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The Effect of *S. cerevisiae*'s Metabolization of Natural and Artificial Sugars on the Rate of Carbon Dioxide Production

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The rate of cellular respiration has been studied for many years. This experiment addresses why different sugars are better for fermentation than others. We proposed that artificial sugars are worse for fermentation than natural sugars because they are manufactured to not have calories, therefore producing less ATP and by extension, carbon dioxide. We conducted five trials to compare the rates of carbon dioxide production using the yeast *S. cerevisiae* to metabolize glucose, saccharine, and water. The findings of this experiment were significant as it proposes data of interest to scientists studying healthier alternatives for alcohol.

Introduction

Alcohol is a popular drink consumed by many people around the globe. What is not popular, however, is how alcohol is made. The process of alcohol production is known as fermentation, which occurs when yeast metabolizes sugar through anaerobic respiration. Anaerobic respiration is a cellular process that uses electron acceptors other than oxygen, as seen in a study where graphite electrodes are used as electron donors (Gregory et al., 2004). Contrary to other studies, it specifically looked at anaerobic respiration. On the other hand, a study on aerobic respiration found that aerobic respiration is a cellular process that has oxygen as a reactant, (Spees et al., 2006).

Both aerobic and anaerobic respiration are also known as cellular respiration. While both types of cellular respiration have multiple products: ATP, NADH, carbon dioxide, and alcohol ethanol (Shaw and French, 2018), anaerobic and aerobic respiration do not produce the same relative amounts of ATP. This statement was corroborated in a study on ATP production, where it was found that the ratio of ATP produced by fermentation to the total amount of ATP produced by fermentation and aerobic respiration is a small number (Raymond et al., 1985), meaning that fermentation produced a small amount of the ATP and aerobic respiration produced a large amount. This is important as ATP is energy, so it powers the cell. This means that fermentation, though it leads to popular alcoholic

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beverages, may not be the best method of providing energy.

There are many factors that affect the rate of fermentation. For example, one experiment studied the effect of the type of sugar on fermentation when there are different kinds of monosaccharides, disaccharides and polysaccharides (Yoon et al., 2003). In another experiment, the type of yeast and its effect on the rate of fermentation was measured. (Lurton et al., 1995). While these two experiments tested different factors for the rate of fermentation, they only tested a few of the potential factors. There are others that have not been thoroughly tested.

One of those factors is the effect of natural and artificial monosaccharide sugars, which is tested in this experiment. Artificial sweeteners are sugars that are manually produced to be "healthier" alternatives to natural sweeteners. A study on type 2 diabetes found that natural sugars are a higher cause of diabetes than artificial sugars (Koning et al., 2011). Additionally, artificial sweeteners are healthier as they do not produce as many calories as natural sweeteners, as stated in a study on the difference between natural and artificial sugars (Niu et al., 2011). Since calories are units of energy, this means that artificial sweeteners do not produce as much energy as their natural counterparts. Therefore as energy, or ATP, is one of the main products of fermentation, it is hypothesized that artificial sugars are worse for fermentation than natural sugars because they produce less ATP and, by extension, carbon dioxide.

It is predicted that, if the hypothesis is supported, then the natural sugar (glucose) would produce more carbon dioxide than the artificial sugar (saccharine), which would produce similar amounts to water. Additionally, if the hypothesis is not supported, then it is predicted that saccharine would produce more carbon dioxide than glucose, or they would produce equal amounts.

Methods

In this experiment, the varying effects of artificial sugars and natural sugars on cellular respiration were measured. This was measured by using a Vernier Carbon Dioxide Gas Sensor to measure the carbon dioxide output in parts per million, and collected on a LoggerPro graphing system. As carbon dioxide is a product of cellular respiration, this process measures the outcome of the cellular respiration occuring.

The effects of the sugars on cellular respiration were measured by observing how different sugars affect the carbon dioxide output of cellular respiration. This was done by making the experimental groups the artificial sugar saccharine and the natural sugar glucose, respectively, and making the control group water. A negative control group was chosen so that the results of the experimental groups could be compared to the control group's data, to see if fermentation occurred.

To start off the experiment, the Canvas protocol for yeast making was adhered to (Shaw and French, 2018). After the alloted time, 10 mL of 0.3M glucose was added to the mixture and left to stir for ten minutes. Ten minutes was chosen as the trial duration to ensure that there was enough time for cellular respiration to fully occur. During this time, the Vernier Carbon Dioxide probe was used to measure the rates of carbon dioxide production in the final mixture, by measuring the amount of carbon dioxide gas produced. Five trials were performed, and then all steps were repeated with 0.3M saccharine. Additionally, all steps were repeated for one deionized water trial in order to compare the fermentation rates between the control group and the experimental group. The trials for glucose, saccharine, and water were kept identical to ensure data accuracy.

Once all data was collected, the rates of the carbon dioxide production were found by dividing the amount, in parts per million, by the time, in

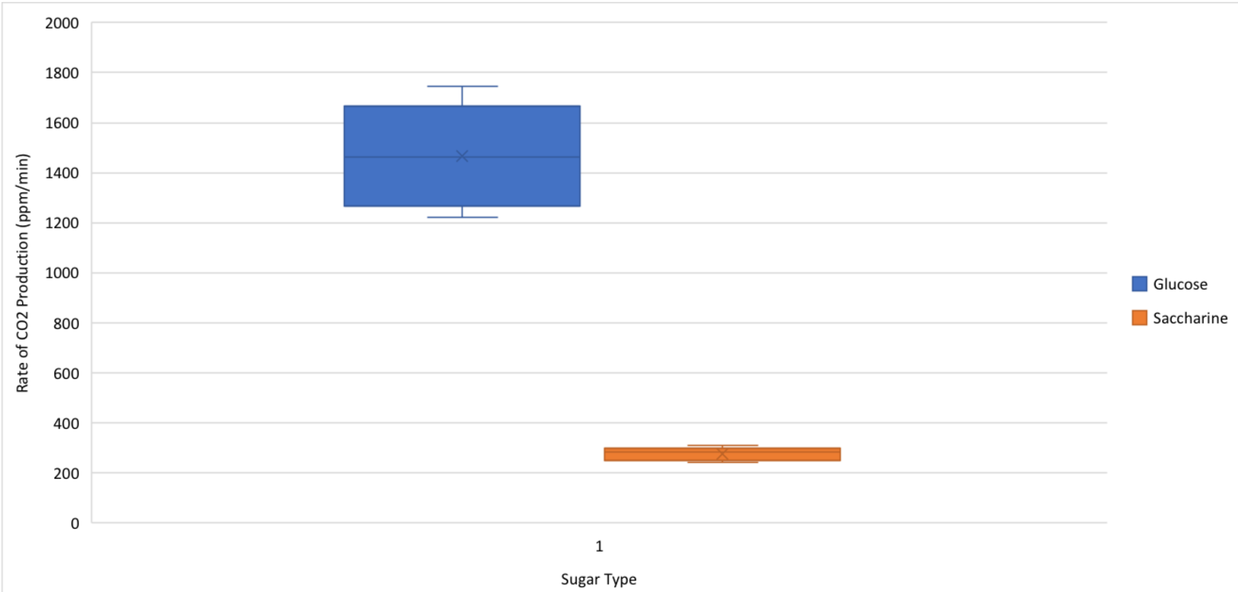

Figure 1. The rate of carbon dioxide production for glucose is higher than the rate of carbon dioxide production for saccharine.

minutes. These rates were then inputted into a box-and-whisker plot (Figure 1) to easily showcase the changes between the production of carbon dioxide for natural and artificial sugars. Then an unpaired t-Test with assumed variance was ran through the use of Microsoft Excel's data analysis tool. An unpaired t-Test was chosen because this experiment contains two groups of nominal and measurement data that are unpaired, and it was done to prove whether or not the data from the trials were statistically different, along with examining if the data was significant or not.

Results

The data collected in this experiment showed a general trend of the natural sugar, glucose, having a higher rate of carbon dioxide production than the artificial sugar, saccharine (Figure 1). For glucose, the average rate of carbon dioxide production was 1465.927 ppm/min. For saccharine, the average rate of carbon dioxide production was 276.176 ppm/min. This shows that the average rate of carbon dioxide production for glucose was significantly higher than it was for saccharine, at 1189.751 ppm/min higher.

Additionally, it was found that the average rate of carbon dioxide production in the control group (water) was 360.622 ppm/min. This rate of carbon dioxide production is much more similar to saccharine's rate of carbon dioxide production than it is to glucose's rate of carbon dioxide production.

As seen in Figure 1, the data gathered for glucose's rate of carbon dioxide production has values varying much more than the data gathered for saccharine's rate of carbon dioxide production.

An Unpaired t-Test was conducted to compare the effect of sugar type on the rate of carbon dioxide production in natural sugar and artificial sugar conditions. There was a significant difference between the two conditions; $t(4) = 2.30600414$, $p = 1.4925 \times 10^{-6}$.

Discussion

The data gathered throughout this experiment supported the hypothesis, as the rate of carbon dioxide production for natural sugars was higher than the rate of carbon dioxide production for artificial sugars. The predictions made at the start of this experiment were also supported, as the prediction for if the hypothesis was supported stated that the natural sugar would produce carbon dioxide at the highest rate, which can be seen in Figure 1, and the rate of carbon dioxide production for saccharine and water were similar. As saccharine and water's carbon dioxide rates were similar, the speculation can be made that neither product actually went through fermentation. This speculation is backed up by a study on creating a new probiotic drink, where it was found that saccharine had no effect on the fermentation process (Angelov et al., 2006).

The results of this experiment are pertinent to alcohol industries, as they prove that saccharine does not go through fermentation. Thus, the formation of a "diet alcohol" can not occur, as alcohols cannot be made without fermentation. Additionally, these results are also important for the baking industry. A study on the process of baking found that the use of yeast in the process led to a higher production of folate, which is produced in the baking process, than would be produced without yeast (Karilouto, 2004). As baking a product also needs fermentation to be successful, saccharine could not be used to create healthier breads, as the dough would not rise. Both of these industries would need to utilize a natural sugar, such as the glucose used here, to create their products.

Alternative interpretations for why the rate of carbon dioxide production for glucose was much higher than the rates for saccharine and water is because water and saccharine do not contain glucose, which is a major component of glycolysis, another name for fermentation. This means that water and saccharine can not even ferment, and thus glucose produces carbon dioxide at a higher rate than the two.

Future experiments could expand on these results by experimenting on different kinds of natural sugar monosaccharides and artificial sugar monosaccharides, such as honey and high fructose corn syrup, to determine if the fermentation rates can accurately be used to represent the general trend of carbon dioxide production rates for artificial and natural sugars.

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