

Sugar Slump: Higher Concentrations of Glucose Lead to Eventual Max Out in Respiration Rates of Yeast

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Fermentation, or the breakdown of substances without the use of oxygen, is an extremely commercially applicable biochemical process due to its ability to yield alcoholic byproducts. Although it is clear that different environments result in different respiration rates within cells, it has yet to be determined why exactly this occurs. We proposed that solutions with higher concentrations of glucose would produce more CO₂ because of the large amount of energy that glucose provides for cellular respiration. We conducted five trials comparing the rate of respiration in different solutions by adding them to activated yeast and then tracking their CO₂ production in parts per million per minute over a period of eight minutes. Interestingly enough, the solution with the greatest concentration of glucose showed a max out in respiration rate, which was not what we expected to observe. We believe this new approach to be of interest to brewery employees seeking information regarding methods of speeding up the fermentation process.

Introduction

The presence of glucose is essential for the successful execution of biochemical processes like cellular respiration and fermentation. In the cellular respiration process, Adenosine Triphosphate (ATP) is created while yielding waste products of carbon dioxide and water. During fermentation, substances are chemically broken down by bacteria, yeasts, and other microorganisms without the presence of oxygen to generate energy in the form of ATP. Due to fermentation byproducts of CO₂, lactic acid, and ethanol, the process has become extremely useful in

the commercial world and plays a huge role in the progression of beer and wine companies across the globe. In the majority of commercial processes, the utilized fermentation medium is a complex mixture of different fermentable sugars (Verstrepen et al., 2004). Our decision to test glucose concentration stems from the entire reaction's dependency on said monosaccharide and the fact that it is one of the most simple forms of sugar (Encyclopedia Britannica, 2018). Despite the ability of mitochondria to provide the cells with constant energy, the synthesis of ATP within cells occurs most quickly during a process called Glycolysis (Koziel et al., 2012). Glycolysis releases energy and

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pyruvic acid through the breakdown of glucose. Before fermentation or cellular respiration can begin, the cells must carry out glycolysis in order to provide adequate energy for the rest of the actions to occur successfully (Brooks, 2010).

The observation of varying respiration rates in cells has sparked curiosity regarding how different concentrations of glucose can affect the speed at which they are fermented in the yeast *S. cerevisiae*. In order to determine the role that sugar levels truly play in respiration rates, we hypothesized that yeast solutions with higher concentrations of glucose also have faster respiration rates because a greater amount of glucose can be broken down into a greater amount of ATP. If our data supports our hypothesis, then the yeast solution with the greatest percentage of glucose will experience faster respiration rates.

Methods

In order to best test our hypothesis, a fermentation reaction was created in a plastic respiration chamber and was then closely observed. To begin, *S. cerevisiae* yeast was prepared as previously described (Shaw and French, 2018). *S. cerevisiae* yeast was used because it is very easily accessible and has been well studied by other scientists. After the yeast had been activated, a solution was added to the chamber. Every trial was

conducted with a different solution, each containing its own concentration of glucose (see Table 1).

Due to our desire to determine the effect of glucose level on respiration rate, this is the only variable that was changed throughout the experiment. Things like type of plastic, amount of yeast, the speed of stir rod, and time of data collection were kept the same so we could be sure that the only thing affecting our results was the percentage of glucose in the solution. The data that was collected was the rate of CO₂ production in parts per million per minute (ppm/m) and this was collected using a CO₂ probe. This probe was given 5 minutes to warm up before it was used in order to ensure an accurate reading of the yeast solution's metabolism.

Since we were interested in the influence that glucose has in the speed of respiration, we included two comparison groups: a solution with 0% glucose and a solution with 100% glucose. The solution with 0% glucose was to provide us with a baseline of respiration rate when there is no sugar present. A 100% glucose solution was tested because we believe glucose to be the most fermentable sugar and in turn best able to increase respiration rates of yeast solutions. Including these two control groups, ten trials total were tested. After the solution was added to the chamber, the CO₂ probe was inserted and began measuring the rate of

| Glucose concentration (%) | 0% | 25% | 50% | 75% | 100% |
|---------------------------|----|-----|-----|-----|------|
| Yeast Solution (mL) | 10 | 10 | 10 | 10 | 10 |
| Water (mL) | 10 | 7.5 | 5.0 | 2.5 | 0 |
| .3 M glucose (mL) | 0 | 2.5 | 5.0 | 7.5 | 10 |

Table 1: Classification of solution based on glucose concentration

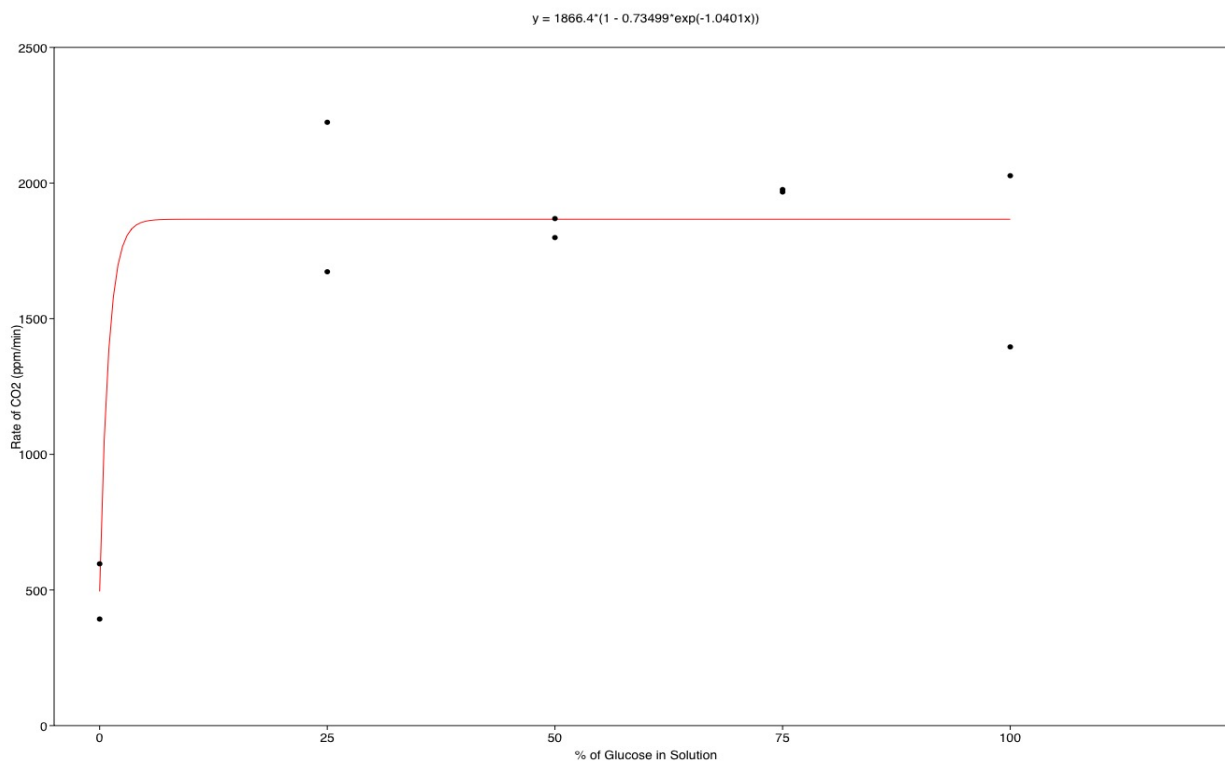


Figure 1: The graph above shows the relationship between the percentage of glucose in a solution in comparison to water and the Rate of CO₂ (ppm/min). The Rate of CO₂ (ppm/min) increases when glucose is added to the solution but maxes around 25% glucose concentration.

CO₂ production once every 15 seconds for eight minutes. Once the trial ended, the LoggerPro software provided us with the rate of CO₂ production in ppm/minute by applying a linear fit line and in turn the slope (LoggerPro, 2016). These steps were repeated for each solution after rinsing out the respiration chamber in between tests. Each solution was tested twice. In order to best represent our data, we used a scatter plot. This type of graph made the differences in trends between each type of solution easier to identify. We used a non-linear model to analyze our data (PAST3, 2013). We recorded the lowest AIC value in order to insert a line of best fit.

Results

The solution with the highest concentration of glucose did not exhibit the quickest rate of

respiration. As shown in Figure 1, the solution with the highest concentration of glucose is actually where the graph began to max out. Before the solution with 100% glucose was used in the fermentation test, the rate of respiration increased slowly, but upon reaching this concentration the rate of respiration stopped climbing.

After inputting our data into PAST3, and running a non-linear fit analysis, we determined that the model that best represented our data was the Von Bertalanffy line (AIC= 4.6E5). Figure 1 shows that the Rate of CO₂ (ppm/min) increases dramatically when glucose is added to the solution, but it maxes out around 2250 ppm/min. The Rate of CO₂ (ppm/min) remains fairly constant even when the glucose concentration increases. Our graph shows that there is a larger spread between the two

trials of 25% and 100% glucose concentration than 50% and 75%.

Discussion

Contrary to our hypothesis, the solution with the highest concentration of glucose did not experience the quickest rate of respiration (Figure 1). We wanted to find out if different concentrations of glucose affected the speed at which yeast becomes fermented to determine the role that varying sugar levels play in respiration rates. Our data suggest that, in general, increasing the amount of glucose in a solution increases respiration productivity up to a certain point at which it then maxes out.

As shown in Figure 1, the solution with the quickest rate of respiration rate was the one with 75% glucose. Our data suggests that metabolic speed is directly related to glucose concentration up to a certain point in which it becomes inverse, consistent with the findings of Lopez, Orlic, Querol, and Barrio (2009). This study consists of much higher concentrations of glucose and shows a strong decrease in yeast growth. The pattern seen in this study is similar to what we started to see occur in the respiration rate of our solution with 100% glucose. Although our respiration rate only maxed out and did not actually start to decrease, we can assume that continued tests with solutions of greater saturation would show said decrease.

The max out of respiration rates can be explained by glucose's ability to trigger unexpected, hormone-like effects including the activation of cellular growth, the mobilization of storage compounds, and the diminution of cellular stress resistance (Verstrepen et al., 2004). These effects can lead to problems like slow or incomplete fermentation (Verstrepen et al., 2004). It is likely that these effects became more apparent as our concentration of glucose increased.

Despite the presence of hormone-like effects within our fermentation chamber, the use of a

variety of yeast types may have resulted in respiration rates that would not have followed the observed trend. Commercial yeast has been cultured by civilizations for thousands of years and as a result has become optimized to conditions like the ones we created in our fermentation chamber. Non-Saccharomyces are a more natural form of yeast and are able to produce more metabolites during the fermentation process (Llexia et al., 2016). *Wickerhamomyces anomalus*, for example, is a type of yeast that has its enzyme used during chemical reactions which plays a big role in medicine and biotechnology (Willaert 2017). Natural forms of yeast like the ones described have not been used as often in the scientific environment and so may or may not have created results that matched our hypothesis more closely.

Based on our data, it is clear that glucose concentration above a certain point will prohibit respiration rate of yeast from increasing, but further research regarding less optimal forms of yeast would need to be conducted in order to determine the role that glucose truly plays in the speed of respiration.

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