Effect of varying temperature on the rate of CO_2 production in baker's yeast (*Saccharomyces cerevisiae*)

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ABSTRACT

We conducted an experiment to find the optimal temperature for cellular respiration of *Saccharomyces cerevisiae*. Respirometers were incubated in water baths at temperatures of 25°C, 30°C and 35°C. The amount of CO_2 gas produced was recorded at five minute intervals for 70 minutes, and cell counts were made before and after incubation. The notable trend in our data was that with increasing temperature, there was less of a lag time before measurable CO_2 appeared, and CO_2 production was more rapid than at lower temperatures. The average CO_2 production rates were $1.66 \times 10^{-9} \pm 6.95 \times 10^{-10}$ mL/cell, $2.31 \times 10^{-9} \pm 6.76 \times 10^{-10}$ mL/cell and $3.02 \times 10^{-9} \pm 6.42 \times 10^{-10}$ mL/cell at 25°C, 30°C and 35°C, respectively. Based on previous research, the lower rate of CO_2 production observed at 25°C is a result of reduced enzyme kinetics and reaction rates at lower temperatures. Additionally, the accelerated rate of CO_2 production seen at 30°C, and even more so at 35°C, can be explained by the increase in enzyme kinetics, membrane fluidity and diffusion rates that accompany higher temperatures. Our results suggest that the optimal temperatures for *S. cerevisiae* growth and metabolism may not be equal.

INTRODUCTION

Saccharomyces cerevisiae, more commonly known as baker's yeast, is a eukaryotic microorganism and a facultative anaerobe. This means that yeast can use sugars to undergo aerobic respiration to produce water and CO_2 gas, or it can undergo fermentation in the absence of oxygen to produce ethanol and CO_2 gas (Berg *et al.* 2012). Due to its ability to form such by-products, *S. cerevisiae* has been widely used in multiple areas of scientific research. For example, in health care research, yeast has been used to identify cancer-causing genes in humans (Sloan and Prize 1992). Moreover, the fermentation by-product of ethanol has been used in biofuel manufacturing as an alternative to fossil fuels (Lin *et al.* 2012).

We were interested in determining which temperature *S. cerevisiae* exhibits the highest metabolic rate while performing aerobic respiration. Literature lists a wide range of optimal growth temperatures for yeast, including 25°C to 30°C (Morano 2012), 30°C to 33°C

(Zakhartsev *et al.* 2015), as well as 25°C to 35°C (Kuloyo *et al.* 2014). Our objective was to determine which temperature, 25°C, 30°C, or 35°C, was closest to the optimal temperature for the metabolism in *S. cerevisiae*. When yeast undergoes aerobic respiration, it produces water, CO_2 gas, and energy in the form of adenosine triphosphate (Berg *et al.* 2012). As this chemical process constitutes a majority of the cell's metabolism, the volume of gas produced over time can be used as an indicator of cell activity. This investigation will add to the body of knowledge regarding metabolism in *S. cerevisiae*, and will enhance the exploitation of yeast by reducing the costs of mass production and increasing the efficiency of manufacturing valuable by-products.

Our first null hypothesis was that temperatures of 25°C, 30°C and 35°C have no effect on CO_2 production per *S. cerevisiae* cell. Our corresponding alternate hypothesis was that temperatures of 25°C, 30°C and 35°C have an effect on CO_2 production per *S. cerevisiae* cell.

Our second null hypothesis was that time has no effect on CO_2 production per *S cerevisiae* cell. Our second alternate hypothesis was that time has an effect on CO_2 production per *S. cerevisiae* cell.

Lastly, our third null hypothesis was that the effect of time on CO_2 production per *S. cerevisiae* cell is the same for 25°C, 30°C and 35°C, whereas our last alternate hypothesis was that the effect of time on CO_2 production per *S. cerevisiae* cell is not the same for 25°C, 30°C and 35°C.

We predicted that temperature would have an effect on CO_2 production in *S. cerevisiae*, as temperature has been found to have the greatest influence on the metabolic rate of yeast compared to other variables such as pH and glucose levels (Arroyo-Lopez *et al.* 2009) (Figure 1). In addition, we believed that time would affect the amount of CO_2 produced, because as the experiment progressed, CO_2 would accumulate. Lastly, we predicted that the effect of time on CO_2 production would be different for each temperature, and that it would take less time for yeast at an optimal temperature to produce the same amount of CO_2 as yeast below or above it. Yeast at or below-optimal temperature would have a lower rate of CO_2 production, as higher temperatures stimulate enzyme kinetics in cell metabolism, up to and including the cell's optimal temperature (Liu *et al.* 2014). Although yeast optimally undergo metabolism in warmer environments, this metabolic rate declines in temperatures above its optimal range (Zakhartsev *et al.* 2015). Enzymes involved in yeast cell metabolism start to denature above-optimal temperatures, resulting in a decrease in both metabolic rate and CO_2 production (Nelson and Cox 2013).

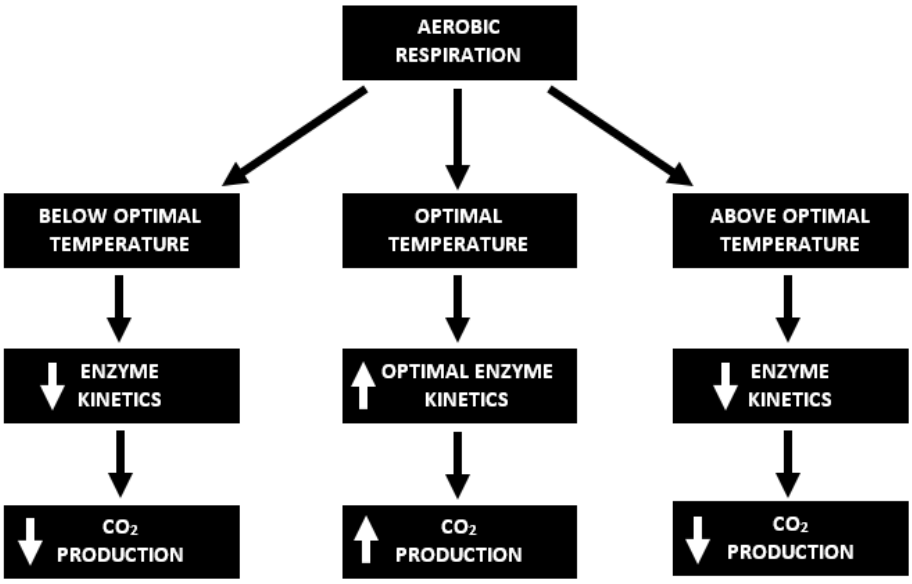

Figure 1. The predicted model *Saccharomyces cerevisiae* will follow when exposed to above optimal temperatures, optimal temperatures, and below optimal temperatures, given all the extraneous variables are kept the same.

METHODS

We obtained 2.2 L of wild-type yeast stock solution with an approximate concentration of 9.0×10^7 cells/mL as well as 4.0 L of yeast extract peptone dextrose (YPD), a medium that facilitates yeast activity. We determined the rate of cellular metabolism by measuring the volume of CO_2 gas produced in respirometers filled with yeast at temperatures of 25°C, 30°C and 35°C. We set the treatment control to be 30°C, because this was the temperature at which most research found the optimal temperature for growth of *S. cerevisiae* (Zakhartsev *et al.* 2015). Each treatment had four replicates (n=4), in addition to four procedural controls that contained only YPD medium.

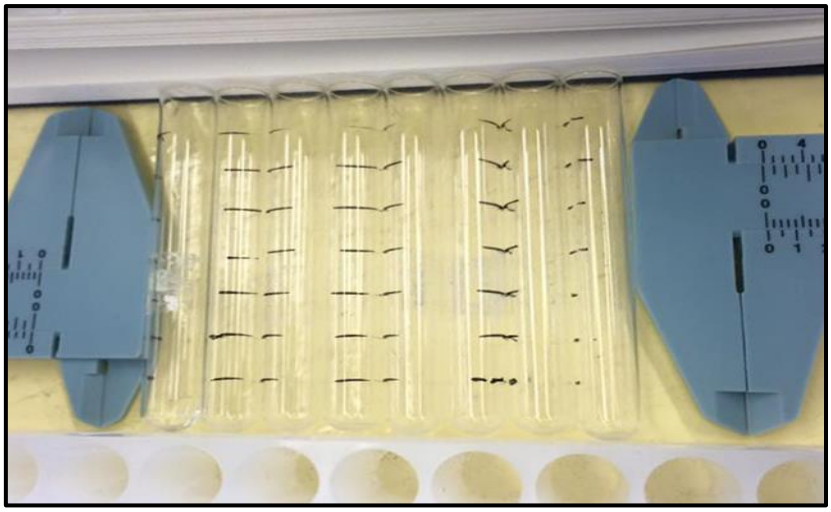

Figure 2. 0.5mL lines were marked in the 4mL test tube for an accurate reading of CO_2 production.

We made marks of 0.5 mL onto the 4.0 mL test tubes of the respirometers to allow for an accurate and efficient reading of CO_2 volume at each time interval (Figure 2). We first prepared 12 procedural control respirometers containing only YPD medium. After preparing the controls, we concentrated the yeast stock to a final concentration of 4.0×10^9 cells/mL by centrifuging and then resuspending the yeast pellets in 200 mL of YPD medium. At this point, we filled the respirometers with the newly suspended yeast and placed four replicates into each water bath of 25°C, 30°C and 35°C. The CO_2 produced by the yeast filled the inside of the inverted respirometer, and we were able to record the volume of CO_2 produced by using the marks we had made on the outside of the tubes.

We collected data every five minutes for a total of 70 minutes. When the yeast solution on the outside of the respirometer obscured the reading of the innermost tube, we pipetted out and discarded the excess liquid for an easier reading (Figure 3).

Figure 3. Pipetting out excess yeast solution.

At the conclusion of our experiment, we withdrew 10 µl from each replicate and added 1 µl of fixative so that the cells would stop budding and we could make accurate cell counts. We determined the final cell concentration of each replicate by counting cells using a haemocytometer that was viewed with an Axio microscope. We divided the CO_2 produced at each five minute interval by the number of cells to determine the CO_2 produced per cell at each time. We analyzed the data using the two-way ANOVA with replication, and the *p*-values were compared with the significance level (α) of 0.05 to determine if there was a significant difference between the treatments with regards to CO_2 production.

RESULTS

We analyzed our data by performing a two-way ANOVA test, and we calculated *p*-values of 3.98×10^{-25}, 2.80×10^{-49} and 1.47×10^{-4}, for our first, second and third hypotheses, respectively. We observed increases in the average cell density from 9.57×10^{7} cells/mL observed before incubation, to 1.05×10^{9} cells/mL, 1.11×10^{9} cells/mL and 9.78×10^{8} cells/mL observed after incubation at 25°C, 30°C and 35°C, respectively.

The CO_2 production rates increased over time at all three temperatures, but each treatment showed different CO_2 production rates (Figure 4). The final volume of CO_2 produced per cell at the end of the 70 minutes was 3.94×10^{-9} mL/cell at 35°C, 3.34×10^{-9} mL/cell at 30°C, and 3.25×10^{-9} mL/cell at 25°C. In addition, the replicates at 25°C began to produce visible amounts of CO_2 gas approximately 10 minutes after the replicates at 30°C and 35°C.

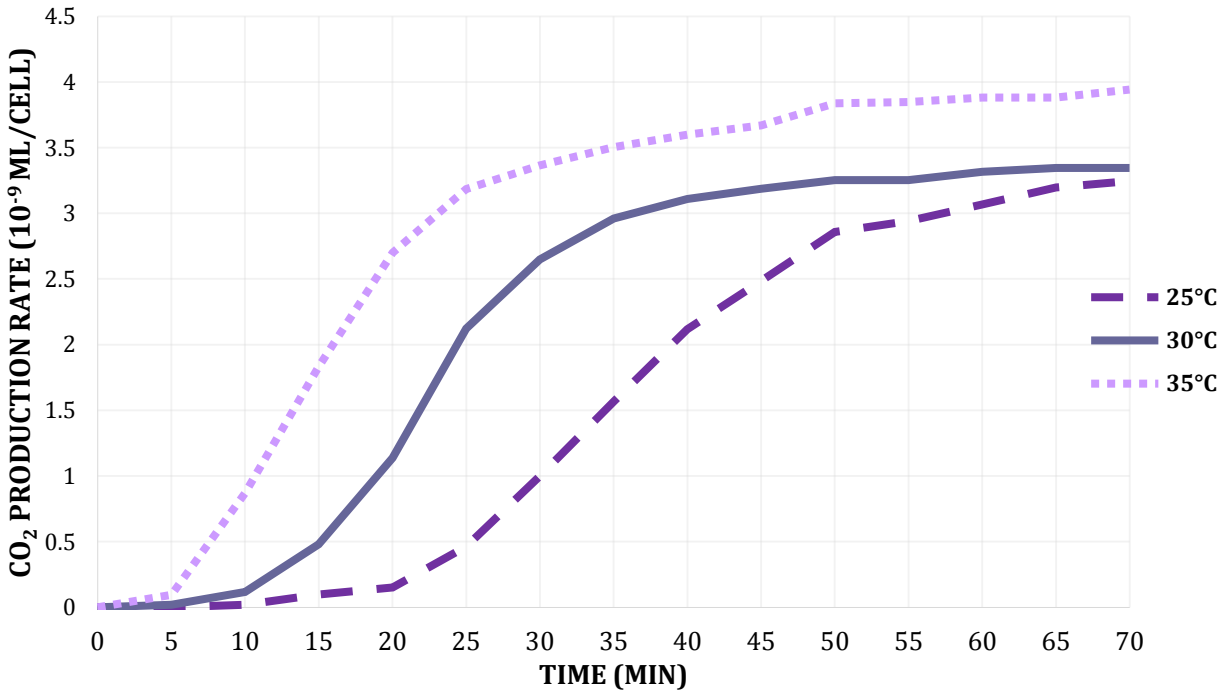

Figure 4. Mean CO_2 production rates (mL/cell) of the 4 replicates *S. cerevisiae* at 25°C, 30°C and 35°C calculated at the end of 70 minutes. (n = 4)

We found that yeast at 35°C entered an exponential rate of CO_2 production quicker than the other two temperatures (Figure 4). An average exponential rate of $1.54 \times 10^{-10} \pm 2.4 \times 10^{-11}$ mL/cell/min was expressed between five and 25 minutes at 35°C, while an average exponential rate of CO_2 production of $1.14 \times 10^{-10} \pm 2.03 \times 10^{-11}$ mL/cell/min was seen at 30°C between 10 and 35 minutes. Finally, there was the largest lag time before the yeast entered an exponential growth at 25°C, as the rate of $7.74 \times 10^{-11} \pm 1.12 \times 10^{-11}$ mL/cell/min was observed between 20 and 50 minutes (Figure 5).

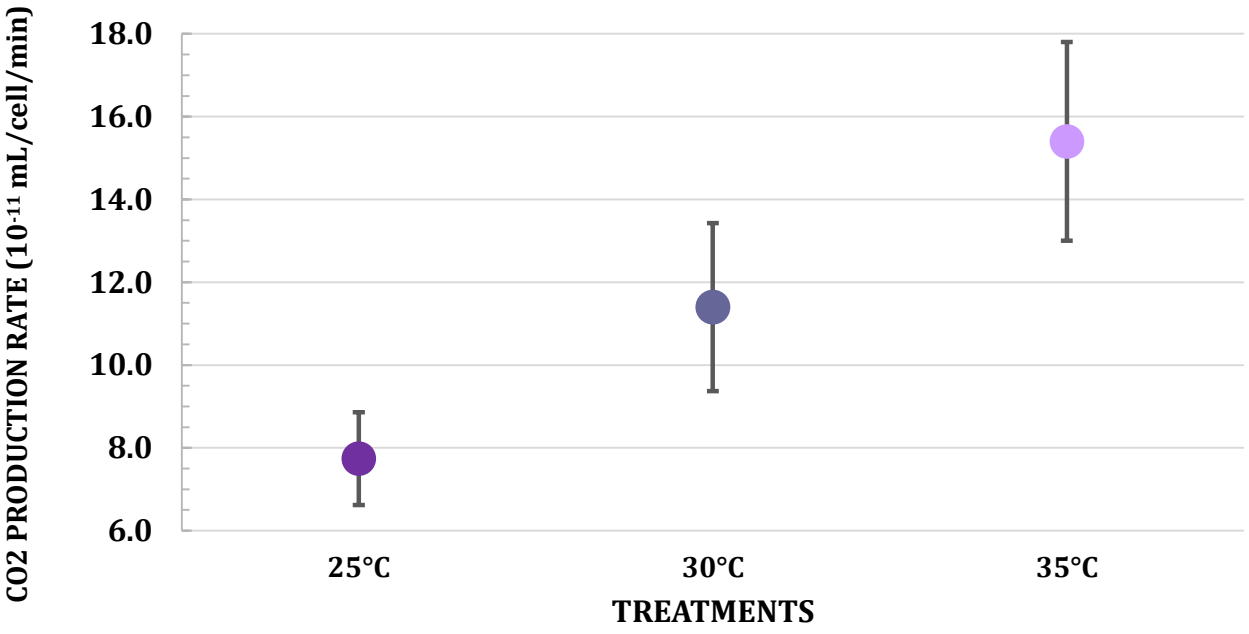

Figure 5. Average CO_2 production rates (mL/cell/min) of the 4 replicates of *S. cerevisiae* at 25°C, 30°C and 35°C. Error bars represent 95% confidence intervals ($\alpha = 0.05$). (n = 4)

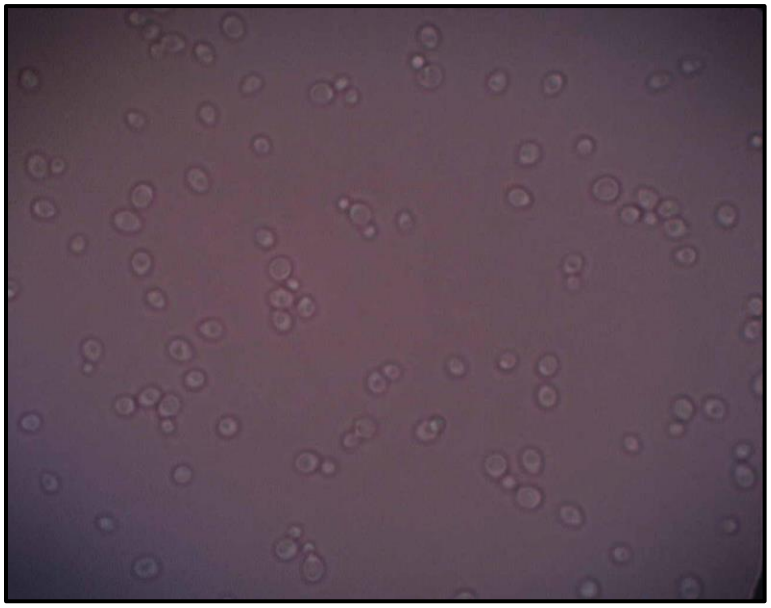

Figure 6. *Saccharomyces cerevisiae* as seen through an Axio microscope with a total magnification of 400x.

We made several qualitative observations throughout this experiment. When *S. cerevisiae* was observed with an Axio microscope at a total magnification of 400x, the yeast cells were transparent, round in shape, and surrounded by a thick cell wall which appeared dark (Figure 6). The yeast stock solution that we obtained was a deep amber color, probably due to the brown YPD medium. As we centrifuged the yeast pellets and removed the supernatant, the cells revealed to be a very pale in colour. Once we resuspended the cells in fresh YPD medium, the solution reverted back to

being deep amber. This colour stayed constant throughout the water bath procedure. Moreover, the CO_2 gas that filled each respirometer was clear and rose to the top of the innermost tube.

DISCUSSION

Based on our statistical analysis, we were able to reject all three null hypotheses, and thus lend support to the alternate hypotheses, due to the fact that our *p*-values were calculated to be less than our significance level of 0.05.

Once we obtained a *p*-value of 3.98×10^{-25}, we rejected our first null hypothesis, which stated that temperatures of 25°C, 30°C, and 35°C have no differing effects on the production of CO_2 gas in *S. cerevisiae*. We were therefore able to support our alternate hypothesis, which stated that temperature does affect CO_2 production in *S. cerevisiae*. This finding also corresponds with our prediction. During our experiment, we observed that as temperature was increased, yeast cells produced more CO_2, with a maximum volume of gas produced at 35°C (Figure 4). Our results are consistent with Arroyo-Lopez *et al.* (2009) who showed that temperature is the variable with the greatest influence on yeast metabolism. The effect of temperature on the rate of CO_2 production will be further discussed with the analysis of our third hypothesis.

After finding the *p*-value relating to our second hypothesis was 2.80×10^{-49}, we rejected the null hypothesis which stated that time had no effect on the production of CO_2 in *S. cerevisiae*. We were able to support to our second alternate hypothesis which stated that time does in fact affect CO_2 production in *S. cerevisiae*. Therefore, our results support our prediction that the rate of CO_2 production varies with time. As the experiment went on, the total amount of CO_2 accumulated. According to Figure 4, it is evident that yeast initially produced CO_2 slowly, and as time passed, the rate of gas production rapidly increased, up to a point where production slowed down. This trend in CO_2 production shown by the yeast follows the model that explains

logistic growth of a population of unicellular organisms. Lag phase, which is the time it takes for cells to adjust to the medium, and in our experiment, the temperature, and begin respiring at a notable rate (Dickinson and Schweizer 2004), is seen by the very slow production of CO_2 initially. This phase was the longest at 25°C, as we found the next phase of yeast growth to commence almost 20 minutes after the yeast were incubated. Exponential growth, which follows the lag phase, is marked by the rapid production of CO_2. During this time, cells are undergoing aerobic respiration, and thus producing waste at an optimal rate (Dickinson and Schweizer 2004). At different temperatures, we found a significant difference in the rate of CO_2 production between 30°C and 25°C as well as between 35°C and 25°C when the yeast were growing exponentially (Figure 5), with yeast exhibiting decreasing rates of CO_2 production with decreasing temperature. Lastly, yeast cells arrest in stationary phase, and thus drastically slow their production of CO_2, when their source of nutrients is depleted (Dickinson and Schweizer 2004).

Lastly, with a *p*-value of 1.47×10^{-4}, we rejected our third null hypothesis, which stated there was no interaction between the effects of time and temperature on CO_2 production in *S. cerevisiae*. We were able to support our alternate hypothesis that stated time has different effects on the production of CO_2 in *S. cerevisiae* at different temperatures. Our results were not consistent with our prediction that 30°C was the optimal temperature for yeast metabolism, as the greatest rate of gas production was at 35°C (Figure 5). This suggests that the optimal temperatures for growth and metabolism are not necessarily equal. This is supported by the trend that we observed in our final cell counts, which was that 30°C resulted in the highest cell density (1.11×10^9 cells/mL), while 35°C showed the lowest cell density (9.78×10^8 cells/mL). As we expected at 25°C, it took a longer time for yeast to start producing a visible amount of CO_2, and

we observed a slower rate of increase in CO_2 production following this initial lag phase. This can be explained by a decreased rate of enzyme kinetics that slows down reaction rates, and thus cellular processes such as metabolism, at below-optimal temperatures (Tai *et al.* 2007). Yeast exposed to higher temperatures began to produce CO_2 noticeably sooner after they were placed in the medium compared to those at lower temperatures, and produced gas at a higher rate once the brief lag phase was complete (Figure 5). In our experiment, the 35°C treatment caused yeast to produce CO_2 faster than the supposed optimal growth temperature of 30°C. Zakhartsev *et al.* (2015) stated that yeast metabolism changes to dissipate more heat when exposed to temperatures that are above optimal, which they defined as being above 31°C. Tai *et al.* (2007) stated that the molecular mechanisms that allow this heat dissipation to occur include increased diffusion rates and increased fluidity of the cell membrane due to changes in phospholipids. A more fluid membrane enables faster transport and thus higher metabolism at higher temperatures (Tai *et al.* 2007). These cellular mechanisms enabled yeast at 35°C to undergo cellular respiration and produce CO_2 at a remarkably higher rate than yeast at 30°C. Salvado *et el.* (2011) found that *S. cerevisiae* has a maximum growth temperature of 45.4°C, which may allow the strain a competitive advantage over other *Saccharomyces* species, which are not able to grow as well or as fast at such high temperatures. They also found that at temperatures that are well above the optimal range, metabolism decreases drastically due to enzyme denaturation and consequent loss of function (Berg *et al.* 2012). This research helps explain why we observed the highest rate of CO_2 production at 35°C, as this is well below the temperature at which enzyme denaturation results in a decline in cellular respiration.

However, we made assumptions throughout our experiment which may have affected our results. We assumed that all the yeast cells were at the same point in their life cycle and that cell

counts remained constant throughout the 70 minutes. We were able to assume this because it has been shown that yeast requires 90 minutes to divide in YPD (Sherman 2002). This source of variation could have affected our CO_2 production rate in certain respirometers; if younger cells were present, the amount of CO_2 produced would be less than a respirometer full of mature yeast cells. As we could not determine the age of the yeast when using the haemocytometer and Axio microscope, we have no way of knowing if this factor had an effect on the number of cells, and thus the CO_2 production, in our experiment.

As well, we assumed that the small amount of water produced by the yeast during aerobic respiration was negligible. As yeast produced both CO_2 gas and water during this process, the water should have ultimately diluted the cell count at the end of 70 minutes when we calculated the cell concentration of each replicate. Replicates that produced more CO_2 gas should have also produced more water than the others, and should have had their cell counts the most diluted. We assumed that the level of dilution was negligible, and if it was not then we would have recorded a higher rate of gas production than the actual rate as we would have divided the total volume of CO_2 gas by fewer cells to produce a larger rate.

As we had three group members measuring the volume of CO_2 produced in each respirometer replicate at each temperature, this may have added error into our data. As we had only marked 0.5 mL differences on each tube, anywhere in between those markings had to be estimated and each group member may have had her own interpretation of the gas levels. This could have caused our data to either be lower or higher than the actual value, depending on the opinion of each group member.

CONCLUSION

Based on the results of our statistical analysis, we rejected all three of our null hypotheses and provided support for our alternate hypotheses. Temperature and time have an effect on CO_2 production per *S. cerevisiae* cell. Additionally, the effect of time on CO_2 production per wild type *S. cerevisiae* cell is not the same for 25°C, 30°C and 35°C. Our results did not support our prediction that we would see the highest rate of CO_2 production at 30°C. We observed this because although 30°C is the optimal temperature for growth of *S. cerevisiae*, it is not necessarily yeast's optimal temperature for cellular respiration. Therefore, our results demonstrate that both temperature and time could have an effect on the cell metabolism and the enzymes involved in *S. cerevisiae*.

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