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Salt concentration increases *Saccharomyces cerevisiae*'s ethanol production during fermentation

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Abstract

S. cerevisiae, a type of yeast typically used in alcoholic brewing, ferments at a normal rate without manipulation. Manipulating it with varying concentrations of sugars or salts allows for variation in fermentation. We proposed that manipulating the salt concentration may result in a slower fermentation process. We conducted 9 trials comparing the increase or decrease of ethanol production as 2 varying salt concentrations were added and compared it to a control group with no added salt concentration. We discovered that salt concentration may increase the production of ethanol within the fermentation process. We expect these new findings to be of interest to those who study the fermentation and the products that are released as fermentation increases, such as marine environmentalists and industrialists.

Introduction

Fermentation is the biological process performed that allows organisms to produce energy without oxygen. Glucose is broken down anaerobically using ATP, water, and pyruvate and releases chemicals such as carbon dioxide and ethanol. Water is essential in fermentation. High substrate concentration has been shown to inhibit yeast growth and results in high osmotic pressure and low water activity (D'Amore, 1991). Salt is hygroscopic, meaning that it attracts moisture. In the presence of salt, the water molecules are moved by osmosis away from the yeast, which slows down the fermentation process (Luchian and Canja, 2010) This study discovered the effect of salt on bread production and the ability to continue to make bread in a salt solution. This inspired us to discover how the concentration of salt affects the ethanol production of yeast fermentation. For our study we used *Saccharomyces cerevisiae* because it is a commonly studied model of eukaryotes due to its basic cellular processes. These processes are similar to those of higher eukaryotic organisms, including humans (Chae et al., 2013). We hypothesize that a high concentration of salt causes the ethanol production to decrease because it

will dehydrate the yeast through osmotic stress. If a solution with a higher salt concentration produces a lower amount of ethanol, then our hypothesis is supported. If a solution with a higher salt concentration yields a lower amount of ethanol or does not change the amount of ethanol produced, then our hypothesis is rejected.

Methods

To test the effect on yeast fermentation, we varied the salt concentration. We prepared three yeast solutions according to the instructions described by Shaw & French (2017). Each solution included 0.6 grams of dry yeast and 10 mL distilled water and received 5 mL of glucose so that fermentation could occur regardless of the salt concentration. Solution 1 served as our control, and therefore received no salt solution. This created a total volume of 15 mL, while the other solutions had a volume of 20 mL. Solution 2 received 5 mL of a 3.0 M salt solution, creating a final molarity of .75 M. Solution 3 received 5 mL of 1.5 M salt solution, creating a final molarity of .375 M. For each trial, the yeast, glucose, salt, and water were placed in the bottom of the respiration chamber. The magnetic stirrer ran for 3 minutes. Then, an ethanol sensor was placed above the solution, and a rubber stopper closed the system. The ethanol sensor, which was connected to LoggerPro, collected data for 7 minutes (LoggerPro3). Three trials were conducted for each solution. The temperature of the three solutions was kept constant at room temperature. After data collection ended, the percent of ethanol in the respiration chamber at the end of each trial was recorded because the amount of ethanol in the chamber indicates the metabolic rate of fermentation. A scatterplot was used to display our data. The concentration of salt was plotted on the x-axis, and the amount of ethanol plotted on the y-axis. We used the scatter plot to find the line of best fit and the R^2 value to analyze the relationship between our variables. Then we used Past3 software to find the correlation.

Results

Our control groups, the group that had no added salt, showed that the average ethanol production of yeast fermentation was 40.924 ppm. The data ranges over 1.8586 ppm. Solution 2, which had a 3M salt solution added, had an average ethanol production of 43.6538 ppm. The data ranges over 13.60 ppm. Solution 3, which had 1.5M salt solution added, had an average ethanol production of 37.581 ppm. The data ranges over 31.257 ppm. Our line of best fit, y=.7002x+41.4, has a correlation coefficient of .0235 (See Figure 1). Ethanol production and salt concentration were weakly, positively correlated, r(8)=.12863, p=.74154 (Hammer and Harper, 2013).

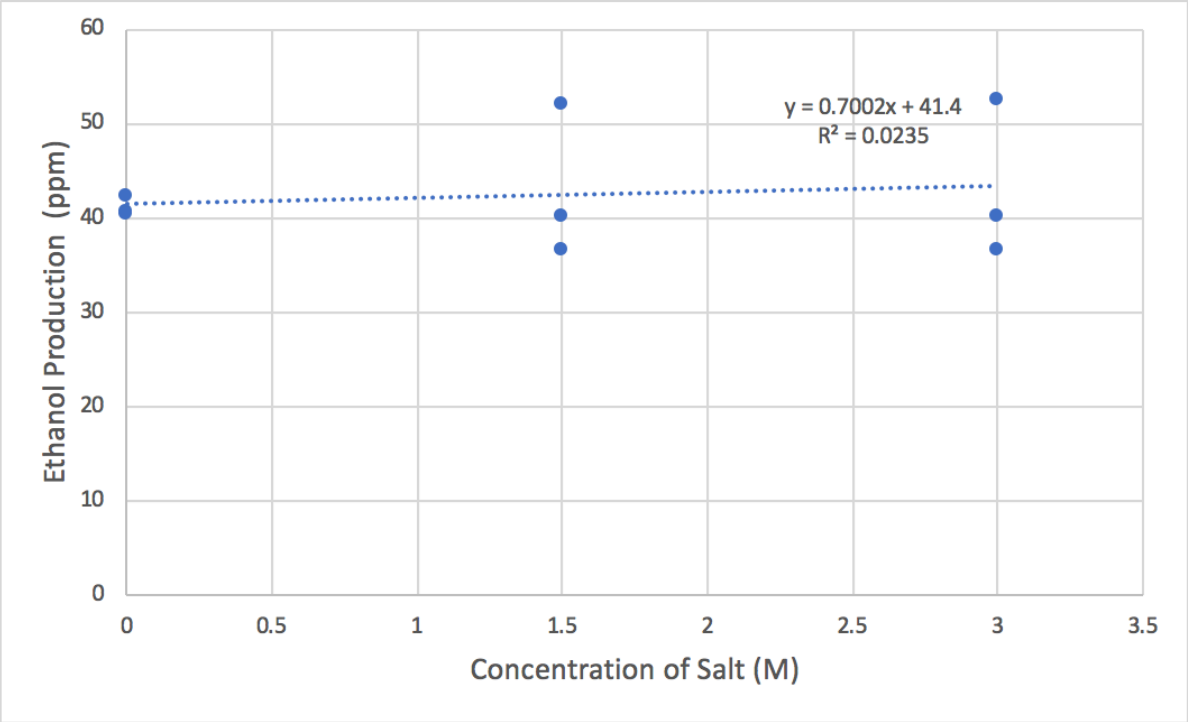

Figure 1: shows the change in ethanol production (ppm) compared to the concentration of the salt solution (M). The salt solution was added to the mixture made of 10 mL distilled water, 5 mL glucose, and .6 grams of *S. Cerevisae*. Solution 1 received no treatment of salt solution.

Discussion

We reject our hypothesis that ethanol production will decrease when subjected to a high concentration of salt, because as our results show (See Figure 1) the concentration of salt on average increases ethanol production. Based on the data collected and the statistical test run, there is not a significant correlation between salt concentration and ethanol production. This suggests that there could be a relationship, but there are external factors that weigh more heavily on the production of ethanol during fermentation. External factors include type of sugar used, differentiation of the yeast sample taken, or human error. We used glucose as our added sugar. Based on previous studies that have been done, glucose is one of the sugars that increases ethanol production the most (Bauer et al., 2016). By changing the type of sugar used, our results could have been altered. Another external factor could be whether or not our yeast used was salt-resistant. Certain yeast strains have been mutated to be salt-resistant or salt-tolerant. This would affect the ethanol production by maintaining the average production as if we hadn't added salt at all. These strains are demanded for certain industrial processes such as brewing and production of yeast biomass (Tekarslan-Sahin et al., 2018). Another field this could be seen in would be the study of marine environments: yeast that has been exposed to or isolated from salt in marine environments have been found to produce different respiration and fermentation rates (Norkans, 1968).

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