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Investigating the Impact of Glucose and Fructose as Carbon Sources on Xylitol-Induced Inhibition of Yeast Fermentation Rate

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ABSTRACT

The process of yeast fermentation plays a huge role in many of the aspects of our world today, but most recently in the way it has more been found to have a profoundly positive impact on wastewater remediation, as well as in biofuel production. The process requires the use of carbon sources, most commonly glucose, fructose, or sucrose, and oftentimes occurs most effectively with the use of glucose. As helpful as this process is, there are quite a few factors that seem to interfere with it in the environments of wastewater treatment plants and biofuel production factors, namely the presence of multiple carbon sources, and xylitol. Xylitol is a known competitive inhibitor in the yeast fermentation process. This paper therefore examined the use of glucose and fructose as a carbon source in yeast fermentation, investigating whether either of these sources slow or inhibit the process when xylitol is present. Yeast fermentation rate was measured through taking the height and circumference of balloons secured over each technical replicate periodically. This approach allowed for the rate to be measured in terms of carbon dioxide production by the yeast as it fermented. Balloon circumference and height were analyzed separately using linear mixed-effects models that accounted for repeated measurements from each tube. Circumference showed a significant carbon source × xylitol concentration × time interaction, whereas height showed a significant carbon source × time interaction but no significant three-way interaction. These results overall supported there being an increasingly negative impact on yeast fermentation rate when glucose and fructose were used as a carbon source while xylitol concentration was increased incrementally. The results provide valuable insight into what factors should be more heavily considered in future wastewater treatment and biofuel production plans, in order to ensure their highest efficiency.

INTRODUCTION

Food fermentation is what acts as a natural preservative for a large majority of the foods we consume, including bread, cheese, and alcohol, keeping them safe and healthy to eat for extended periods of time (Nicula et al.). From a biochemical stance, fermentation is simply a metabolic process used when organisms need to produce energy (in the form of ATP) in the absence of oxygen. It's a process used by

many organisms in oxygen-deficient environments, including humans through the use of lactic acid fermentation, and bacteria to survive in anaerobic environments. Fermentation can be broken down into steps, the first of which is a process known as glycolysis. The process begins with a glucose molecule. In heterotrophs, glucose is obtained from food, while in autotrophs, glucose is synthesized from CO_2 through processes like photosynthesis or chemosynthesis (Chaudry et. al). One glucose molecule is then broken down into two pyruvate molecules, which are intermediate metabolic molecules. Next, two ATP molecules are produced, which are the most usable energy currency of the cell due to their high-energy bonds and ability to be reconverted back into ATP from ADP, quickly. Additionally, during this process, two NADH molecules are produced, which act as electron carriers within the cell, and play a critical role in the continuation of glycolysis, and other metabolic cycles. Further, these NADH molecules are then oxidized back into NAD+, through the acceptance of an electron (Chandel). Organisms are able to use this incredibly versatile process to create energy, in various different environments, even when conditions aren't ideal.

The biochemical metabolic processes that occur after glycolysis vary based on the organism. For instance, humans can undergo lactic acid fermentation (often leading to the sore feeling in muscles after strenuous exercise), or alcoholic fermentation in organisms such as yeast. In lactic acid fermentation, pyruvate is then reduced by NADH to form lactate and NAD+ (Aguirre-Garcia et al). Meanwhile, in alcoholic fermentation, pyruvate is instead converted to acetaldehyde, which releases CO2 as a byproduct, leading to the bubbles often seen in fermented products. Acetaldehyde is then reduced by NADH to form ethanol and NAD+. Overall, the goal of any cycle of fermentation is to continuously regenerate NAD+, in order to keep the process of glycolysis going to produce energy – in the form of ATP – for the organism to survive (Maicas).

Although glycolysis yields a significantly lower amount of ATP per glucose molecule, compared to the electron transport chain (which requires oxygen), it's nonetheless an extremely important metabolic process for organisms to survive in the absence of oxygen. The process of yeast fermentation is one such example of this anaerobic condition. In circumstances such as baking, beverage production, and even in processes (Attfield et al.) such as manufacturing biofuel. This last use is especially key for our world today, as we are constantly on the search for an energy resource that isn't damaging to our already deteriorating environment, and is also sustainable and efficient to use. It is due to this need, that scientists have begun looking into the potential of yeast fermentation to help create a biofuel that would achieve this purpose. The most common biofuel that is most widely used is bioethanol, due to its significant impacts in reducing crude oil consumption and environmental pollution. Bioethanol is also known as ethyl alcohol, chemically C2H5OH and can be used as pure ethanol or used in blends such as "gasohol" (a blend of gasoline and ethanol). Microorganisms such as yeasts play a key role in the production of bioethanol, as they are able to ferment a very wide range of sugars into ethanol.), and second-generation bioethanol uses other lignocellulosic biomasses, including wood, straw and grass (Mohd Azhar et al.). These forms of lignocellulosic biomass often create by-products when being used in the fermentation process, these by-products often include products such as xylitol, which can originate from hemicellulose (from wood and other agricultural waste).

Certain yeast strains can also be used to metabolize pollutants such as those in Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) conditions which are very common in wastewater. In these anaerobic conditions, yeast strains such as Saccharomyces cerevisiae use these pollutants in the wastewater as carbon sources (Nicula et al.). The use of yeast for fermentation in these plants is especially helpful, due to the typically high sugar content of the pollution in food-production wastewater. Since yeast is able to ferment most successfully with the use of the glucose sugar, it can be especially key in being able to continuously break down these sugars, and therefore reduce pollution in the wastewater of food and beverage production.The fermentation process overall results in ethanol, CO2, and biomass as products, which have much less of a harmful impact on the environment and can further be used in biofuel production, as discussed previously. It's therefore rather important in this aspect that we both understand what factors affect yeast fermentation, and know how to best optimize the rate and outcomes of yeast fermentation, to continue to provide these benefits to our environment.

As many benefits as yeast fermentation provides us, there are still challenges that seem to be limiting the success of this process in becoming as efficient and widespread as possible. One issue seems to be the other chemical factors or by-products that tend to be produced during the process of biofuel production. The main impactful by-product being the chemical compound xylitol (Deng et al.). Xylitol is a five-carbon sugar alcohol derived from xylose, which is a major component of hemicellulose in lignocellulosic biomass. This is the same material used in biofuel production, and in that process, the breakdown of the material releases xylose, which may be partially converted into xylitol through reduction reactions (Aeini et. al). Unlike the carbon sources yeast tends to prefer, such as glucose and fructose, xylitol can not be used very effectively by yeast strains, and can instead have the reverse effect by accumulating and slowing the fermentation process. In environments with multiple of these preferable carbon sources then, xylitol may act as a competitor and therefore interfere with the yeast fermentation process, decreasing its positive effects on the decrease of pollution in our environment. It's extremely important that we understand exactly what factors may interfere with each other in the yeast fermentation process, to ensure the most efficiency and success possible in biofuel production and wastewater treatment. There is little known about the true effects of xylitol on yeast fermentation when in an environment with different carbon sources. Therefore, the study in this paper will examine if the use of glucose and fructose as a carbon source would cause xylitol to slow or stop yeast fermentation, using the indirect measure of CO2 provided by the measurement of balloon circumference and height over time.

MATERIALS AND METHODS

Strains

Dry baker's yeast produced by LaSaffre was used throughout the study (Global identification number: 00017929159108).

Preparation of stock solutions

A 10% glucose (Modernist Pantry, UPC: 852473004838, 819543012964) stock solution was made by combining 50.0 grams of glucose with 500 mL H2O. The glucose solution was slightly heated to fully dissolve the glucose into solution. A 10% sucrose (Modernist Pantry, UPC: 852473004722) stock solution was made by combining 50.0 grams of sucrose with 500 mL H2O. A 10% fructose (Modernist Pantry, UPC: 852473004494) stock solution was made by combining 50.0 grams of fructose with 500 mL H2O. The low xylitol solution (1%) was obtained by diluting 8 mL of the 10% stock solution into 72 mL of H2O. The high xylitol solution (3%) was obtained by diluting 24 mL of the 10% stock solution into 56 mL of H2O.

Measurements of yeast fermentation

For each condition, 25 mL of the appropriate sugar stock solution was added to a 50 mL conical tube. For conditions requiring xylitol, 25 mL of the prepared xylitol solution (1% or 3%) was added. For control conditions without xylitol, distilled water was added to maintain a consistent total volume of 50 mL per tube. All samples were equilibrated in a water bath maintained at approximately 30–32°C for 5 minutes prior to yeast addition. Following equilibration, 0.50 g of active dry yeast was added to each tube. Balloons were immediately secured over the opening of each conical tube to capture carbon dioxide produced during fermentation. T0 was measured immediately following the addition of yeast. The circumference and height of the balloon of each test condition was taken at 10 minute intervals. Protocol was followed across three biological replicates, across three testing days. Balloon height and circumference were used as semiquantitative proxies for relative fermentation activity, and were recorded in 10 minute intervals. During alcoholic fermentation, yeast produces carbon dioxide, which accumulates in the balloon secured over each tube. Therefore, expansion of the balloon over time was interpreted as reflecting greater cumulative gas/CO2 production. This interpretation was supported by the minimal inflation observed in the control tubes lacking a fermentable carbon source. Since the same balloon type, tube volume, solution volume, yeast mass, incubation temperature, and measurement schedule were used across all experimental conditions, the differences in balloon dimensions provided a standardized basis for comparing the relative CO2 accumulation, and therefore yeast fermentation rate, among treatments.

Statistical Analysis

Statistical analyses were conducted in jamovi using the GAMLj linear mixed-model module. Figures displaying estimated marginal means and 95% confidence intervals were generated from the fitted models.

RESULTS

The overall trend seems to be that as xylitol concentration increases, for the carbon sources of both glucose and fructose, the balloon height and circumference begins to increase at a slower rate, resulting in an overall lower value for the balloon height and circumference. There seems to also be a considerably

more noticeable effect on the balloon circumference when compared to the height. It is to be noted that balloon height and circumference were used as indirect measures of CO2 accumulation during yeast fermentation. Fructose being used as a carbon source with no addition of xylitol tended to result in a higher rate of yeast fermentation when compared to sucrose and glucose, as previously assumed, but both glucose and fructose seemed to have been affected around the same amount by the increasing concentrations of xylitol. The use of sucrose as a carbon source with and without the addition of xylitol did not reveal the typical results expected. There seemed to have been little to no change in both balloon height and circumference throughout the time periods, and despite other changing factors such as concentration of xylitol. Sucrose, although not ideal, is a viable carbon source for yeast fermentation, yet the experimental results did not align with this. This may be due to the texture and type of the sucrose itself. While both glucose and fructose were in the forms of salt-like grains, the sucrose was in the form of a fine white powder, causing the texture of the sucrose when dissolved in water to be thick and gelatinous, rather than the clear liquid that both glucose and fructose were. This may have possibly resulted in yeast fermentation being unsuccessful when sucrose was used as a carbon source. The correlation between xylitol concentration and the balloon height and circumference still remains however, for glucose and fructose. Since both the balloon height and circumference decreased as xylitol concentration increased, this can serve as a possible indicator that the rate of yeast fermentation decreased as well. It can therefore be reasonably interpreted from this that xylitol may have a negative effect on yeast fermentation when glucose or fructose are used as carbon sources. The results then support the overall hypothesis of this paper, which stated that xylitol would slow or stop yeast fermentation when glucose or fructose were used as carbon sources.

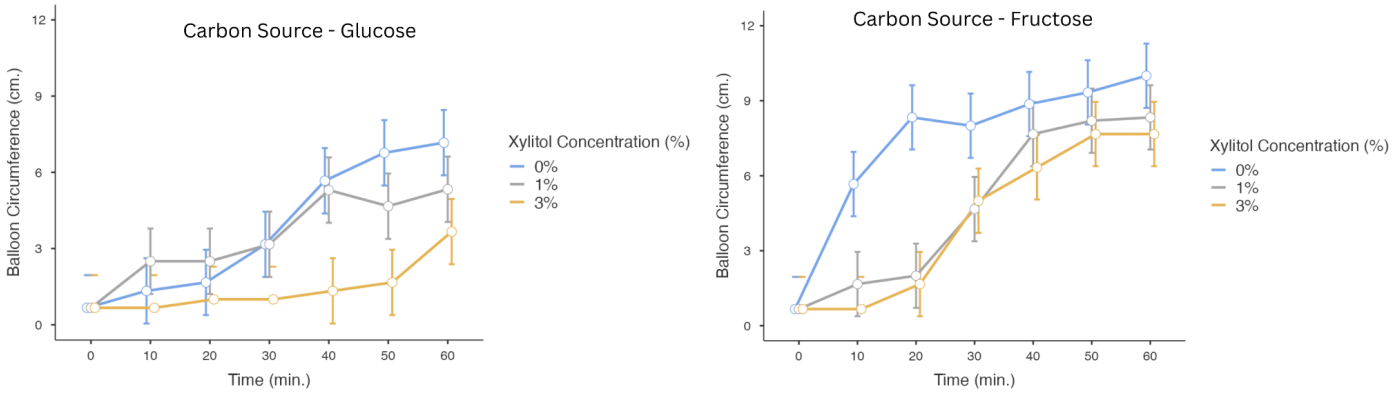

Figure 1. (Left Panel) Balloon circumference growth rate with the use of glucose decreases with higher concentrations of xylitol (Right Panel) A similar trend can be observed for balloon circumference with the use of fructose, as the overall balloon circumference grows at a slower rate, as xylitol concentration and time increase.

The left panel shows the glucose conditions, and the right panel shows the fructose conditions. In both carbon-source conditions, the xylitol-treated groups generally showed less balloon inflation over time than the 0% xylitol control, especially at the 3% concentration. Since balloon circumference was used as one of the indirect measures of carbon dioxide accumulation, this pattern is consistent with the hypothesis that xylitol would slow yeast fermentation when glucose or fructose was used as the carbon source. The points and connecting lines represent estimated marginal means, and error bars represent 95% confidence intervals. Three independent experimental replicates were included per condition (n = 3), with no within-run technical replicates.

Fixed Effects Omnibus Tests

	F	df	df (res)	p
Run	0.144	2	10.00	.867
Carbon Source	52.057	1	10.00	<.001
Xylitol Concentration (%)	19.576	2	10.00	<.001
Time (min.)	133.280	6	72.00	<.001
Carbon Source ✻ Xylitol Concentration (%)	3.415	2	10.00	.074
Carbon Source ✻ Time (min.)	12.610	6	72.00	<.001
Xylitol Concentration (%) ✻ Time (min.)	3.837	12	72.00	<.001
Carbon Source ✻ Xylitol Concentration (%) ✻ Time (min.)	7.132	12	72.00	<.001

Table 1. Fixed-effects omnibus tests for balloon circumference.

A linear mixed-effects model tested the effects of experimental run, carbon source, xylitol concentration, time, and their interactions with balloon circumference. Carbon source, xylitol concentration, and time were significant, as were the carbon source × time, xylitol concentration × time, and carbon source × xylitol concentration × time interactions, as indicated by the p values depicted in the table. The significant three-way interactions indicate that the relationship between xylitol concentration and balloon circumference over time differed between the glucose and fructose conditions. This, along with the patterns shown in Figure 1, supports the effects xylitol has on yeast fermentation with the increase of time, along with its tendency to have stronger inhibition on glucose as a carbon source. Experimental run and the carbon source × xylitol concentration interaction meanwhile, seemed to not be significant. Each condition included three independent experimental replicates (n = 3 per condition), with no within-run technical replicates.

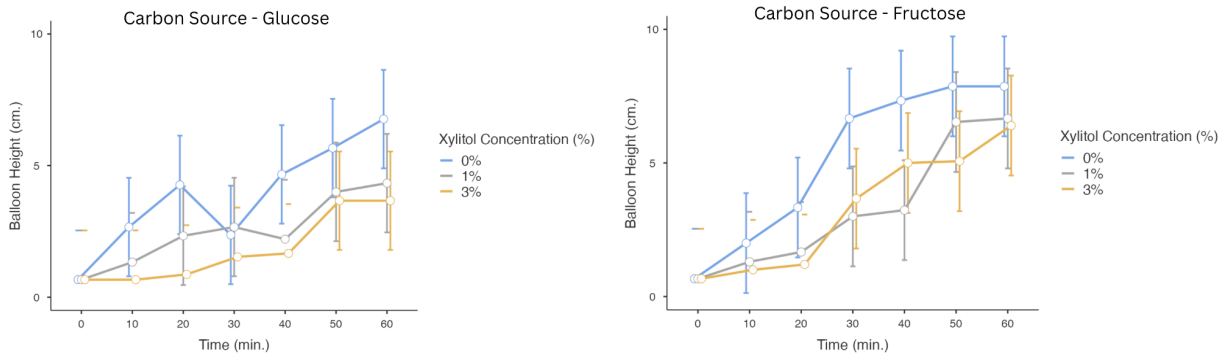

Figure 2. (Left Panel) Balloon height shown in relation to glucose as a carbon source with the addition of xylitol, with a suggested negative impact on height as time goes on (Right Panel) A similar pattern exists for balloon height in relation to fructose as a carbon source, as balloon height is shown to increase slower, and end in a lower value at the end of the time period

The left panel shows the glucose conditions, and the right panel shows the fructose conditions. In both conditions, the xylitol-treated groups generally showed lower balloon height than the 0% xylitol control during much of the measurement period, and seemed to be especially low when xylitol concentration was highest (3%). As balloon expansion was used as an indirect measure of carbon dioxide accumulation, this pattern is once again consistent with the way in which xylitol has been hypothesized within this paper to slow yeast fermentation when glucose or fructose are used as the carbon source. The points and connecting lines represent estimated marginal means, and the error bars represent 95% confidence intervals. Three independent experimental replicates were included per condition (n = 3), with no within-run technical replicates.

Fixed Effects Omnibus Tests

	F	df	df (res)	p
Run	12.626	2	10.00	.002
Carbon Source	7.779	1	10.00	.019
Xylitol Concentration (%)	8.151	2	10.00	.008
Time (min.)	32.613	6	71.09	<.001
Carbon Source ✻ Xylitol Concentration (%)	0.226	2	10.00	.802
Carbon Source ✻ Time (min.)	3.326	6	71.09	.006
Xylitol Concentration (%) ✻ Time (min.)	0.969	12	71.09	.486
Carbon Source ✻ Xylitol Concentration (%) ✻ Time (min.)	0.717	12	71.09	.730

Table 2. Fixed-effects omnibus tests for balloon height.

The linear mixed-effects model once again tested the effects of experimental run, carbon source, xylitol concentration, time, but focused on their interactions on balloon height. Experimental run, carbon source, xylitol concentration, and time had significant effects on balloon height. The carbon source × time interaction was also significant. However, the carbon source × xylitol concentration × time interaction was not significant, which indicated that the relationship between xylitol concentration and balloon height over time did not differ significantly between glucose and fructose. Therefore, the results from measuring the height of the balloon provided somewhat more limited support for the hypothesis proposed than those provided from measuring balloon circumference results. Each condition included three independent experimental replicates (n = 3 per condition), with no within-run technical replicates.

DISCUSSION

The mixed-effects analyses showed that balloon-inflation patterns varied across carbon-source, xylitol, and time conditions. For balloon circumference, the significant Carbon Source * Xylitol Concentration * Time interaction indicated that the relationship between xylitol treatment and circumference over time differed between glucose and fructose conditions. This three-way interaction was not quite as significant for balloon height, although height showed a significant carbon source × time interaction. These differences indicate that circumference and height didn't capture treatment-related changes equally. This could indicate that circumference was a more sensitive or consistent measure of balloon inflation - and indirect measure of CO2 production - than height. Balloons may expand outward without increasing uniformly in height, and height measurements may have been more affected by the balloon position, shape, and elasticity. The significant effect of experimental run in the height analysis also suggests that height varied more across experimental days than circumference. This indicates that circumference may have been a slightly more reliable measure of gas accumulation than height, although the height measurements aren't to be neglected. On a different note, in terms of experimental results, the inhibition xylitol seems to have caused in the fermentation process seemed to have taken a more drastic effect when glucose was used as a carbon source, in comparison to when fructose was used. This specific difference between fructose and glucose was not one directly explored within this study, however, a proposed explanation for this may be due to the way in which the two sugars differ in transport, phosphorylation, and metabolic regulation in yeast. It is possible that due to the way glucose is able to be metabolized directly, xylitol is able to act as a stronger, more efficient inhibitor. It is again important to note that research on the specific cause of xylitol acting as a stronger inhibitor for glucose is rather limited, and more research may need to be done to establish the true cause for this distinction between how glucose and fructose were affected. There are limitations to note when experimental results are interpreted. There was a singular yeast strain being tested within the experiment, along with a limited range of sugar and xylitol concentrations. Furthermore, although total solution volume, yeast mass, incubation temperature, and measurement schedule were standardized across conditions, pH, dissolved oxygen availability, and yeast viability were not independently measured during the experiment. As for the method of measurement, balloon height and circumference were indirect measures of CO_2 production, not a direct

representation or calculation of it. The changes in the balloon's elasticity, nonuniform expansion, and possible dissolved CO_2 may have weakened the relationship between the balloon dimensions and the actual volume of gas produced. Although the height and circumference of the balloon provide reliable proxies for measurement, they should be interpreted as relative differences in CO2 accumulation rather than precise fermentation rates. These limiting factors may have influenced fermentation rate independently of carbon source and xylitol concentration. The overall findings therefore may not be directly generalizable to industrial fermentation systems such as wastewater treatment plants and biofuel production centers. The results do however, support an overall trend of lower fermentation as time and xylitol concentration increases, for the limited range that was tested. The results of this experiment also support previous studies that have shown the role of xylitol as a competitive inhibitor in yeast fermentation, and suggest the increased impact it can have when certain carbon sources are used in this fermentation process, namely glucose and fructose.

CONCLUSION

Overall, it can be shown that xylitol may have a negative impact on the success and rate of fermentation in the presence of glucose or fructose as a carbon source, with a more drastic impact when glucose is used. This is an important factor to keep in mind as new technological advancements and wastewater treatment adaptations are created, as we may need to be more mindful of carbon sources being used for these techniques. It is important that studies continue to be conducted, on the possible effects of mixed-carbon environments in the presence of xylitol, a subject this paper's experiment didn't delve deep into, as well as further research conducted using direct CO2 measurements.

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