HIGH SCHOOL EDITION
Journal of Student Research
Volume 12 Issue 3 (2023)

Efficient Biofuel Generation of Plants via Enzymes

Vega Mani

Taylor Allderdice High School

ABSTRACT

The purpose of this research is to observe the Efficiency of Biofuel Generation of Plants Using Enzymes. The question being asked is at which concentration of glucose will yeast fermentation rates be the most efficient to produce Ethanol through fermentation? It was predicted glucose concentrations and ethanol production rates would increase simultaneously. Ethanol is a renewable transportation fuel made from plants. Methods for researching this topic includes studying the environmental impacts of Ethanol, how it is produced, the process of enzyme digestion, the differences between cellulose and cellulase and the process of yeast alcohol fermentation. The methodology required for this experiment includes creating eight samples: Cellulase + Yeast, Cellulose + Yeast, Cellulase + Cellulose + Yeast, Cellulase + Cellulose + No yeast, and all four concentrations of glucose in separate samples (0.5%, 1%, 3% and 5%). The apparatus used to collect the data is made up of a syringe attached to a pipette, each sample started at a given volume of mL then as the samples began fermenting and releasing CO2 data was recorded for 15 minutes in 1 minute intervals. The volume of carbon dioxide released corresponds to the amount of sugar (glucose) fermented. The results of this experiment show a correlation between the increased rate of ethanol production as glucose concentrations increase. The interaction between cellulose and cellulase demonstrates the process of producing glucose through enzyme digestion.

Introduction

Plant-derived fuels are emerging and are extremely beneficial to improving sustainability throughout our society. Ethanol is a type of biofuel commonly found in gasoline. There is a significant environmental impact of ethanol produced from edible plants with high sugar content, such as corn and sugarcane. Scientists and chemical engineers are interested in using green chemistry to solve the issue of biofuel generation because of the major environmental impacts. However, this poses several technical challenges paired with the energy requirements of growing more crops. The process of generating Ethanol begins with yeast cells which can convert carbohydrates into ethanol through fermentation. Yeast cells convert carbohydrates into ethanol through fermentation. Plants produce cellulose in their cell walls, which would be a crucial resource for ethanol, but yeast cells do not have the enzymes necessary to break down cellulose therefore the process of enzyme digestion and alcohol fermentation are both necessary to produce Ethanol. Cellulase is the enzyme that can digest Cellulose. Cellulase is a biological catalyst that has been found to speed up the conversion of cellulose to ethanol as it breaks down cellulose into monosaccharides such as glucose. It is usually found in cellulose-digestive bacteria such as fungi. Cellulose is a natural organic compound carbohydrate which makes up about half of a plant's biomass, located in a plant's cell wall, as shown in the figure 1.

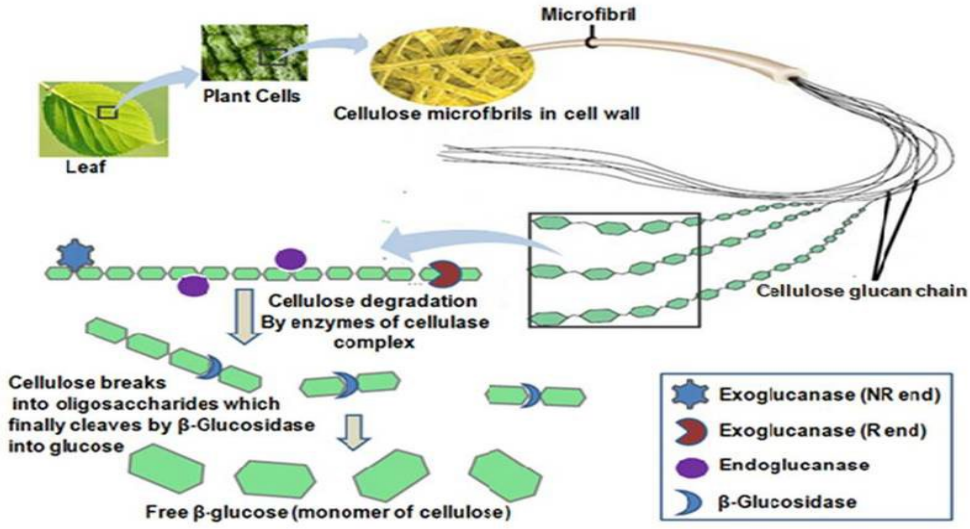

Figure 1. Enzyme digestion process
*Source: Diagrammatic Overview of Cellulose Metabolism by Cellulase System (*https://www.researchgate.net/figure/Diagrammatic-overview-of-cellulose-metabolism-by-cellulase-system-During-cellulose_fig1_288889789)

Rationale

This project is topical because of all the environmental attempts that we are making to preserve our earth. Ethanol is a renewable transportation fuel produced from plants. It helps reduce emissions and transportation costs for consumers and businesses. Ethanol also improves air quality by helping reduce air pollution caused by organic fuels like gasoline. It is easily available as it is made from raw plant materials leading to an increase in the construction of jobs in rural areas. Ethanol helps provide an expanding performance level to engines such as cars, improving engine efficiency.

Past Studies

One of the past studies researched relating to this experiment was conducted by a group of students from the University of Oklahoma, Department of Biology. They used an Ethanol probe, connected to a Logger pro to measure the ppm of Ethanol every 15 seconds. Next, they set up their different concentrations of glucose, since Ethanol is an output of cellular respiration, they were able to measure its rate. Lastly, they compared the output of ethanol in ppm/min to the glucose concentration and got results displaying that the rate of ethanol increased along with the increased glucose concentration.

Current Research Goals

The purpose of this experiment is to determine at what concentration of glucose, derived from cellulose will yeast fermentation rates be most efficient in order to produce Ethanol? The hypothesis was that if cellulose is broken down into different concentrations of glucose through enzyme digestion, then the yeast fermentation rates will be most efficient at the highest concentration of glucose because as yeast performs alcohol fermentation it uses glucose to produce Adenosine Triphosphate (ATP) which is a main energy carrier in cells, therefore necessary for Ethanol production.

Methods

To begin the process of Enzyme digestion, the catalyst used was Cellulase. To perform the enzyme digestion Cellulose is necessary because their interaction makes it possible to produce glucose. Instead of physically deriving glucose from the Cellulose since that is difficult to measure, an already constructed glucose concentration was used. Four concentrations of glucose were chosen- 0.5%, 1%, 3% and 5%. For the yeast fermentation process heated yeast is a necessary component. To build the apparatus in which the experiment was conducted, a syringe and a pipet were needed for each sample. Lastly, the pH 5 buffer solution was needed to resist pH levels in the enzyme to prevent alternative reactions.

An eight-step methodology was used to conduct the experiment through two necessary parts of Ethanol production including Enzyme Digestion and Yeast Alcohol Fermentation.

1. Combine two separate solutions: pH 5 buffer solution + Cellulase and pH 5 buffer solution + Cellulose. Next prepare four solutions, each containing one of the four glucose concentrations mixed with pH 5 buffer solution- let these solutions sit overnight
2. Prepare eight samples with 1 mL buffer solution in each sample containing yeast and 3mL of buffer solution in the one sample without yeast.
3. These eight samples include Cellulase + Yeast, Cellulose + Yeast, Cellulase + Cellulose + Yeast, Cellulase + Cellulose + No yeast, and all four concentrations of glucose in separate samples (0.5%, 1%, 3% and 5%).
4. Attach tubing to end of the syringe connecting the syringe and pipette, draw air into syringe and choose 1 mL as the starting point.
5. Put the buffer solution in each sample first, next add the yeast and lastly add the experimental value- the total volume of each sample should be 5mL.
6. The change in CO2 is the difference between the start and end points.
7. Collect data for 1-minute intervals over 15 minutes.
8. After data is collected calculate the fermentation rate through this formula:

final C02 volume - initial C02 volume/ final time - initial time

The following images show a visual of the apparatus set up to conduct the yeast fermentation process along with a diagram which depicts the yeast fermentation process resulting in the production of Ethanol. The volume of carbon dioxide released corresponds to the quantity of sugar (glucose) fermented and ethanol produced. If the sugar was fermented, then the carbon dioxide was released pushing the droplet upward in the pipette.

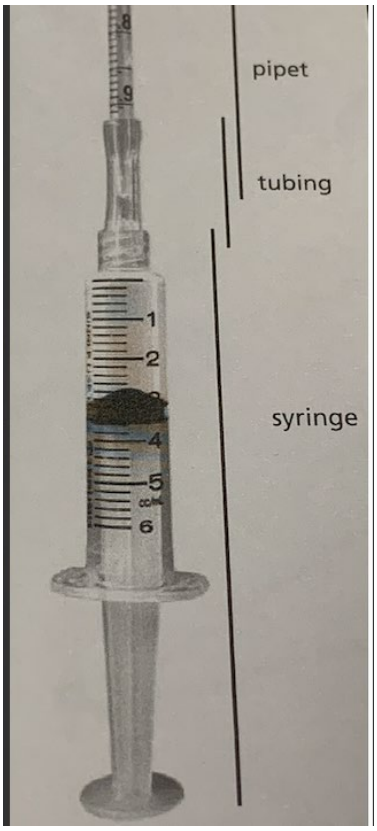

Figure 2. Apparatus used throughout experiment.

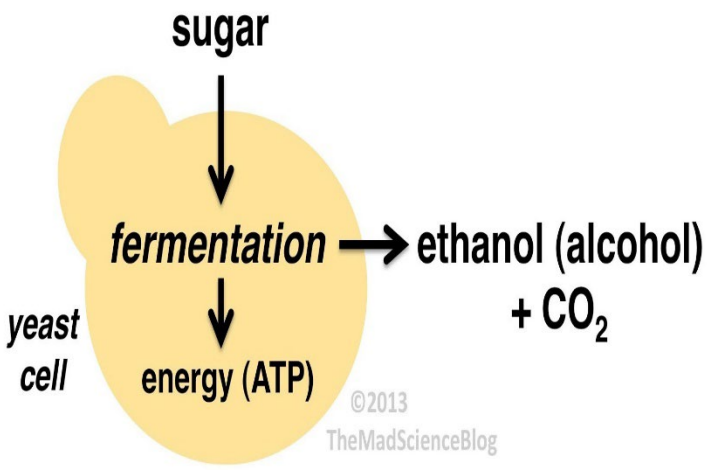

Figure 3. Yeast fermentation process.

Results

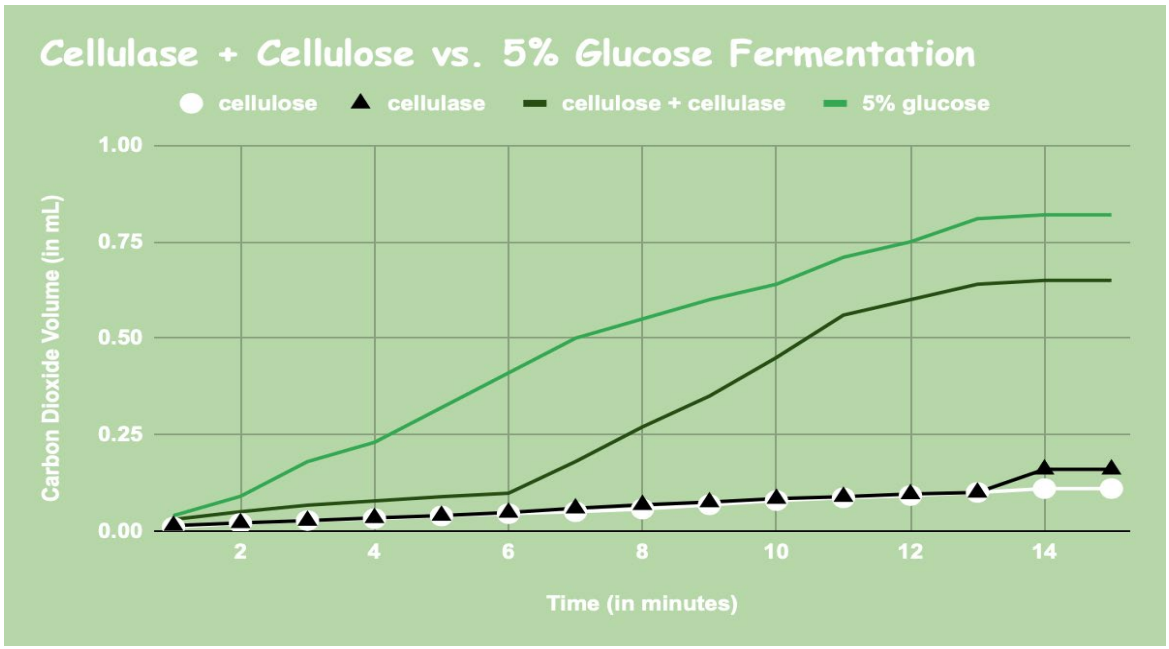

Figure 4. Interaction between Cellulose and Cellulase.

Their interaction is mirrored by glucose production. Enzyme digestion is what breaks down and produces monosaccharides such as glucose. This is why the CO2 volume on the Y-axis for 5% glucose and the combination of Cellulose + Cellulase are close in number in their fermentation rates. Cellulose and Cellulase cannot properly ferment alone because both an enzyme and a carbohydrate are requisite for glucose construction.

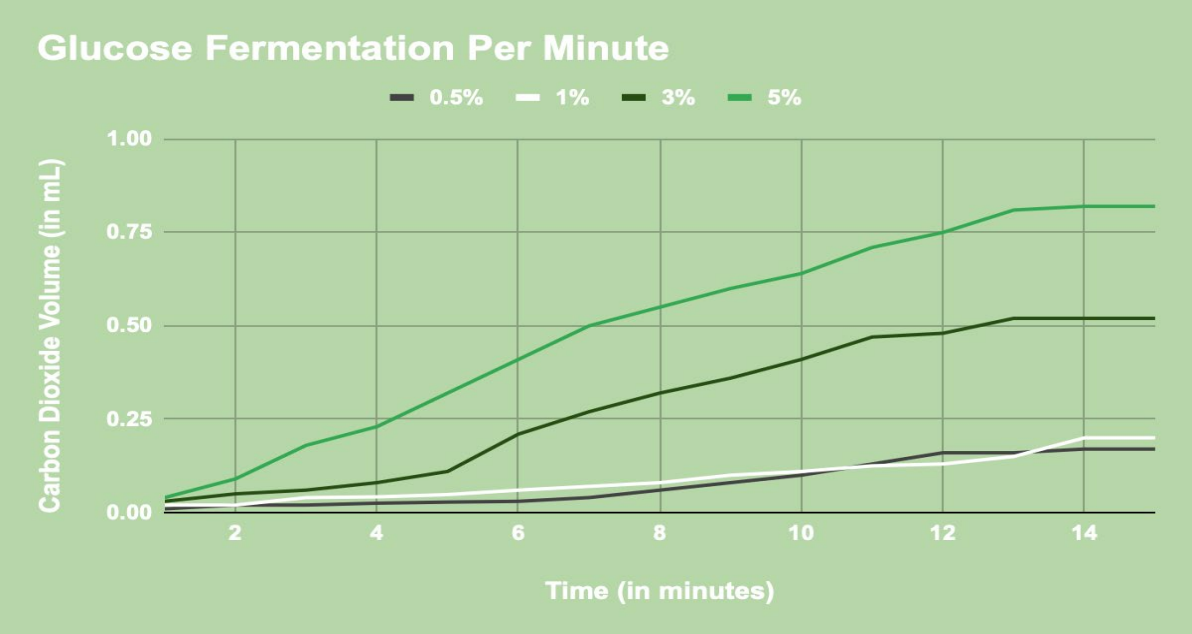

Figure 5. Glucose Fermentation Rate (average between two trials)

Figure 5 represents the fermentation rate per minute for each glucose concentration. (0.5%, 1%, 3% ,5%) Five percent glucose had the most efficient fermentation rate at around 0.06% per minute reaching a volume of 0.82 mL by minute 14. Each glucose concentration seemed to plateau towards the end of the 15-minute period meaning that the fermentation was slowing down. 0.5% and 1% glucose had similar rates as they overlapped twice throughout the process after minute 10.

Table 1. The data table shows the average fermentation rate per minute for each concentration of glucose. 0.5% and 1% glucose had similar average fermentation rates while 5% glucose was significantly more efficient. Since all the samples seemed to plateau towards the end of the fifteen-minute period, a graphed best fit line was unable to be accurately formed.

Glucose Concentrations	Average Fermentation (per minute) CO2 Volume
0.5%	0.01 mL
1%	0.01 mL
3%	0.03 mL
5%	0.06 mL

Discussion

The data displays that as the concentration of glucose increases, the fermentation rate increases along with it. Glucose concentrations at 5% and 3% had a significantly faster fermentation rate than the lower concentrations. In the first graph, you can see how the interaction between Cellulose and Cellulase produced a fast fermentation rate, each by itself had around the same slow gradual rate at less than 0.25 mL by minute fifteen. The sample of Cellulase + Cellulose + No yeast did not end up fermenting at all which was expected because yeast is necessary for the fermentation process. When comparing the two graphs you can see that 3% glucose and the interaction of Cellulase and Cellulose had similar fermentation rates which means that the specific amount of Cellulose and Cellulase used throughout this experiment produced a bit over 3% glucose. The main confounding variable throughout this experiment was temperature, because temperature plays a huge role in impacting fermentation rates. This experiment was done at room temperature and the yeast was heated at around 42 degrees Celsius. Past studies show that increased heat seems to speed up fermentation rates because the yeast can activate quicker.

Conclusion

The results of the experiment show a significant correlation between the increase in the rate of ethanol production and glucose concentrations increase. The reasoning behind this is that oxygen is not present, so yeast must use glucose to obtain the energy used to produce ethanol. Therefore, increased glucose implies increased energy causing the fermentation process to speed up. These results do in fact support the hypothesis stated, which expressed that the higher the glucose concentration the faster the fermentation process because glucose forms ATP necessary for ethanol production. Although this study may be described as a pilot study, since only two trials were conducted to produce these results. Based on background research the results from this study correspond to many other past studies based on biofuel generation via enzymes. Hopefully in the future biofuel generation will become more common and efficiently produced using methods such as enzyme digestion and yeast fermentation as performed in this experiment.

Limitations

The materials used, cellulose and cellulase, are expensive, which impacts their availability, making it hard to obtain. The experiment was conducted in a high school chemistry lab, so there was no access to high-tech materials like Ethanol probes and C02 Probes.

Extensions

This project's relevancy makes it possible to extend it in multiple ways:

1. Testing the accuracy of the fermentation rates from this experiment in comparison to real observations of carbon dioxide and ethanol levels using an Ethanol probe or CO2 probe.
2. Testing enzymatic digestion of cellulose on other sources of cellulose and non cellulose such as sugar cane, cotton, wood, grass, cornstarch.
3. Testing enzymatic digestion via different enzymes. (Biological catalysts)
4. This project was conducted at room temperature, therefore testing the fermentation rate at different temperatures will produce alternative results.
5. This project used small concentrations of glucose; therefore, it would be interesting to see how fermentation rates may differ using higher concentrations of glucose.

References

1. "Why Is Fermentation of Cellulose to Produce Biofuel and Nutrients so Difficult?" *Chemistry Stack Exchange*, 1 Oct. 1964, https://chemistry.stackexchange.com/questions/83926/why-is-fermentation-of-cellulose-to-produce-biofuel-and-nutrients-so-difficult.
2. "Ethanol Fuel Basics." *Alternative Fuels Data Center: Ethanol Fuel Basics*, https://afdc.energy.gov/fuels/ethanol_fuel_basics.html.
3. Maicas, Sergi. "The Role of Yeasts in Fermentation Processes." *Microorganisms*, U.S. National Library of Medicine, 28 July 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7466055/.
4. Vasić, Katja, et al. "Bioethanol Production by Enzymatic Hydrolysis from Different Lignocellulosic Sources." *Molecules (Basel, Switzerland)*, U.S. National Library of Medicine, 1 Feb. 2021, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7867074/.
5. "Ethanol Benefits and Considerations." *Alternative Fuels Data Center: Ethanol Benefits and Considerations* https://afdc.energy.gov/fuels/ethanol_benefits.html.