The effect of temperature on the carbon dioxide production of *Saccharomyces cerevisiae* as measured by the change in volume of carbon dioxide produced

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Abstract

Cellular respiration occurs in *Saccharomyces cerevisiae* cultures in the presence of oxygen as glucose is oxidized and carbon dioxide is produced as a result of the process. For *Saccharomyces cerevisiae*, the optimal temperature for respiration should be close to 40ºC (Slaa *et al.* 2009), and we predicted that temperatures closer to 40ºC would result in a greater amount of carbon dioxide produced by *Saccharomyces cerevisiae.* The effect of different temperatures was tested by putting respirometers with *Saccharomyces cerevisiae* in 20ºC, 30ºC, 35ºC, and 40ºC. The volumes of carbon dioxide produced were calculated using the height measured at each respirometer and were compared. At 40ºC and 35ºC, there was a significantly higher amount of carbon dioxide produced than at 20ºC and 30ºC. *Saccharomyces cerevisiae* produced more carbon dioxide at temperatures closer to 40ºC.

Introduction

Saccharomyces cerevisiae is the most well studied yeast; it is known for its involvement in fermentation for wine and bread production. *Saccharomyces cerevisiae* are facultative anaerobes, and they can grow both aerobically and anaerobically. Aerobic respiration or cellular respiration is the process in which cells breaks down organic molecules for energy. This chemical reaction involves sugar and oxygen metabolizing to yields carbon dioxide, water and energy. Aerobic respiration begins with glycolysis; the oxidation of glucose to pyruvate producing 2 ATP. ATP, Adenosine-5'-triphosphate, transport chemical energy. The energy we can obtain from the hydrolysis of one ATP is 30.5 kJ/mol or 7.3 kcal/mol (Wendell *et al.* 2010). Next, pyruvate is oxidized by the TCA Cycle (Kreb Cycle) and the Electron Transport Chain; this results in a total of 34 ATP, energy equivalent form. In the absence of oxygen, yeast can undergo anaerobic respiration. There are two types of anaerobic respiration: lactic acid fermentation and alcohol fermentation; alcoholic fermentation process is widely used in the baking industries, the beer industries, and the wine industries.

Through this research, we wish to gain a better understanding of the effect of the optimal temperature and the respiration rate on *Saccharomyces cerevisiae*. Slaa *et al.* (2009) state that there is maximum growth at the optimal temperature of 35°C. Salvadó *et al.* (2011) also state that *Saccharomyces cerevisiae* has maximum growth in temperature ranges from 32.3°C to a maximum 45.4°C (Salvadó *et al.* 2011). We predicted for our alternative hypothesis that there will be an increase in CO_2 as it approaches the optimal temperature of 35°C (Slaa *et al.* 2009). Our null hypothesis is that there will be an increase or no effect in CO_2 production as the temperature shifts away from the optimal temperature, and our alternative hypothesis is that there will be a decrease in CO_2 production as the temperature shifts away from the optimal temperature.

Methods

In order to test the respiration rate of *Saccharomyces cerevisiae* culture, we recorded the amount of carbon dioxide produced in a certain period of time. Thus, we measured the volume of *Saccharomyces cerevisiae*'s carbon dioxide production using respirometers under different temperatures. We had a total of four temperature treatments, 20°C (room temperature), 30°C, 35°C, and 40°C, achieved by placing cultures in four water baths. We used regular tap water for the room temperature treatment water bath and there was no insulation. The latter three treatments were put into three individual water baths at the specific temperatures.

The original *Saccharomyces cerevisiae* culture in our experiment was clear brown in color, and the culture medium was darker clear brown than the yeast culture. We used a haemocytometer and a compound microscope to count the number of cells/ml. In order to obtain a more concentrated *Saccharomyces cerevisiae* culture, we centrifuged the culture at speed 5 for

5 minutes. For every spin, we kept only the pellet, and we added 25ml of culture medium to each tube after we collected all the pellets. We then resuspended the *Saccharomyces cerevisiae* culture using a Vortex mixer. In the end, we obtained cream white in color concentrated *Saccharomyces cerevisiae* culture and we poured it into a flask and covered the opening immediately with foil.

The respirometer we used in this experiment was set up by having a small test tube (7.5 cm high) containing solution (yeast culture or culture medium) inverted inside a large one (15 cm high). To set up a respirometer, we first filled up at least half of the large test tube with solution, and put the inverted small test tube into the large one. We inverted the large tube to let solution run into the small test tube with our thumb blocking the opening of the large test tube to avoid any leaking. We kept adding solution to the large test tube and inverting until the filled up small tube had no bubbles. We then pipetted out the extra solution left in the large test tube to the 1 centimeter mark at the bottom. We labeled every respirometer with a piece of masking tape with the temperature and replicate number as shown in Figure 1.In the experiment, every respirometer containing *Saccharomyces cerevisiae* culture was one replicate. We had three replicates for each of the four temperature treatments. The control replicate contained the same amount of culture medium that had no yeast cells, and we had the same number of controls as replicates at each treatment level.

Figure 1. The setup of a respirometer with a small test tube filled with solution inverted inside a big test tube.

After a respirometer was set up, we put it into the plastic test tube racks in the corresponding water bath and started timing. We looked at each replicate every 10 minutes to track any volume change in the small test tube by measuring the height of the clear space with a ruler. Once the bottom of clear part was below the white *Saccharomyces cerevisiae* solution in the large test tube, we had to pipet out solution from the large test tube to have an accurate measurement. Only temperatures in water baths were monitored with thermometers, other factors, such as light intensity and water pH, were not monitored as we assumed they would stay constant.

After collecting our data, we converted all the height recordings to volume using a micropipette and determined how much gas the yeast produced over 60 minutes. We also calculated the averages, standard deviations, and 95% confidence intervals (C.I.) for replicates at each temperature.

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Results

The calculated height average for replicates in each treatment level is shown in Table 1. All control replicates had no height changes (0mm height) so they were not included in the following figures.

Therefore, we were able to convert all of our data in Table 1 from height to volume, and the average volume change for replicates in each temperature treatment is shown in Table 3.

	10min	**20min**	**25min**	**30min**	**40min**	**50min**	**60min**
20C	**200**	**325**	**433.3**	**483.3**	**783.3**	**1200**	**1950**
30C	**295**	**483.3**	**600**	**650**	**650**	**650**	**650**
35C	**775**	**1766.7**	**2400**	**2450**	**2550**	**2616.7**	**2666.7**
40C	**616.7**	**1883.3**	**2416.7**	**3123.3**			

Table 3. The average volume of carbon dioxide produced in µL by *Saccharomyces cerevisiae* in the respirometer for each temperature at different time periods.

We graphed our results to compare the carbon dioxide produced by *Saccharomyces cerevisiae* at different temperatures.

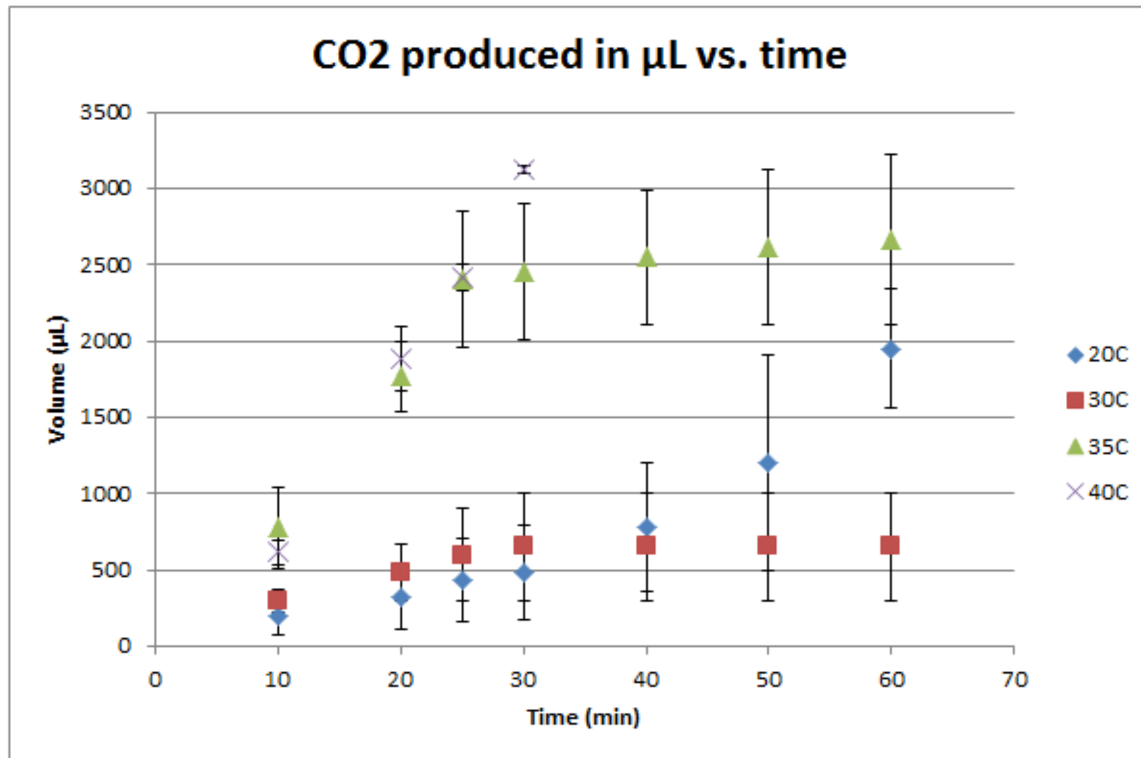

Figure 2. The carbon dioxide production of *Saccharomyces cerevisiae* (µL) at 20ºC, 30ºC, 35ºC, and 40ºC for different time observed. Bars represent 95% C.I.

Discussion

According to our results, the confidence intervals of high temperatures (35ºC, 40ºC) do not overlap with low ones (30ºC, 20ºC), so our alternative hypothesis that there is less carbon dioxide produced as temperatures shift away from the optimal temperature is supported, and the null hypothesis is therefore rejected. At 40ºC, the optimal temperature, *Saccharomyces cerevisiae* produced the maximum amount of carbon dioxide as it has the highest height reading. The carbon dioxide produced at 40ºC is also significantly higher than carbon dioxide produced at 20ºC and 30ºC at all times as the confidence intervals do not overlap. We could also see a trend that the volume increased as *Saccharomyces cerevisiae* as time increased, which indicated that respiration was a continuous process.

The cellular processes in living organisms can be impacted by the outside environment, such as temperature, so organisms respond to decreasing or increasing temperature by kinetic rate changes (Tai *et al.* 2007). In this case, *Saccharomyces cerevisiae* responded to temperature lower than optimal level by showing reduced rates of respiration. Since there is oxygen present in our experiment, the yeast culture continuously undergoes oxidation of glucose in the respirometers as $C_6H_{12}O_6(s) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l)$ (Slaa *et al.* 2009). They also conducted a similar experiment by measuring the mass of carbon dioxide produced by glucose-fed *Saccharomyces cerevisiae.* They found the greatest amount of carbon dioxide production around 35ºC, which provided the optimal condition for enzyme functions.

However, Slaa *et al.* (2009) found yeast cultures at 30 ºC had high carbon dioxide productivity as the mass of carbon dioxide produced at 30 minutes actually exceeded the production at 40 ºC. This finding contradicts our results, so it indicates that there are possible errors in our experiment. Since we had to prepare the respirometers one by one using pipettes, it was a slow process. The 30 ºC replicates were the last treatment group to be put into the water bath as the first 30 ºC respirometer was not set up until 30 minutes after the first 40 ºC one. *Saccharomyces cerevisiae* in the flask might already undergo the respiration during the half hour period as it is a natural activity. Therefore, these yeasts became less active when they were put into the water bath and the carbon dioxide production was very low. It is possible we could obtain better results from the 30 ºC replicates if they were put in earlier.

Another source of error could be that the air temperature over the water bath could have been altered when we opened the cover every 5 or 10 minutes to measure the height of carbon dioxide in respirometers. The room temperature was around 23ºC and was lower than three of our

treatment levels, so the cooler room air could alter the temperature of the yeast culture when we opened the water bath lids for measurement. The volume measured at 30 ºC and 40 ºC plateaued before reaching the 60 minutes mark. At 40 ºC, the yeast culture plateaued after 35 min in the respirometers as the respiration occurred very fast. At 30 ºC, there were no more changes in the respirometers after 30 minutes due to low activity, and we stopped recording. Thus, each treatment levels have different numbers of data points, which may cause variance in our result.

The experiment was repeated two times so there were three trials over the period of two weeks to collect the final data. In the first week of Oct.29, we were unsure about the yeast concentration to use for this type of experiment and did not centrifuge to concentrate the cells. Therefore, there were no differences among different temperatures as there was no change in most respirometers with a yeast concentration of 5.1x10^6 cells/ml. We centrifuged the yeast culture in the week of Nov.5 to concentrate the yeast culture to 1.5x10^7 cells/ml. However, we still could not obtain any statistically significant production, and we decided to conduct the experiment one more time. In our last attempt, we had 8.9 x 10^8 cells/ml and followed the same procedures except that we put the respirometer into the water bath as soon as it was set up instead of waiting for all respirometers of the same treatment level to be completed. We timed every individual respirometer rather than all of them in each water bath, and we saw more significant differences among our data. Even though we tried to eliminate errors in the experiment, they still occurred as some of our findings were contradicted with literature.

Conclusion

We were able to find significant differences in carbon dioxide produced by *Saccharomyces cerevisiae* at different temperatures. Our alternate hypothesis that there is a

decrease in carbon dioxide production as temperatures shift away from 40ºC is supported. Therefore, in the temperature ranges between 20ºC and 40ºC, more carbon dioxide is produced by *Saccharomyces cerevisiae* as the temperature approaches 40ºC, which indicates more *Saccharomyces cerevisiae* respiration.

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References

Hester, A. 2006. A Fresh Approach to US Energy Security and Alternative Fuels: The Western Hemisphere and the Ethanol Option. CIGI Working Paper No.10.

Salvado, Z., Arroyo-Lopez, F.N., Guillamon, J.M., Salazar, G., Querol, A., and Barrio, E. 2011. Temperature adaptation markedly determines evolution within the genus *Saccharomyces*. Appl. Environ Microbiol., **77** (7): 2292-2302.

Salvado, Z., Arroyo-Lopez, F.N., Barrio, E., Querol, A., Guillamon, J.M. 2011. Quantifying the individual effects of ethanol and temperature on the fitness advantage of *Saccharomyces cerevisiae*. Food Microbiol., **28** (6): 1155-1161.

Slaa, J., Gnode, M., and Else, H. 2009. Yeast and fermentation: the optimal temperature. Journal of Organic Chemistry, **134**: a-c.

Tai, S.L., Daran-Lapujade, P., Walsh, M.C., Pronk, J.T., and Daran, J.M. 2007. Acclimation of to low temperature: a chemostat-based transcriptome analysis. Molecular Biology of the Cell, **18**: 5100-5112.

Wendell, D., Todd, J., and Montemagno, C. 2010. Artificial photosynthesis in ranaspumin-2 based foam. Nano letters, **10** (9): 3231-3236.