. The rate at 45 minutes was measured at 0.022+/-0.001cm/min.

In the second trial of our experiment, the highest rates for yeast at each temperature treatment occurred during the first 15 minutes. In our 25°C treatment, the yeast had an average RFD of 0.029+/-0.001cm/min during the first time interval. For our 30°C, the RFD obtained at 15 minutes was 0.1+/-0.005cm/min. The positive control yielded an RFD with the value of 0.12+/-0.005cm/min at 15 minutes. Once again, our negative control yielded RFDs of 0cm/min for all time intervals. The 40°C treatment had RFDs of 0.14+/-0.006cm/min (Figure 8).

Based on comparisons of 95% C.I.s we were able to find that the yeast produced carbon dioxide to displace fluid fastest during the first 15 minute interval. In the first trial, the highest RFD at the first 15 minute interval was produced by the yeast at 30°C. In the second trial, the 40°C treatment produced the highest RFD. In general, for most yeast at the differing temperatures, RFDs decreased as time went by. In the first trial however,

the 40°C treatment produced an RFD during the third time interval which exceeded the RFD value during the first time interval (Figure 7).

Example Calculation of Mean:

$$\text{Mean Fluid Height Displacement} = \frac{(0.5\text{ cm}) + (0.5\text{ cm}) + (0.3\text{ cm})}{3}$$

$$= 0.43\text{ cm}$$

Example Calculation of Variance

$$\text{Variance} = \frac{(0.5\text{cm}-0.43\text{cm})^2 + (0.5\text{cm} - 0.43\text{cm})^2 + (0.3\text{cm} - 0.43\text{cm})^2}{(3-1)}$$

$$= 0.013$$

Rates of fluid displacement (RFD) were also obtained for each treatment. We calculated RFDs by calculating the difference in displacement between 15-minute intervals and dividing the resulting value by 15 minutes.

Example Calculation of Rates of Fluid Displacement (RFD)

$$\text{RFD}_{(0-\ 15\ \text{minutes})} = \frac{\text{Fluid Height Displaced}_{(\text{time} = 15\ \text{minutes})} - \text{Fluid Height Displaced}_{(\text{time} = 0\ \text{minutes})}}{15\ \text{minutes}}$$

$$= \frac{(0.4\text{cm}) - (0.0\text{cm})}{15\ \text{minutes}}$$

$$= 0.027\text{cm/min}$$

Discussion

Based on comparisons of the 95% C.I.s of the RFDs we calculated, we fail to reject our null hypothesis which states that higher temperatures will have either no effect or decrease the respiration rate of *S. cerevisiae*. The highest RFD from the first day came from the 30°C treatment, during the first interval we measured. On the second day, the highest RFD came from the 40°C treatment, during the first interval we measured. Despite having overlapping C.I.s, a decreasing trend of RFDs was apparent as temperatures were increased to 40°C for the first day (Figure 7). This was not consistent for our second trial. In this case, decreasing the temperature from 40°C was accompanied with a lower RFD. This however, can be attributed to bubble-formation, which became a major problem resulting in the need for more replicates. The increased occurrence of bubbles made it difficult to obtain accurate displacement readings. Having a maximum respiration rate not at 35°C was surprising, since Salvadó *et al.* (2011) found that temperature to be optimal for growth of their yeast.

An explanation for this occurrence may be due to the fact that it is possible we have a wild type strain of *S. cerevisiae* other than BY741a. Torija *et al*. (2003) found that certain strains of yeast performed better at lower temperatures. In that same study, they found that as temperatures increased, the number of viable yeast cells decreased.

There exists an optimal temperature for *S. cerevisiae* respiration rates due to simple thermodynamics of metabolic pathways (Berg *et al.* 2012). These metabolic pathways rely on enzymes in order to occur, thus the rate of respiration will be limited by enzyme activation and function. In our study, the temperatures of 30°C and 40°C provided optimal environments for the enzymes involved to have the lowest activation

energy and fastest reaction rate possible. Departure from this optimal temperature will result in unfavorable conditions for these enzymes (Berg *et al.* 2012). Temperatures lower than that of the optimal conditions will result in higher activation energies for the enzymes, as well as slower rates of reaction. Alternatively, higher temperatures might result in denaturation, thus leading to the inactivation of the enzymes involved in aerobic respiration (Berg *et al.* 2012). Since respiration relies on functional enzymes to occur at greater rates, denaturation inhibits enzyme function, which in turn results in a drop in respiration rate.

From our results it was also apparent that as time passed, yeast at most temperatures experienced decreased respiration rates. This observation can be attributed to an ever decreasing nutrient pool for the yeast to use as the experiment wore on. We did not keep the nutrient concentrations constant. During the process of aerobic respiration, *S. cerevisiae* utilize oxygen to oxidize the nutrients acquired from their environment to produce energy to fuel cellular processes. Werner-Washburne *et al.* (1993), stated that under nutrient limiting conditions, yeast transition into a stationary phase to survive such conditions. It is noted that under a starvation-induced stationary phase, most cellular processes slow in order to accommodate for the stress applied (Werner-Washburne *et al.* 1993). In our experiment, we pre-allocated a certain volume of YPD media for yeast in the respirometer. As the experiment progressed, the nutrients available in the YPD media decreased as respiration occurred. The nutrients must have decreased to a certain concentration which forced the yeast to start transition into a stationary state, thereby decreasing respiration rates as well. This may have been observed because the yeast cell no longer has sufficient nutrients to maintain regular cellular processes that occur in

situations with an abundance of nutrients.

Despite most of our yeast showing decreased respiration rates as time progressed, there are a few interesting observations to be made for the cultures subjected to 40°C. *S. cerevisiae* placed in 40°C conditions, exhibited a spike in respiration rates after 30 minutes (Figure 6). This sudden increase in respiration rate could have been caused by an acclimation of yeast to their present environment. This can occur if the organism is exposed to nonlethal stimuli for period of time. They can accomplish this by expressing and suppressing certain genes to cope with the environment at hand (Tai *et al.* 2007). During the period of acclimatization, yeast cells enter a stationary-phase like state where respiratory rates drop. Upon adaptation to the environment, respiration rates increase as cellular processes return to nearly full functionality (Tai *et al.* 2007).

Despite our 30°C treatment yielding one of the higher RFDs, Inoue *et al*. (2000) propose that higher temperatures which can induce a state of heat shock in yeast cause higher oxygen respiration rates. We speculate that 40°C could yield a higher RFD if we extended the time in which we executed the experiment. This would give it enough time to acclimate to the warmer temperature. The hypothesis of Inoue *et al*. (2000) was confirmed when measured oxygen respiration and intracellular oxidation levels went up after being introduced to temperatures above their optimal growth temperature of 34°C. When *S. cerevisiae* enter a heat-shocked state, certain transcription factors are present which cause heat sensitive genes to be expressed. The mitochondrial oxygen respiration rates increased as well (Inoue *et al.* 2000). Although higher temperatures do not foster optimal growth in yeast, respiration rates are definitely affected.

A drop in respiration rates was observed in yeast at 20°C. We speculate that a

similar spike had a possibility of occurring past the 60-minute mark. However, we were unable to observe one due to time constraints placed on our experiment.

Sources of error were present in both the preparation and execution of the experiment. During the second day of the study, an extra milliliter of YPD media was added to our concentrated yeast culture prior to the experiment. This did not alter the cell concentration greatly and still produced a count which was well above the predetermined minimum value of cells needed to conduct the experiment.

During both trials of the study, bubbles were present in the respirometer chambers throughout the experiment. The transient presence of bubbles could have skewed readings of fluid height displacement. During the times we recorded displacement, the presence of bubbles would yield results suggesting a larger displacement than what actually occurred. This error had the most effect on the replicates placed in water baths that required a constant influx of heat to maintain set temperatures; bubbles did not form as much in the baths at 20 and 25°C. Having bubbles present would exaggerate the carbon dioxide production rates of the yeast present in the chamber since fluid displacement would inherently increase. To minimize this source of error, final readings were only recorded once all bubbles in the measuring vessel settled.

In future experiments, it may be worth extending the observation period to longer than an hour. This would allow the yeast to achieve sufficient acclimatization for observation. It would be interesting to see if the expression of certain heat sensitive genes causes an increase in metabolic demand, thereby increasing respiration rates.

Conclusion

Based on the results from our experiment on the effect of temperature on respiration rates of *S. cerevisiae*, we failed to reject the null hypothesis because higher temperatures did not increase the amount of carbon dioxide produced. While the failure to reject our null hypothesis was expected from the review of past studies, it was interesting to observe how *S. cerevisiae* behaved at the upper limits of its growth temperature range. The results of this study are vital for both the commercial applications of the organism, as well as for the study of the organism itself.

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