EFFECT OF GLUCOSE CONCENTRATION ON RESPIRATION IN YEASTS

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Abstract

The experiment conducted involved quantifying the impact of glucose solution concentration on the rate of anaerobic yeast fermentation. The investigation was completed by taking initial and final measurements of solution pH, to quantify the release of carbon dioxide, following the incubation of yeast in solutions of 0%, 2.5%, 5%, 7.5%, and 10% glucose by mass. It was observed that the rate of fermentation increased between glucose concentrations of 0% and 5% and stabilized for more concentrated samples. The trend in results aligned well with the hypothesis, however the point of pH change stabilization may have been impacted by systematic errors in the introduction of yeast samples to the glucose solution. Hence, while the trend in fermentation rate can be seen as reliable, the point at which glucose's impact is diminished may have been recorded inaccurately.

Introduction

The prolonged use of coal and gas as fuel sources has been the primary driving cause of global warming and thus wider climate change. Despite the controversies surrounding the use of fossil fuels, the enduring financial benefits of using them as opposed to emerging renewable technologies have made them a defining aspect of modern energy generation. However, the use of biofuels, fuels synthesised from existing plant matter, has become an increasingly appealing option as governments worldwide continue to phase out traditional fossil fuels.

Biomass partially offsets the carbon dioxide production from traditional fossil fuels as it is derived from plants that have been carbon sinks, instead of their fossilized remains. Additionally, as biofuels are produced through organic processes (such as alcoholic yeast fermentation), they lack many of the toxic byproducts of fossil fuel burning, including sulfur amongst other impurities. Biofuels allow the biomass from the waste products of food (e.g., husks) to be recycled and used for energy instead of being discarded, aiding the construction of circular economies.

The production of biofuels from biomass is often catalyzed through cellular respiration. In particular, the alcoholic fermentation that occurs in yeasts yields ethanol from glucose, as below:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Carbon dioxide reacts in aqueous solution to form carbonic acid ($CO_2 + H_2O \rightarrow H_2CO_3$). Carbonic acid is a weak acid that undergoes reactions with water ($H_2CO_3 + H_2O \rightarrow HCO_3^- + H_3O^+$) to form hydronium ions, reducing the solution pH. Thus, the fermentation of yeasts in an aqueous solution can be quantified by measuring the change in pH over a period.

The process of alcoholic fermentation in yeasts is an alternative anaerobic pathway to aerobic cellular respiration that occurs in poor-oxygen environments. Multiple factors can impact the rate of the anaerobic respiration pathway. Glucose concentration, enzyme concentration and temperature are among these factors. An increasing temperature would likely first increase the rate of fermentation before reducing it, as the enzymes that catalyse the alcoholic fermentation pathway would denature. Increasing the glucose concentration would be expected to result in more collisions between particles, increasing the fermentation rate. Similarly, increasing the enzyme concentration would also be expected to increase the fermentation rate. However, increasing the concentration of a single reactant would be expected to, after a saturation point, have minimal impact on the rate of fermentation, as the rate of reaction is bounded by a limiting factor, or the reactant with lower concentration.

Aim and Hypothesis

The investigation being conducted intends to establish the impact of a changing biomass concentration on the rate of anaerobic fermentation in yeast specimens. By placing yeasts in different concentrations of glucose and measuring the release of carbon dioxide through 2 pH readings, the rate of anaerobic fermentation can be measured due to its proportional relationship to CO_2 release. A control group (0% glucose) will be included to test the validity of the experiment.

It is hypothesized that increasing the glucose concentration will increase the rate of anaerobic fermentation in the yeasts until a point of glucose saturation, after which the fermentation rate will plateau. This is as, prior to the saturation point, increasing the mass of $C_6H_{12}O_6$ in the same volume will increase the quantity of collisions between glucose molecules and fermentation-catalysing enzymes in the yeasts, as per collision theory, heightening the rate of reaction. However, at and past the point of saturation, glucose concentration will no longer be the limiting factor in the reaction, and the rate of fermentation will not increase further.

Methodology

Materials

1. 150mL 10% yeast solution
2. 150mL 10% glucose solution
3. Distilled water
4. 5 test tube racks
5. 25 test tubes (50mL)
6. 25 test tube stoppers
7. 5 beakers (100mL)
8. 1 measuring cylinder (10mL)
9. 1 measuring cylinder (50mL)
10. 1 Pasco pH meter
11. 1 stopwatch
12. 1 pair of gloves
13. 1 pair of safety glasses
14. 1 lab coat

Method

1. 30mL stock solutions of 0%, 2.5%, 5%, 7.5%, and 10% glucose were prepared in 5 100mL beakers.
2. 5 test tubes were placed in the test tube rack, and 6mL of 0% glucose solution was added to each.
3. 6mL of 10% yeast solution was added to each test tube.
4. The pH reading of the solutions was measured and recorded, and the test tubes were stopped, with a stopwatch set for 30 minutes.
5. After 30 minutes, the test tube stoppers were removed, and the final pH reading was measured and recorded.
6. Steps 2-5 were repeated for the 2.5%, 5%, 7.5%, and 10% glucose solutions.

Risk Assessment

Type of Risk	Hazard	Level of Risk	Cause of Risk	Safety Precautions
Biological	Yeast Samples	Low Risk	Minor injury due to ingestion.	Care should be taken not to ingest the yeast during the investigation.
Physical Equipment	pH Meter	Low Risk	Exposure to electrodes should be avoided.	Care should be taken to use the pH meter in a responsible and precise manner.
Physical Equipment	Glass Beaker/Measuring Cylinder	Low Risk	Potential cuts due to chipped rims or glass fragments.	Glass equipment should be used with caution to prevent breakage. All broken glass should be without physical contact (e.g., with a brush).

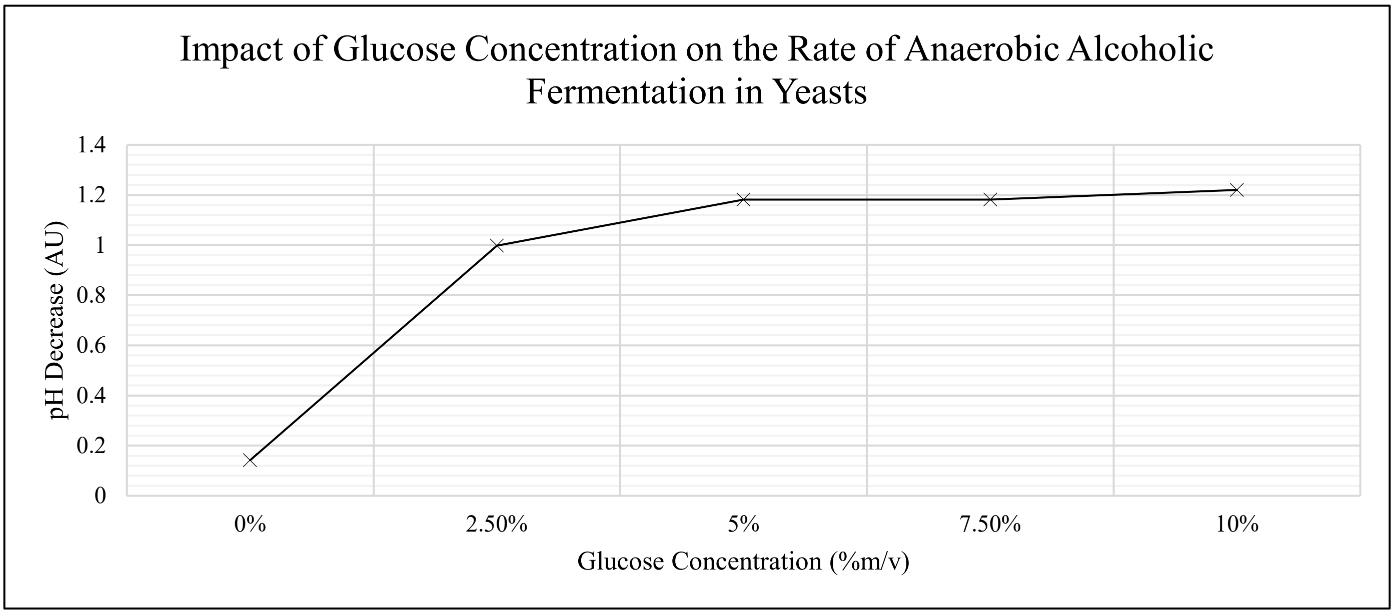
Results

Tabular

Impact of Glucose Concentration on the Rate of Anaerobic Fermentation in Yeasts

		Glucose Concentration									
		0%		2.50%		5%		7.50%		10%	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH Readings	T1	5.77	5.6	5.63	4.57	5.67	4.55	5.59	4.35	5.63	4.35
	T2	5.75	5.64	5.61	4.62	5.69	4.57	5.5	4.38	5.57	4.4
	T3	5.74	5.62	5.62	4.57	5.7	4.43	5.54	4.37	5.62	4.42
	T4	5.75	5.6	5.6	4.64	5.65	4.42	5.56	4.38	5.6	4.41
	T5	5.76	5.6	5.55	4.62	5.7	4.53	5.59	4.39	5.62	4.36
	Avg.	5.754	5.612	5.602	4.604	5.682	4.5	5.556	4.374	5.608	4.388
Average pH Decrease		0.14		1.00		1.18		1.18		1.22	

Graphical



Discussion

The data collected in the experiment showcased an initial increase in the average pH change due to carbon dioxide release with increasing glucose concentration between 0% and 5%, followed by a stabilization at concentrations between 5% and 10%. The average pH change at a 5% glucose concentration was approximately -1.18, while the average pH change at a 0% concentration of glucose was 0.14. The pH changes between 5% and 10% glucose differed only by approximately 0.04.

The stabilization of the graph of pH change at high glucose concentrations reflects the principle of saturation of reactants, the point at which the addition of a particular reactant is no longer sufficient to greatly increase the rate of a reaction, as another factor (possibly enzyme concentration) is limiting. The initial increase in the pH change aligns well with the results that would be expected from collision theory. The results collected supported the hypothesis, as the trend observed was composed of an initial increase in fermentation rate followed by a plateau beginning at a 5% glucose concentration.

However, there was a potential systematic error in the investigation, in that the carbon dioxide released by the yeasts may have escaped from the yeast solution in the test tube. While this was somewhat alleviated by stopping the test tube to encourage escaped carbon dioxide to again dissolve into solution, the final pH readings may still have been overestimated due to a lower carbon dioxide concentration than expected. Conducting the experiment in a test tube containing a greater volume of solution may assist in ensuring carbon dioxide does not escape in future investigations.

Additionally, the process of measuring the initial pH of the solution may have resulted in another systematic error. As the yeast solution was added to the glucose prior to the measurement of pH, some of the yeasts likely began fermenting prior to the pH reading, resulting in an underestimation of the initial pH and thus a lower observed pH decrease. Future investigations could first measure pH prior to the addition of yeasts in order to limit this systematic error.

The investigation ultimately showed a clear relationship between the rate of fermentation in yeast samples and the concentration of glucose solution. Though there were likely errors in the measurement of pH and hence inaccuracies in the observed location of the saturation point, it appears that the most economical glucose concentration is at 5%m/v, as further increases in the glucose concentration fail to increase the pH decrease due to fermentation beyond 1.18, excluding minor fluctuations at 10%m/v glucose.

Conclusion

Overall, the aim of the experiment in identifying a relationship between a variable glucose concentration and the rate of anaerobic yeast fermentation was achieved. The hypothesis was supported in that the pH change due to CO_2 release initially increased before plateauing at a point of saturation at approximately 5% glucose and a pH decrease of 1.18. However, systematic errors in the timing of yeast addition and potential release of carbon dioxide from the yeast solution may have had an impact on the observed point of saturation. Hence, while the trend in results can be seen as reliable and valid, the point of saturation was likely measured inaccurately. In future investigations, additional trials could be conducted at more distinct glucose concentrations with a lower difference between trial concentrations in order to better ascertain the concentration of saturation and the behaviour of the pH change prior to saturation. Indeed, more trials could be conducted at each concentration as well as a means of further improving the accuracy of the pH readings. Nevertheless, the fermentation of yeast to produce biofuels from biomass is a key technique being considered for future energy production initiatives globally and presents a new way to sustainably provide for Earth's future energy needs.

References and Acknowledgements

1. Azhar, S. H. M., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Faik, A. a. M., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports*, *10*, 52–61. https://doi.org/10.1016/j.bbrep.2017.03.003
2. *Biofuels and the Environment | US EPA*. (2024, April 22). US EPA. https://www.epa.gov/risk/biofuels-and-environment
3. The Influence of Fermentation Conditions on Ethanol Yields from Whey Permeate on JSTOR. (n.d.). *www.jstor.org*. https://www.jstor.org/stable/25558162
4. University of Oklahoma, Bryan, A., Hart, C., Howell, A., Wise, M., & Roberts, B. (2018). Glucose Concentrations Effect on Rate of Fermentation in Yeast. *Journal of Undergraduate Biology Laboratory Investigations*. https://undergradsciencejournals.okstate.edu/index.php/JUBLI/article/download/8738/1870