Effect of Fructose Concentration on Fermentation Rate of *Saccharomyces cerevisiae*

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Introduction

Canada is known for its unique food and beverages, and ice wine is one of them. When I visited Toronto four years ago, my family visited a famous winery near Niagara falls. It was my first time visiting a winery and everything there was new to me. According to a winemaker at the winery, ice wine uses frozen grapes that are fermented at a very low temperature to give its unique sweet flavor. However, it takes at least three to five months to ferment ice wine which requires a longer time than other typical wines that take approximately 10 days. I was shocked that it takes so much time and care to make a single bottle of ice wine. I wondered if there is any method to shorten the fermentation time so the wine brewing process is more efficient.

As I studied more about molecular biology and cellular respiration, I noticed that yeast is responsible for the fermentation of many alcoholic beverages. During wine fermentation, yeast uses sugar molecules to produce ATP in an anaerobic condition which produces CO_2 and ethanol as a by-product. When I learned about this fact, I immediately wondered what would be the optimum sugar concentration that could speed up the fermentation process. Since fructose is the major monosaccharide in green and red grapes (approximately 12.4%), I have decided to find out the optimum fructose concentration for wine yeast (*Saccharomyces cerevisiae*) and the alcohol percentage that starts to reduce yeast's respiration rate (Sruthi, 2021).

Research Question

How does increasing fructose concentration (0, 50, 100, 150, 200, 250g/dm^3 ±1.27) affect the fermentation rate of *Saccharomyces cerevisiae* (ppm/minute), and at what concentration will the respiration rate reach its maximum due to high ethanol percentage (v/v) at 32°C for 15 minutes of respiration?

Background Information

Saccharomyces cerevisiae is a unicellular fungus that has extensive use in food and beverage fermentation, which is commonly used in the production of wine, beer, and even vodka (Parapouli, 2020). Unlike other yeasts which are very sensitive, *S. cerevisiae* is commonly found in harsh environments due to its unique biological characteristics. It is resilient to adverse conditions such as low pH, high alcohol concentration, and high osmolarity (Stanley, 2009).

Although high sugar concentration is often hostile to many yeasts, *S. cerevisiae* shows high tolerance to high osmolarity as it can do the fermentation process by overcoming the fermentation stresses (Parapouli, 2020). The major pathway for ATP production in *S. cerevisiae* is known to be anaerobic respiration. Due to its unique gene expression, *S. cerevisiae* prefers anaerobic respiration to produce ATP through glycolysis even under aerobic conditions (Gasmi, 2014). When the carbohydrate source is scarce, ethanol produced during fermentation is used to continue ATP production which requires a shift to aerobic respiration. Since *S. cerevisiae* is highly alcohol resistant, it can continue respiration in a condition with high alcohol concentration. However, if the alcohol concentration exceeds its tolerance level, *S. cerevisiae* fermentation rate starts to decrease (Stanley, 2009).

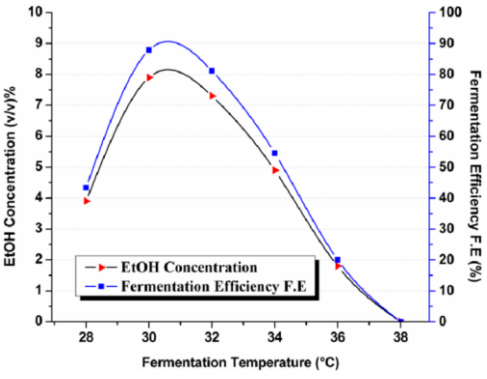


Figure 1 - Effect of fermentation temperature (S. cerevisiae) (Khoja,

Ethanol is toxic to many organisms and it can be toxic to *S. cerevisiae* as well. This is because ethanol can influence cells' metabolism as intracellular proteins and enzymes denature and their activity decreases, and alter *S. cerevisiae*'s membrane structure which can disrupt its membrane fluidity (Stanley, 2009). Although *S. cerevisiae* is highly ethanol tolerant, when the ethanol concentration exceeds 14%, its growth gradually decreases until it becomes completely prevented (Ghareib, 1988).

Temperature is another limiting factor that influences *S. cerevisiae*'s fermentation. Although many of the winemakers set their temperature low around 10 to 15°C, the optimum temperature for *S. cerevisiae* is known to be around 32°C (López-Malo, 2013). As shown in Figure 1, *S. cerevisiae*'s fermentation efficiency peaks between the temperature of 30 to 32°C. If the temperature continues to increase, the fermentation efficiency decreases drastically. Hence, the temperature should be set at 32°C to ensure *S. cerevisiae*'s optimum growth.

Hypothesis

As the fructose concentration keeps increasing, the fermentation rate of *S. cerevisiae* will rise since there are more carbohydrates available for anaerobic respiration. At the concentration of 200g/dm^3, *S. cerevisiae* will reach its maximum fermentation rate (ppm/minute) and start to plateau because of increasing ethanol percentage (v/v) which is toxic and kills *S. cerevisiae* (Figure 2). Also, Both fermentation rate and ethanol concentration by *S. cerevisiae* will show statistically significant differences with fructose concentration since the trend is not caused by random chance. Hence, the null hypothesis (H_0) will be rejected and the alternative hypothesis (H_a) will be accepted.

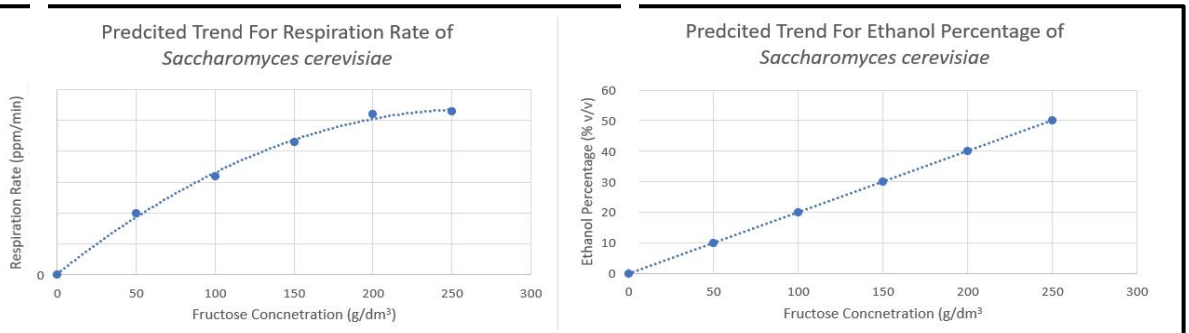


Figure 2 – Theoretical trend for respiration rate and ethanol concentration of S. cerevisiae

Variables

Independent	Range	Unit	Method	Justification
Fructose Concentration	0 50 100 150 200 250	g/dm^3 (±SD)	Fructose solution will be used to provide *S. cerevisiae* carbohydrates for anaerobic respiration. 500ml of distilled water will be used to create each solution and fructose will be added. For example, to create a solution of 50g/dm^3, 25g of fructose will be added to 500ml of water.	Typically, the optimum sugar concentration for yeasts is between 150g/dm^3 and 225g/dm^3 (Beigbeder, 2021).

Dependent	Trials	Unit	Method	Significance to The Research Question
CO_2 Concentration Per Minutes	5×3	Parts per million per minute (ppm/min) (±SD)	CO_2 concentration will be used to measure the *S. cerevisiae*'s fermentation rate. Vernier CO_2 sensor will be connected to the laptop through LabQuest 2, and data will be collected using software called Logger Lite (Figure 4).	CO_2 is produced during anaerobic fermentation to restore NADH for glycolysis (Cornell, 2016). Hence, the fermentation rate is high when more CO_2 is produced by *S. cerevisiae.*

Dependent	Trials	Unit	Method	Significance to The Research Question
Ethanol Percentage	6×3	Volume per volume (% v/v)	Ethanol percentage will be measured to identify at what point yeast activity will slow down. It will be measured using an alcohol refractometer (Figure 6), which shows the percentage of alcohol in a solution in volume per volume.	High ethanol concentration can reduce cell vitality and influence cell metabolism (Stanley, 2009). Hence, by comparing ethanol concentration and fermentation rate of *S. cerevisiae*, optimum fructose concentration can be identified.

Controlled	Value	Unit	Method
Temperature	32	°C (±0.5)	To make sure the temperature is the optimum temperature for fermentation, a container containing yeast will be maintained at 32°C. This condition can be maintained using a warm water bath. A plastic container containing fructose solution and *S. cerevisiae* will be placed inside the water bath which will be set at 32°C and maintain temperature.
Time	15	min	To make sure every experiment is done for the same period, every experiment will last only for 15 minutes. A 15-minute timer will be set using a smartphone to make sure the experiment does not exceed 15 minutes.
The volume of fructose solution	100	ml (±0.5)	For every experiment, 100ml of fructose solutions will be used. If too much solution is used, it might contact the CO_2 sensor which leads to inaccurate measurement. During the preliminary trial, 150ml of fructose solution was used and bubbles created by yeast touched the sensor. To prevent this, only 100ml of fructose solution will be used instead. To accurately measure the volume of the fructose solution, 100ml graduated cylinder will be used.

Uncontrollable	Possible Impact
Anaerobic condition	Yeast fermentation only occurs in an anaerobic condition. However, it is impossible to make the bio chambers into an anaerobic condition. Due to the remaining oxygens in the bio chambers, *S. cerevisiae* may perform aerobic respiration instead of anaerobic respiration which does not produce ethanol as a byproduct. Hence, the concentration of ethanol may be lower, and *S. cerevisiae* may not be affected by the accumulation of ethanol significantly.

Apparatus

Chemicals and consumables	Glassware	Other equipment	
-Fructose (25, 50, 75, 100, 125g for each solution) - Red Star wine yeast (*S. cerevisiae*) (2.5g for each trial) -Distilled water	-5 500ml (±25ml) conical flasks -2 100ml (±0.5ml) graduated cylinder -1 100ml (±0.5ml) beaker -1 thermometer (±0.5°C)	-1 water bath -3 250ml plastic container -1 magnetic stirrer -1 magnetic bar -1 measuring spoon -3 Vernier CO_2 gas sensor -3 stands	-1 Vernier LabQuest 2 -1 laptop -1 digital balance pan (±0.01g) -5 weighing boats -1 alcohol refractometer (±0.5%) -6 clamps

Safety Measures

Table 1: Risk Assessment for Lab Equipment

Lab Equipment	Risks	Precaution	Disposal
Magnetic stirrer	-When the magnetic stir bar is spinning at high speed, it can cause damages such as scratches to the container. -Having physical contact with a heated magnetic spinner may cause skin burn (*Precautions*, 2018).	-Avoid contact with the hotplate while it is operating. -Check whether the container has scratches to prevent possible leakage. -Put a lid on the container when speeding up the stirring speed to prevent possible leakage (*Precautions*, 2018).	-After using the magnetic stirrer and magnetic bar, cleanse the magnetic stir bar using flowing water (*Precautions*, 2018).
Glassware	-Forcing glass tubing into stoppers may cause cuts. -Touching broken glass pieces may cause cuts (*SAFE*, n.d.).	-Never use laboratory glassware to serve foods or drinks. -Discard or repair any chipped or cracked items. -At least 10% of air space should be left. -Never pick up broken glass pieces with bare hands (*SAFE*, n.d.).	-Wear gloves and use a brush and dustpan to clean up broken glasses. -Remove broken glasses in the sink (*SAFE*, n.d.).
Water bath	-Electrocution of operator due to electrical malfunction or the operation of the equipment with wet hands. -Fire hazard is possible when it is overheated. -Mechanical injury may happen when hands, hair, or clothes get caught in a water circulation impeller (*RISK*, n.d.).	-Before handling the machine, dry both hands. -Unplug the machine before moving it. -Make sure that the machine is not continuously used for an extended period. -Keep hands, clothes, and hair away from the machine (*RISK*, n.d.).	-Clean the bath using detergent to prevent pathogenic growth which can cause electrical failure (*RISK*, n.d.).

Table 2: Risk Assessment for Chemical Substances

Chemical	Hazards	Precaution	Disposal
Fructose	-Fructose powder may cause eye irritation when directly contacted but low hazard. -Low hazard level for skin contact, inhalation, and ingestion (*Material*, 1997).	-Wear a mask or extra protection to avoid breathing dust, and close the lid tightly when not in use. -Wear appropriate eyeglass wears while using.	-Vacuum or sweep up spilled material into appropriate disposal container (*Material*, 1997).
Ethanol	-It is highly flammable. -May cause eye irritation through contact. -Cause organ damages when inhaled or ingested (*SAFETY*, 2009).	-Wear personal safety wears (eye and face) before handling ethanol. -Keep the chemical away from flames. -Always keep the container tightly closed.	-Dispose the chemical substance into an appropriate container. -Follow regional regulation when it is being released (*SAFETY*, 2009).

Environmental Safety

Environmental Safety	Explanation
Fructose Solution	According to the material safety data sheet, it is known that fructose is not toxic, and there is no known environmental issue when it is released into the environment (*Material*, 1997)
Production of CO_2	Currently, the atmospheric CO_2 level is increasing, contributing to global warming and the enhanced greenhouse effect. Since this experiment is producing additional CO_2 to measure the fermentation rate, it can contribute to an increase in CO_2 levels. Hence, when disposing the water with CO_2, it should be diluted with flowing water.
Ethanol	Since ethanol is created during anaerobic respiration, fructose solution will be slightly alcoholic after the experiment. It is known that ethanol is toxic to many aquatic organisms; hence, ethanol is hazardous when it is released into the environment. To minimize the effect of ethanol, it should be diluted with distilled water to lower the concentration before it is being disposed.

Ethical consideration

Saccharomyces cerevisiae is used for this investigation to find out the optimum fructose concentration for efficient fermentation. To find out the optimum condition for *S. cerevisiae*, it will be exposed to harsh conditions such as high sugar concentration or high alcohol percentage which can be toxic to the organism. Hence, it involves the killing of an organism. However, *S. cerevisiae* is already intentionally killed while baking and brewing to control fermentation. Therefore, killing *S. cerevisiae* will not have a huge ethical issue.

Experiment Setup

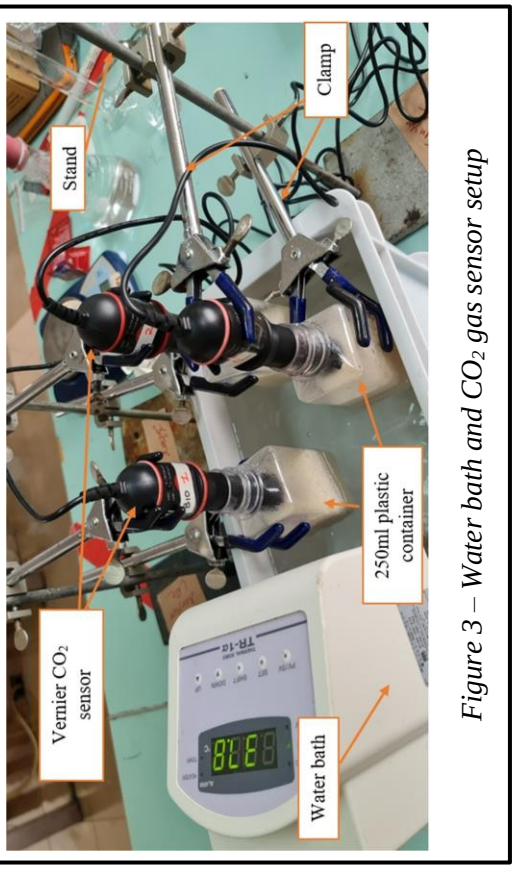


Figure 3 – Water bath and CO_2 gas sensor setup

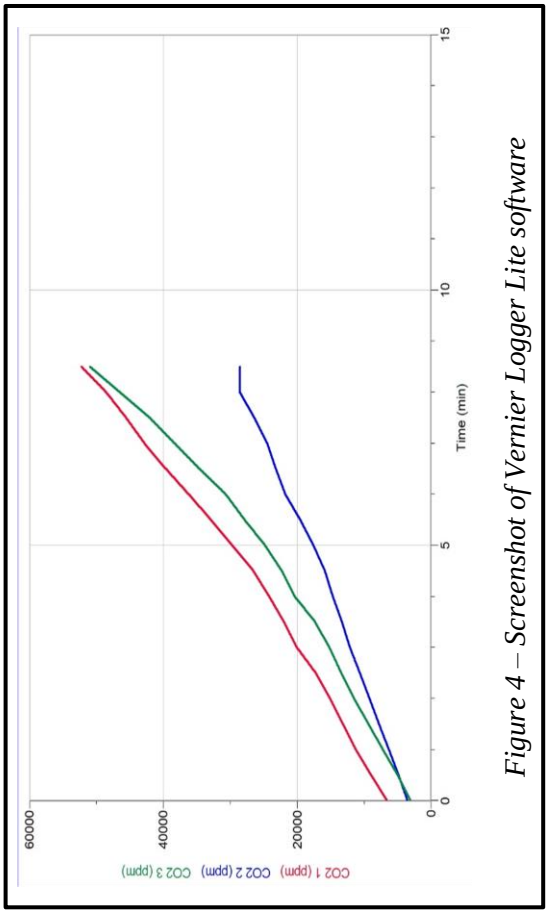


Figure 4 – Screenshot of Vernier Logger Lite software

Figure 5 – Filtering setup

Figure 6 – Refractometer

Procedure

Step 1. Making fructose solutions

1. Use 100ml (±0.5ml) graduated cylinder to pour 500ml distilled water into a 500ml (±25ml) conical flask.
2. Weigh 25g of fructose using a digital balance pan (±0.01g)and a weighing boat.
3. Add 25g fructose inside the conical flask containing 500ml of distilled water.
4. Set the magnetic stirrer at 60°C and 1000 rpm.
5. Place the conical flask on a magnetic stirrer and put a stirring bar inside the flask.
6. Turn on the magnetic stirrer and wait until all fructose molecules are dissolved.
7. When completely dissolved, put a stopper on and place the fructose inside the refrigerator.
8. Repeat steps 1 to 7 for the other four fructose solutions (100, 150, 200, 250 g/dm^3).

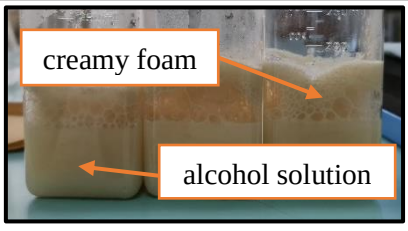
Step 2. Measuring fermentation rate

1. Pour 100ml of fructose solution into a 250ml plastic container using a 100ml (±0.5ml) graduated cylinder.
2. Connect a clamp to a stand and hold the container and place it inside a water bath with a temperature of 32°C (Figure 3). Use a thermometer (±0.5°C) to measure the temperature.
3. Using digital balance pan (±0.01g) and weighing boat, weigh 2.5g of *S. cerevisiae*.
4. When the fructose solution reaches 32°C, pour 2.5g of *S. cerevisiae* inside the container and stir the solution using a stirring rod.
5. Place the CO_2 sensor inside the bio chamber and fix it in the air using another clamp (Figure 3).
6. Connect the CO_2 sensor to the laptop using LabQuest 2 (Figure 3).
7. Measure the CO_2 concentration every 30 seconds using Logger Lite software (Figure 4).
8. After 15 minutes, take out the CO_2 sensor and remove the plastic container from the clamp.
9. Repeat steps 1 to 9 for the other four fructose solutions.

Step 3. Measuring alcohol percentage

1. Place a funnel on top of a 100ml conical flask and put a filter paper inside the funnel (Figure 5).
2. Slowly pour the yeast solution on the filter paper.
3. Filter the solution to prevent yeasts from damaging the display of the alcohol refractometer.
4. Using a pipette, place two drops of alcoholic solution on the alcohol refractometer's display (Figure 6).
5. Close the cover and look into the refractometer to measure the ethanol percentage (Figure 6).
6. Repeat steps 1 to 5 for the remaining four fructose solutions.

Qualitative Data

Observation		Significance
Alcohol solution made by *S. cerevisiae* has a sweet and alcohol-like scent. The sweet and alcohol smell gets stronger as the concentration of fructose increases. It also has a similar scent as bread dough.		The alcohol-like scent suggests that *S. cerevisiae* is doing anaerobic respiration instead of aerobic. Ethanol is produced as a byproduct of anaerobic respiration.
After 15 minutes of fermentation, the solution made by yeast had two distinct layers. The bottom layer was the alcoholic solution, and the top layer was a creamy foam on the surface of the solution. Both layers showed a beige color.	 *Figure 7 – Two distinct layers of alcohol solution*	The creamy foam on top of the solution suggests that *S. cerevisiae* is alive and activated. The bubble gets bigger over time which suggests that CO_2 gas is being produced (Thiel, n.d.). Hence, bigger foam indicates more CO_2 is being produced.

Raw Data

Table 3 – Representative sample table for CO_2 concentration (used 3-minute interval) (full table on Appendix 1)

Conc.			CO_2 Concentration (PPM) (±0.1 ppm)					
g/dm³	Trial	Time (min)	0 min	3 min	6 min	9 min	12 min	15 min
Controlled (0 g/dm³) (±0)		1	480.2	485.7	486.5	487.3	490.3	493.3
		2	540.5	545.0	546.8	547.6	550.6	553.6
		3	530.5	535.9	536.8	537.6	540.6	543.5
50 (±0.27)		1	2059.9	7406.2	15979.8	28059.0	44551.8	61462.4
		2	2103.8	6073.0	12466.4	21091.5	31248.1	46529.8
		3	2460.5	9029.4	19287.1	36199.6	59904.1	84186.6
100 (±0.52)		1	6612.8	20086.3	36251.1	54327.0	72687.1	88199.6
		2	3519.1	12144.1	21776.2	30223.8	44561.4	52354.8
		3	3128.1	15216.8	30733.1	54706.6	73421.5	97951.9
150 (±0.77)		1	2500.5	12844.1	25388.7	44084.5	63201.9	82109.5
		2	2374.6	11034.0	21614.1	34334.2	51977.2	73453.9
		3	1098.6	9492.9	21776.2	43520.0	72929.4	99388.1

Table 4 – Raw data of ethanol percentage produced by *S. cerevisiae* in five different fructose solutions (Appendix 2)

Ethanol Percentage (% v/v) (±0.5 v/v)						
Trials/*Conc.*	0 g/dm³ (±0)	50 g/dm³ (±0.27)	100 g/dm³ (±0.52)	150 g/dm³ (±0.77)	200 g/dm³ (± 1.02)	250 g/dm³ (±1.27)
1	0%	13%	21%	39%	48%	72%
2	0%	13%	22%	33%	49%	69%
3	0%	12%	22%	34%	51%	76%

Calculations

All sample calculations used data from fructose concentration of 50g/dm³.

Calculation 1: Concentration Calculation

To calculate the concertation (g/dm³) of the fructose solution, the weight (g) of the fructose was divided by the volume of the distilled water. For example, to make a fructose solution with a concentration of 50g/dm³, 25g of fructose was added to 500ml (0.5dm³) of distilled water.

$$\text{Concnetration (Conc.)} = \frac{\text{weight } (g)}{\text{volume } (dm^3)} = \frac{25g}{0.5dm^3} = 50\ g/dm^3$$

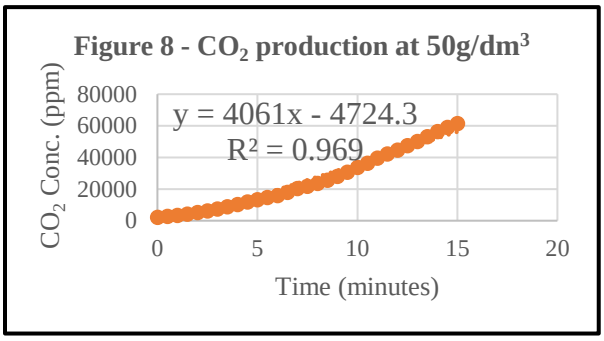
For uncertainty of fructose concentration, measurement uncertainty was used to calculate concentration uncertainty. The uncertainty for 25g of fructose was ±0.01g, and the uncertainty for 0.5dm³ of water was ±0.5cm³. To convert cm³ into dm³, the uncertainty (±0.5cm³) should be divided by 1000. Then the uncertainty for the volume of water should be multiplied by 5 since the water was poured into a conical flask five times as a 100cm³ graduated cylinder was used to measure the volume. Then both measurement uncertainties should be converted to percentage uncertainty because only percentage uncertainties are added when multiplying or dividing quantities (Owen, 2014). Then, the percentage uncertainty for fructose concertation is converted to absolute uncertainty by dividing it by 100 and multiplying the concentration value which is 50g/dm³.

$$\text{Percentage uncertainty} = \frac{\text{absolute uncertainty}}{\text{value}} \times 100$$

$$\text{Percentage uncertainty}_{\text{Conc.}} = \left(\frac{5 \times 0.0005}{0.5} + \frac{0.01}{25}\right) \times 100 = \pm 0.54\%$$

$$\text{Absolute uncertainty}_{\text{Conc.}} = \frac{0.54}{100} \times 50 = \pm 0.27\ g/dm^3$$

Calculation 2: Fermentation Rate Calculation



To calculate the value for the fermentation rate of *S. cerevisiae*, the gradient of the linear regression line derived from the CO_2 production graph was used. To derive the best fit line, software called Excel was used. For instance, according to Figure 8, the best-fit line has an equation of $y = 4061x - 4724.3$. In this equation, the gradient is 4061 which indicates that the fermentation rate at fructose concentration of 50g/dm³ is approximately 4061ppm/min. This assumption is accurate since the coefficient of determination (R^2) is 0.969 which is very close to 1. Hence, the correlation shown in figure 8 is very strong.

Calculation 3: Average Fermentation Rate Calculation

To calculate the mean fermentation rate at each concentration, fermentation rate from each trial, derived from calculation 2, were added and divided the number of terms. For this investigation, the number of terms is 3 since a total of three trials were done for each fructose concentration. For sample calculation, data from appendix 3 are used.

$$\text{Mean}_{\text{fermentation rate}} = \frac{\text{sum of terms}}{\text{number of terms}} = \frac{4061.0 + 2927.5 + 5581.4}{3} \approx 4190.0 \text{ ppm/min}$$

For the uncertainty, the standard deviation of each fermentation rate was calculated using software called Microsoft Excel. After selecting data from each trial, STDEV.P function was used to calculate the standard deviation for a sample set of data.

$$\text{Standard deviation} = \text{STDEV.P}(4061.0 : 2927.5 : 5581.4) \approx \pm 1087$$

Calculation 4: Average Ethanol Concentration Calculation

To calculate the mean ethanol concentration at each fructose concentration, the same methodology as calculation 3 was used. For sample calculation, data from table 2 was used.

$$\text{Mean}_{\text{ethanol Conc.}} = \frac{\text{sum of terms}}{\text{number of terms}} = \frac{13 + 13 + 12}{3} \approx 12.7\%$$

For the uncertainty, standard deviation of each ethanol concentration was calculated using same method as calculation 3.

$$\text{Standard deviation} = \text{STDEV.P}(13 : 13 : 12) \approx \pm 0.5$$

Processed Data

Table 5 – Average Respiration Rate by *S. cerevisiae* (appendix 4)

C (g/dm³)	0 (±0)	50 (±0.27)	100 (±0.52)	150 (±0.77)	200 (± 1.02)	250 (±1.27)
Rate (ppm/min ±SD)	0.6811 ($\pm 9.441\times10^{-3}$)	4190 (±1087)	5155 (±1286)	5696 (±952.6)	4517 (±886.8)	4271 (±670.4)

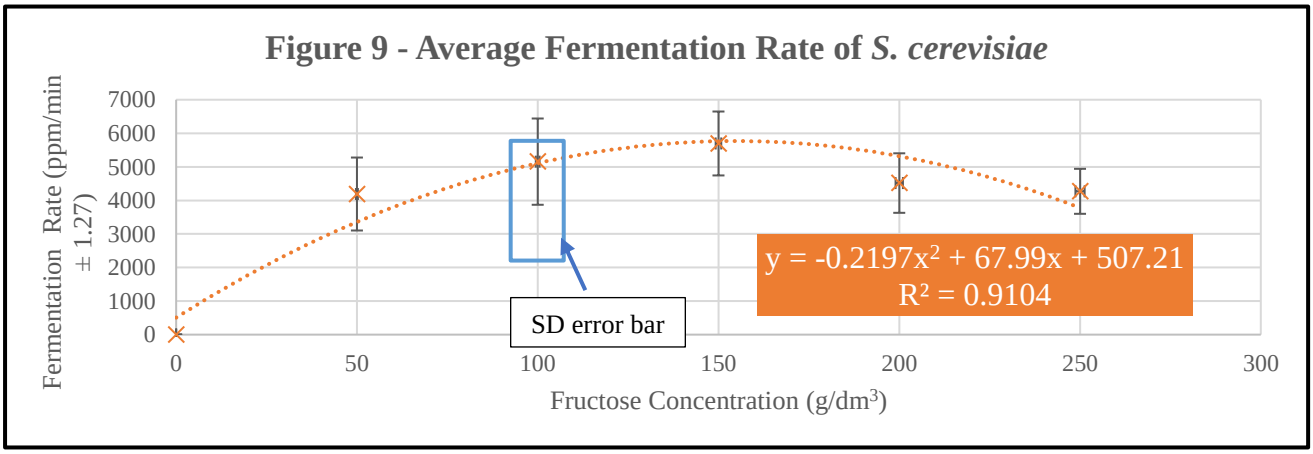
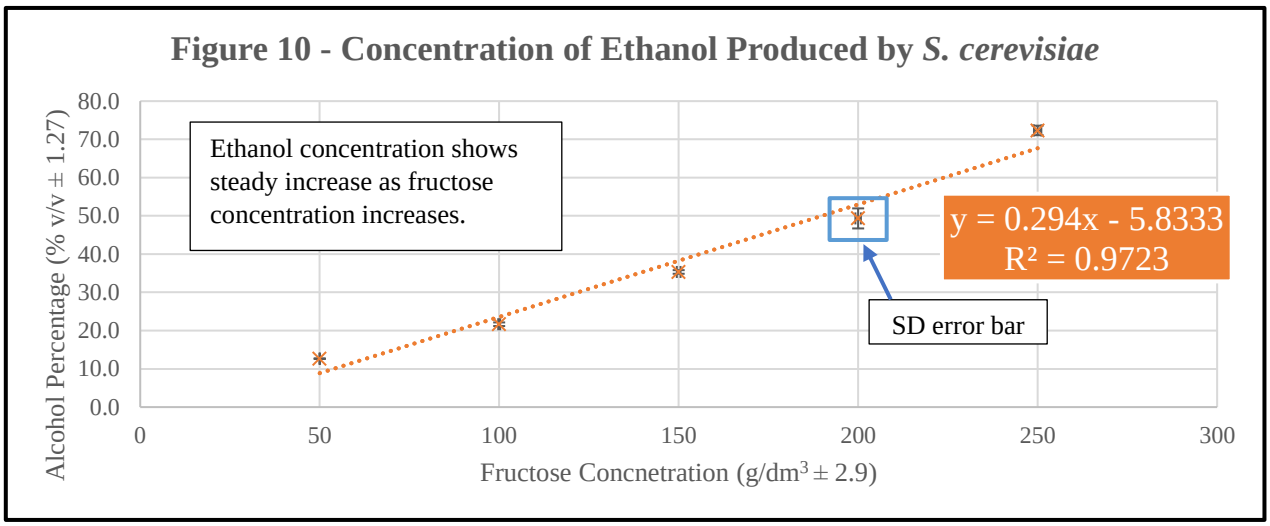


Table 6 - Average concentration of ethanol produced by *S. cerevisiae* (appendix 5)

C (g/dm^3)	0 (±0)	50 (±0.27)	100 (±0.52)	150 (±0.77)	200 (± 1.02)	250 (±1.27)
Ethanol Concentration (% v/v) (±SD)	0% (±0)	12.7% (±0.471)	21.7% (±0.471)	35.3% (±2.62)	49.3% (±1.24)	72.3% (±2.87)



Analysis

The respiration rate of *S. cerevisiae* showed a trend of both increase and decrease. At first, as the fructose concentration increased from 0 to 150g/dm^3, the respiration rate showed a positive correlation (Figure 9) as the rate increased from 0.6811 ppm/min (±9.441×10^{-3}) to nearly 5696 ppm/min (±952.6) (table 5). However, as the fructose concentration increased to 200g/dm^3 and 250g/dm^3, the respiration rate of *S. cerevisiae* started to decrease after reaching the maximum respiration rate at 150g/dm^3 (Figure 9). Although there were more carbohydrates available for *S. cerevisiae* to do cellular respiration, the rate started to decline which suggests that *S. cerevisiae* was not able to do the fermentation.

As the fructose concentration increased from 0 to 250g/dm^3, one condition that has changed is the concentration of ethanol produced by *S. cerevisiae*. According to Figure 10, ethanol concentration showed a very strong positive correlation with an R^2 value of 0.9723. It showed a steady increase with a gradient of 0.294 indicating that every 1g/dm^3 increase in fructose concentration will result in a 0.294% increase in alcohol concentration (figure 8). Due to the accumulation of ethanol, which is a byproduct of anaerobic respiration, the respiration rate of *S. cerevisiae* has declined after 150g/dm^3 since ethanol is toxic.

To check the reliability of the experiment results, measurement uncertainty was used for fructose uncertainty as standard deviation (SD) could not be calculated since there is only one data for concentration. For respiration rate and ethanol concentration, SD value has been calculated (calculation 3&4). According to both tables 5 and 6, the measurement uncertainty for fructose concentration is extremely low as the highest uncertainty value is only ±1.27g/dm^3 which is only ±0.508% when converted to percentage uncertainty. Similarly, the SD value for ethanol concentration was very low as well. According to table 6, the highest SD value was 2.9 which can barely be seen on the graph as an error bar (Figure 10). This indicates that each value gathered from each trial is very close to the mean value, suggesting that data for ethanol concentration are precise. However, the SD value for respiration rate was relatively very high. According to table 5, the lowest SD value was 670 and the highest was 1286. As shown in figure 7, the error bar for SD value is very wide which suggests that data for respiration rate are far apart from the mean value and are not precise compared to the SD value for ethanol concentration.

Overall, the *S. cerevisiae*'s respiration rate showed a positive correlation until the fructose concentration of 150g/dm^3. Concentration above resulted in a decline in respiration rate, and one possible reason is due to accumulation of ethanol. Although *S. cerevisiae* can resist up to 14% of ethanol (Ghareib, 1988), ethanol is still toxic and may cause stress which will result in decreased yeast activity. However, the data for respiration rate may not be an accurate representation of effect of fructose concentration on yeast activity due to high SD value.

Statistical Analysis

To determine the statistically significant difference among the data, a one-way ANOVA test will be used to investigate if mean values for both respiration rate and concentration of ethanol produced by *S. cerevisiae* are equal or not. One-way ANOVA test is often associated with two statistical hypotheses: null hypothesis (H_0) and alternative hypothesis (H_a) (*One-Way*, n.d.).

H_0 = There is no statistically significant difference between the value for respiration rate (or ethanol concentration). Any differences are the result of random chance.

H_a = There is a statistically significant difference between the value for respiration rate (or ethanol concentration). The differences are too large to be due to random variation.

To determine which hypothesis should be accepted, F-statistic and P-value will be calculated using a one-way ANOVA test. The F-value in the one-way ANOVA test is used to check if the variance between means is significantly different. It is often used along with P-value which is the probability that the mean values have occurred by random chance (Glen, n.d.). The null hypothesis is rejected when the P-value is smaller than the alpha value (level of significance), and when the F-value is larger than the F-critical value (ibid).

The one-way ANOVA test will be done using Microsoft Excel. To run the test, a data analysis tool kit in Microsoft Excel will be used, and the "ANOVA: Single Factor" function will be selected for the test. For this investigation, the alpha value was 0.05.

Table 7 – Results of one-way ANOVA test for respiration rate of *Saccharomyces cerevisiae*

One-way ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F-value*	*P-value*	*F-critical value*
Between Groups	61732223	5	12346445	9.920279	0.000609	3.105875

Based on table 7, the P-value for respiration rate of *S. cerevisiae* was 0.000609 which is much lower than the alpha value (0.05). Moreover, the F-value for the respiration rate (9.92) was higher than its F-critical value (3.11). Hence, for the respiration rate of *S. cerevisiae*, H_0 hypothesis is rejected and H_a hypothesis is accepted instead.

Table 8 – Results of one-way ANOVA test for ethanol percentage

One-way ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F-value*	*P-value*	*F-critical value*
Between Groups	10328.44	5	2065.689	482.8883	2.13E-13	3.105875

Similarly, according to table 8, the P-value for ethanol concentration is 2.13E^{-13}, which is smaller than the alpha value (0.05). Also, the F-value for ethanol concentration (483) is larger than its F-critical value (3.11). Hence, for ethanol concentration, the H_0 hypothesis is rejected and the H_a hypothesis is accepted as well. This means that there is a statistically significant difference between the mean values of both respiration rate and ethanol concentration and their trends are not caused by random chance. Thus, this suggests that there is a trend for both respiration rate and ethanol percentage.

Conclusion

As the fructose concentration of the solution increased from 0 to 150g/dm^3, the overall respiration rate by *S. cerevisiae* has increased from 0.6811 to 5696 ppm/min. However, the respiration rate reached its maximum at 150g/dm^3 (5696 ppm/min) and started to decline as the concentration increased to 200 and 250g/dm^3. In contrast, ethanol concentration showed a steady increase from 0% to 72.3% with a steady increasing rate of 0.294% per 1g/dm^3 of fructose concentration. However, this trend weakly supports my hypothesis. Although it showed an increase in both respiration rate and ethanol concentration, my hypothesis predicted that the respiration rate will peak at 200g/dm^3 and start to plateau after that instead of declining. Instead, the respiration rate peaked at 150g/dm^3 and started to decrease as the concentration increased to 250g/dm^3.

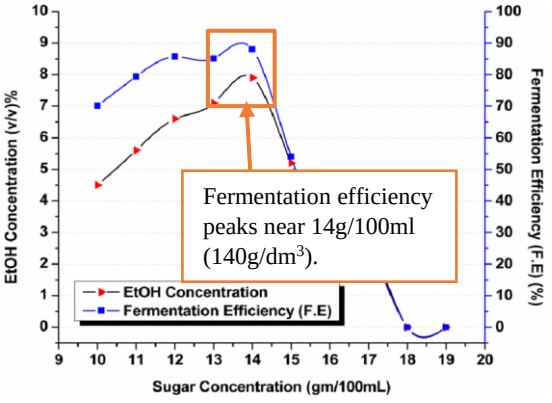


Figure 11 - Effect of sugar concentration on yeast (S. cerevisiae) fermentation (Khoja, 2015)

One possible reason why the respiration rate dropped is because of the increasing concentration of ethanol. Although *S. cerevisiae* is known to have high alcohol tolerance, relatively high ethanol concentration inhibits its metabolism and cell growth (Stanley, 2009). This is because ethanol acts as an inhibitor to yeast growth. At high concentrations, ethanol inhibits cell division within *S. cerevisiae* and decreases its cell volume (Stanley, 2009). It also influences the cell's metabolism by stimulating the production of "heat shock-like protein," which lowers the rate of RNA and protein accumulation within *S. cerevisiae* (Ghareib, 1988). Hence, the respiration rate of *S. cerevisiae* decreases when the sugar concentration is too high due to the accumulation of ethanol.

To test the accuracy, the investigation results were compared with similar data from published data. According to a research paper by Asif H. Khoja, the fermentation efficiency of *S. cerevisiae* reaches its maximum at a sugar concentration of 14gm/100ml (Figure 11). After the fermentation efficiency reached its peak near 14gm/100ml, the efficiency rapidly drops (Figure 11). The sugar concentration of 14gm/100ml in dm^3 would be 140g/dm^3. Considering that the respiration rate of *S. cerevisiae* peaked at 150g/dm^3 for this investigation, the trend for fermentation efficiency also shows a very similar trend. Although Khoja's experiment did not use fructose and used a different dependent variable (fermentation efficiency), it still shows a similar trend with a low percentage error of 7.14%. Thus, although the experimental results did not fully support my hypothesis as *S. cerevisiae*'s fermentation rate declined after its peak at fructose concentration of 150g/dm^3 due to high ethanol concentration produced, the result is reliable since the percentage error of the experiment is 7.14% which is relatively low.

$$\text{Percentage error} = \frac{|\text{Observed value} - \text{expected value}|}{\text{expected value}} = \frac{|150 - 140|}{140} \times 100 \approx 7.14\%$$

Evaluation

Strengths	Significance to the results
Filtering alcohol solution before measurement	Before measuring the concentration of ethanol, the solution was filtered using filter paper. This was because the alcoholic solution had a foam on top which contains impurities such as *S. cerevisiae*. The operation manual of the alcohol refractometer (Figure 6) explicitly states that abrasive substances can damage the measuring prism's coating and should not be measured as it will lead to inaccurate measurement. By filtering the solution, excess *S. cerevisiae* was removed from the solution to prevent it from damaging the refractometer. As a result, the concentration of ethanol has a very low standard deviation value (table 6) suggesting that the data are very precise.

Strengths	Significance to the results
Low percentage error	The percentage error for this investigation was approximately 7.14%. This shows that this investigation's results for respiration rate were close to the theoretical value. The low percentage error suggests that the results gained from this investigation are reliable to some extent as the accuracy is high.

Limitations	Impacts	Suggestions
Number of trials	Due to limited time, only three trials were done for each concentration. Although there were no significant outliers, the standard deviation (SD) values for respiration rate were very high (Table 5). This shows the data for respiration rate has low precision. Hence, the trend shown in Figure 9 may not be an accurate representation of the trend.	To lower the SD value, more trials should be performed. For example, at least 5 trials should be done for higher precision.
Alcohol refractometer	The alcohol refractometer used for this investigation is analog and the smallest unit is only 1% with an uncertainty of ±0.5. Thus, it is difficult to measure accurate data as the measurement heavily relies on eye judgment and it cannot measure to decimal places, which can lead to huge human error.	Instead of using an alcohol refractometer, a Vernier alcohol sensor can be used to measure more accurate data.
Anaerobic condition	Fermentation occurs when the *S. cerevisiae* is in anaerobic condition. Although *S. cerevisiae* prefers anaerobic respiration, there is a possibility that it performed aerobic respiration since it was difficult to make an anaerobic condition using equipment in the school laboratory. As a result, the ethanol concentration can be lower than the actual value because less ethanol may have been produced due to aerobic conditions.	It is impossible to make an anaerobic condition in a school laboratory since the equipment required is not affordable.

Further Investigation

According to this investigation's results, the respiration rate of *S. cerevisiae* reached peaked at a fructose concentration of 150g/dm^3 and declined due to the accumulation of ethanol. However, the difference between each independent variable is 50g/dm^3, and this result does not show the accurate trend of respiration rate between each fructose concentration. Therefore, as an extension of this investigation, I would like to investigate the trend for fermentation rate near 150g/dm^3. Instead of using 50g/dm^3 intervals, I would like to use 10g/dm^3 intervals with a range of 100g/dm^3 and 150g/dm^3 concentration. By doing this, I would be able to investigate if the respiration rate reached its maximum before 150g/dm^3. Although a higher fermentation rate does not necessarily mean a higher quality of wine, the result from this study can be used in real life to control the concentration of sugar used in wine as the yeasts necessary for wine production may die before the wine is fully fermented. I would also like to investigate how different types of carbohydrate monomers, such as glucose and galactose, affect the fermentation rate of *S. cerevisiae*. Although researchers claim glucose is a more efficient monosaccharide as it is consumed at a higher rate (Lamon, 2018), the major sugar molecule in most grapes is fructose (Sruthi, 2021). Hence, I would like to investigate which sugar monomers are best for *S. cerevisiae*'s respiration as well.

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Appendices

Appendix 1 –Raw data for CO_2 concentration in ppm

CO_2 concentration (ppm)									
Conc.	Controlled (0 g/dm^3)			50 g/dm^3			100 g/dm^3		
Time (min)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0.0	480.2	540.5	530.5	2059.9	2103.8	2460.5	6612.8	3519.1	3128.1
0.5	480.2	540.5	530.5	2672.2	2615.0	3374.1	8998.9	4871.4	5044.9
1.0	483.9	544.2	534.1	3427.5	3160.5	4343.0	11243.8	6330.5	7219.3
1.5	483.2	543.5	533.5	4255.3	3770.8	5300.5	13208.4	7837.3	9359.4
2.0	483.3	543.6	533.6	5153.7	4425.0	6387.7	15161.5	9220.1	11518.5
2.5	484.8	545.0	535.0	6139.8	5214.7	7690.4	17232.9	10681.2	13393.4
3.0	485.7	545.9	535.9	7406.2	6073.0	9029.4	20086.3	12144.1	15216.8
3.5	486.7	546.9	536.9	8869.2	6958.0	10438.9	21993.6	13343.8	17410.3
4.0	485.8	546.1	536.0	10253.9	8083.3	11936.2	24190.9	14688.5	20349.5
4.5	486.0	546.3	536.2	11793.1	9185.8	13460.2	26559.8	15909.2	22338.9
5.0	486.3	546.6	536.6	13176.0	10164.3	14965.1	29703.1	17644.9	24864.2
5.5	486.7	547.0	537.0	14652.3	11455.5	16550.1	32873.2	19523.6	27874.0
6.0	486.5	546.8	536.8	15979.8	12466.4	19287.1	36251.1	21776.2	30733.1
6.5	486.6	546.8	536.8	17940.5	13780.6	21543.5	39703.4	23210.5	34683.2
7.0	487.2	547.5	537.5	20402.9	14720.9	23605.3	42821.9	24528.5	38375.9
7.5	487.2	547.5	537.5	21854.4	15932.1	26193.6	45616.1	26472.1	42076.1
8.0	487.5	547.7	537.7	23590.1	17156.6	28780.0	48606.9	28585.4	46545.0
8.5	487.8	548.0	538.0	25600.4	18770.2	32159.8	52251.8	28570.2	51019.7
9.0	487.3	547.6	537.6	28059.0	21091.5	36199.6	54327.0	30223.8	54706.6
9.5	488.3	548.6	538.6	30616.8	22365.6	39630.9	57659.1	31707.8	58311.5
10.0	489.1	549.4	539.4	33641.8	23517.6	43893.8	60766.2	33428.2	59162.1
10.5	489.3	549.6	539.6	36415.1	25205.6	48669.8	63823.7	35596.8	58691.0
11.0	489.1	549.4	539.4	39548.9	27484.9	52471.2	67159.7	38158.4	63142.8
11.5	489.3	549.6	539.6	42247.8	29825.2	55845.3	69829.9	41202.5	68698.9
12.0	490.3	550.6	540.6	44551.8	31248.1	59904.1	72687.1	44561.4	73421.5
12.5	490.3	550.6	540.5	47498.7	33275.6	64415.0	74951.2	46464.9	77638.6
13.0	491.2	551.5	541.5	50041.2	36546.7	69376.0	78331.0	48303.6	81827.2
13.5	491.8	552.1	542.1	53127.3	39995.2	72637.6	80530.2	49114.2	85981.4
14.0	492.3	552.6	542.6	56362.2	42907.7	75775.1	83374.0	49844.7	90339.7
14.5	492.3	552.6	542.6	58925.6	44626.2	79666.1	85659.0	51544.2	94078.1
15.0	493.3	553.6	543.5	61462.4	46529.8	84186.6	88199.6	52354.8	97951.9

(Appendix 1 continued)

CO_2 concentration (ppm)									
	150 g/dm^3			200 g/dm^3			250 g/dm^3		
Time (min)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0.0	2500.5	2374.6	1098.6	1077.7	1951.2	2134.3	1148.2	1632.7	1188.3
0.5	3803.3	3459.9	1804.4	1810.1	2725.6	3183.4	1661.3	2292.6	1878.7
1.0	5464.6	4810.3	2862.9	2828.6	3830.0	4554.7	2363.2	3112.8	2821.0
1.5	7352.8	6290.4	4316.3	4156.1	5029.7	6242.8	3295.9	4098.9	4091.3
2.0	9193.4	7909.8	5914.7	5674.4	6389.6	8228.3	4373.6	5155.6	5434.0
2.5	11047.4	9517.7	7741.9	7345.2	7978.4	10072.7	5571.4	6336.2	6862.6
3.0	12844.1	11034.0	9492.9	8935.9	9510.0	11978.1	6849.3	7597.0	8445.7
3.5	14644.6	12617.1	11375.4	10433.2	10898.6	13610.8	8365.6	8859.6	9973.5
4.0	16292.6	14215.5	13139.7	11961.0	12336.7	15192.0	9700.8	10187.1	11608.1
4.5	18985.7	15512.5	14844.9	13290.4	13860.7	17114.6	11171.3	11280.1	13071.1
5.0	21381.4	17253.9	16721.7	14703.8	15033.7	19735.3	12535.1	12651.4	14534.0
5.5	23248.7	19212.7	19630.4	16010.3	16513.8	21722.8	13879.8	13790.1	15918.7
6.0	25388.7	21614.1	21776.2	17919.5	18186.6	23820.9	15214.9	15092.8	17971.0
6.5	28173.4	23141.9	24335.9	20013.8	20465.9	26147.8	16723.6	16031.3	20513.5
7.0	31143.2	24631.5	27223.6	21631.2	22153.9	28507.2	19050.6	17690.7	22182.5
7.5	34374.2	26907.0	30582.4	23164.7	23284.9	31829.8	21135.3	19683.8	24202.3
8.0	37406.9	29922.5	34721.4	25047.3	24829.9	35348.9	22491.5	21108.6	26613.2
8.5	41122.4	32238.0	38499.8	26968.0	27473.4	38312.9	24501.8	22701.3	29365.5
9.0	44084.5	34334.2	43520.0	29228.2	29642.1	41954.0	26523.6	24198.5	31776.4
9.5	46991.3	37494.7	48656.5	31902.3	31381.6	46176.9	29394.1	25577.5	34725.2
10.0	49928.7	41713.7	53051.0	34553.5	33334.7	49816.1	31900.4	27463.9	38839.3
10.5	53417.2	45408.2	58080.7	37200.9	36037.4	52757.3	34660.3	30212.4	42499.5
11.0	57037.4	48027.0	63415.5	40220.3	39270.4	56259.2	37902.8	33083.0	45906.1
11.5	60201.6	49995.4	68653.1	42848.6	42308.8	60625.1	41317.0	35001.8	50123.2
12.0	63201.9	51977.2	72929.4	45000.1	44523.2	64737.3	44445.0	36975.9	54361.3
12.5	66581.7	54452.9	78544.6	47611.2	46281.8	68290.7	47918.3	39926.5	58689.1
13.0	69257.7	57157.5	83780.3	50479.9	48080.4	71714.4	51881.8	43479.9	61994.6
13.5	72618.5	60913.1	89820.9	52990.0	49852.4	75101.9	55511.5	47424.3	65765.4
14.0	75958.3	64754.5	93641.3	55568.7	51981.0	78805.9	58050.2	50546.6	69915.8
14.5	79532.6	69915.8	97330.1	58542.3	54485.3	82826.6	61313.6	52875.5	74716.6
15.0	82109.5	73453.9	99388.1	60964.6	57916.6	86809.2	64193.7	54765.7	78594.2

Appendix 2 – Raw data for ethanol concentration

Ethanol Percentage (% v/v) (±0.5 v/v)						
Trials/ Fructose Conc	Controlled (0 g/dm^3)	50 g/dm^3	100 g/dm^3	150 g/dm^3	200 g/dm^3	250 g/dm^3
1	0%	13%	21%	39%	48%	72%
2	0%	13%	22%	33%	49%	69%
3	0%	12%	22%	34%	51%	76%

Appendix 3 – Respiration rate by *S. cerevisiae*

C	Respiration Rate (ppm/minute)		
	Trial 1	Trial 2	Trial 3
Controlled	0.6884	0.6872	0.6678
50	4061.0	2927.5	5581.4
100	5673.1	3386.7	6405.3
150	5488.3	4647.6	6953.0
200	4031.4	3757.8	5760.7
250	4217.4	3477.3	5116.9

Appendix 4 – Average Respiration rate by *S. cerevisiae*

C (g/dm^3)	0 (±0)	50 (±0.27)	100 (±0.52)	150 (±0.77)	200 (± 1.02)	250 (±1.27)
Rate	0.6811 (±0.0094)	4190 (±1087)	5155 (±1286)	5696 (±952)	4517 (±887)	4271 (±670)

Appendix 5 – Average Alcohol Percentage

C (g/dm^3)	0 (±0)	50 (±0.27)	100 (±0.52)	150 (±0.77)	200 (± 1.02)	250 (±1.27)
Ethanol Concentration (% v/v)	0 (±0)	12.7% (±0.5)	21.7% (±0.5)	35.3% (±2.6)	49.3% (±1.2)	72.3% (±2.9)