Extended Essay

Research title:

The relationship between fermentation and plasmolysis in yeast due to a varying glucose concentration

Research question:

Is there any correlation between the process of fermentation and plasmolysis in yeast when exposed to varying glucose concentrations?

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Table of Content

Introduction

Yeasts are single-celled fungi, which identify as eukaryotic organisms. There are 1,500 species of yeast that have been found where most of them are in the phylum Ascomycota and few are in Basidiomycota. Yeasts belong to the fungi kingdom in which they make up 1% of all fungal species (Springer, 2006). They are crucial in the making of beer, wine, and bread and are generally found in soil as well as on plant surfaces such as flowers, leaves, and fruits.

A scientific phenomenon carried out by all yeasts is fermentation. These organisms have the ability to promptly convert sugars into ethanol and carbon dioxide which helps yeasts, among other things, to outcompete other microorganisms.

The development and survival of larval has been known to be assisted by the availability of ripe fruits due to the fact that they grant wild yeasts to grow and flourish which furthermore attracts insects (Becher et al., 2012). Yeasts can also have nutritional benefits for humans and animals. Because of the fact that they don't use up calories in uneconomic activities, they can and have been successfully used in treating vitamin B deficiencies. The results of various tests showed an increase in overall growth in rats and children who had more food yeast in their diet than others that didn't ("Food Yeast").

In order for yeasts to perform fermentation, sugars such as glucose and sucrose have to be broken down at aerobic or anaerobic conditions to produce carbon dioxide and ethanol. In alcoholic fermentation under anaerobic conditions, the organic compound acetaldehyde is the

final electron acceptor and gets converted, under fermentative growth, into ethanol. Under aerobic conditions, however, oxygen is present and therefore takes over the place of the final electron acceptor. Yeasts such as *Saccharomyces cerevisiae* exhibit alcoholic fermentation until the sugar is broken down fully from the medium. The biochemist Herbert Grace Crabtree discovered a process called the Crabtree effect which describes the ability of Crabtree-positive yeasts to simultaneously use respiration and fermentation to produce ATP (Hagman and Piškur).

Another process carried out by cells is plasmolysis which is a response of cells when they're exposed to hyperosmotic stress. When the surrounding solution of a cell has a higher solute concentration and therefore lower water potential, osmosis occurs and results in water flowing from the inside of the cell to the cell's surrounding solution. This is called hyperosmotic stress. The cell starts to shrink due to the loss of water. Cell death can result from this if the cell shrinks to the point that the cell membrane collapses. Deplasmolysis, the reversal of plasmolysis, can occur if the solute concentration outside of the cell is decreased. Water will then reenter the cell by osmosis, which can then lead to a full restoration of the cell and its membrane but only if it hasn't been severely damaged (Lang et al).

The experiments carried out in this research paper measured the rate of fermentation and the rate of plasmolysis in yeast cells when exposed to different glucose concentrations. The aim of the experiments is to find out whether a correlation between these two processes can be observed in the cells of *Saccharomyces cerevisiae*.

Hypothesis

I believe that there will be a relationship between the process of fermentation and the process of plasmolysis in cells of *Saccharomyces cerevisiae*. This is because the essential input in the fermentation process is sugar, generally glucose being converted into carbon dioxide and ethanol. In nature, the sugar concentration outside of the cells can vary from very high to very low which will not only affect the production of carbon dioxide and ethanol but also the size of the cells. This is due to the fact that cells experience plasmolysis when in exposure to a solute concentration higher than inside of the cell which could lead to cell death and a decrease or halt of fermentation.

Methods of Investigation

Fermentation experiment

The fermentation experiment was designed to show the change in fermentation performance in *Saccharomyces cerevisiae* cells when exposed to varying glucose concentrations. The possible methods for this investigation were restricted due to limited available equipment which will be elaborated in greater detail in the evaluation. The independent variable of this experiment will be the differing glucose concentrations of the yeast-glucose solution. The concentrations are: 1 M, 1.5 M, 2 M, 2.5 M, and 3 M. These were chosen after various trial experiments in which the glucose concentrations ranged from for instance 0.2 M to 1 M and showed little difference in CO_2 production. The dependent variable will be the volume of CO_2 produced. The reason for

the choice of this variable, rather than the amount of ethanol produced or amount of glucose taken up by the cells over time, was the accessible gas collection tubes already provided in the laboratory. These were filled up with water, attached to a clamp stand with the opening of the tube dipped into a bowl filled with water. Two water baths were set to a goal temperature of 37°C. The rubber tubes attached to the rubber stoppers were placed under each gas collection tube. The test tubes were put in a test tube rack which was placed into the water bath. 10 ml of each differently concentrated glucose solution was measured and poured into separate beakers which were then placed into the water bath half covered with water. This was done to ensure that each solution has the same temperature when the yeast is added. The glucose solutions were then poured into the test tubes. 1 g of yeast powder was measured for each test tube, added to it, and the stoppers were put on. The volume of CO_2 produced was measured every two minutes for 32 minutes. This whole process was repeated four times. The controlled variables within this investigation include the temperature of the water bath which was controlled by placing a thermometer in it and adjusting the temperature when it fluctuated slightly. Additionally, the yeast condition should be the same throughout the whole investigation in order to accurately compare the cells' ability to ferment in each glucose concentration. Only one package of yeast powder from a single producer was used, therefore the age and condition of the yeast were assumed to be the same.

Plasmolysis experiment

The second investigation that will be carried out is the plasmolysis experiment which was constructed to offer findings regarding the cells' size when exposed to varying glucose

concentrations. Again, the independent variable of this investigation will be the changing glucose concentration. The same glucose concentration as in the fermentation experiment will be used because it will allow for direct observation and comparison between the volume of CO_2 produced at each concentration and the size of the cells at each concentration. This is essential in providing an answer to the research question. The dependent variable of this investigation will be the size of the yeast cells. In order to find if cells, as hypothesized, become plasmolyzed in solutions with excessive amounts of glucose, the cells have to be measured to see if a decrease in size occurs. 250 *Saccharomyces cerevisiae* cells were measured using a microscope with an oil immersion lens which has a magnification of 100x. Before this was done, 10 ml of each glucose concentration solution was measured and poured into separate beakers before 0.08 g of yeast were added to each of them. This method was chosen after various trial experiments. 5 yeast cells were measured in a 0M glucose solution and a 1M glucose solution. 1 g of yeast powder was added to both solutions before any measurements were taken. The solutions were left for 30 minutes. A 40% difference between cell sizes in both solutions was observed while noting that the excessive number of yeast cells made it hard to measure cells. 0.05 grams of yeast was mixed with a 2M glucose solution, observing a 10% decrease in cell size from the 1M solution. This time, the number of yeast cells was too little. Various variables will be controlled during the experiment. These include the condition of the yeast which was again was assumed to be the same as it came for all cells from the same package. The same microscope and oil immersion lens were used to measure the cells throughout the whole measuring process.

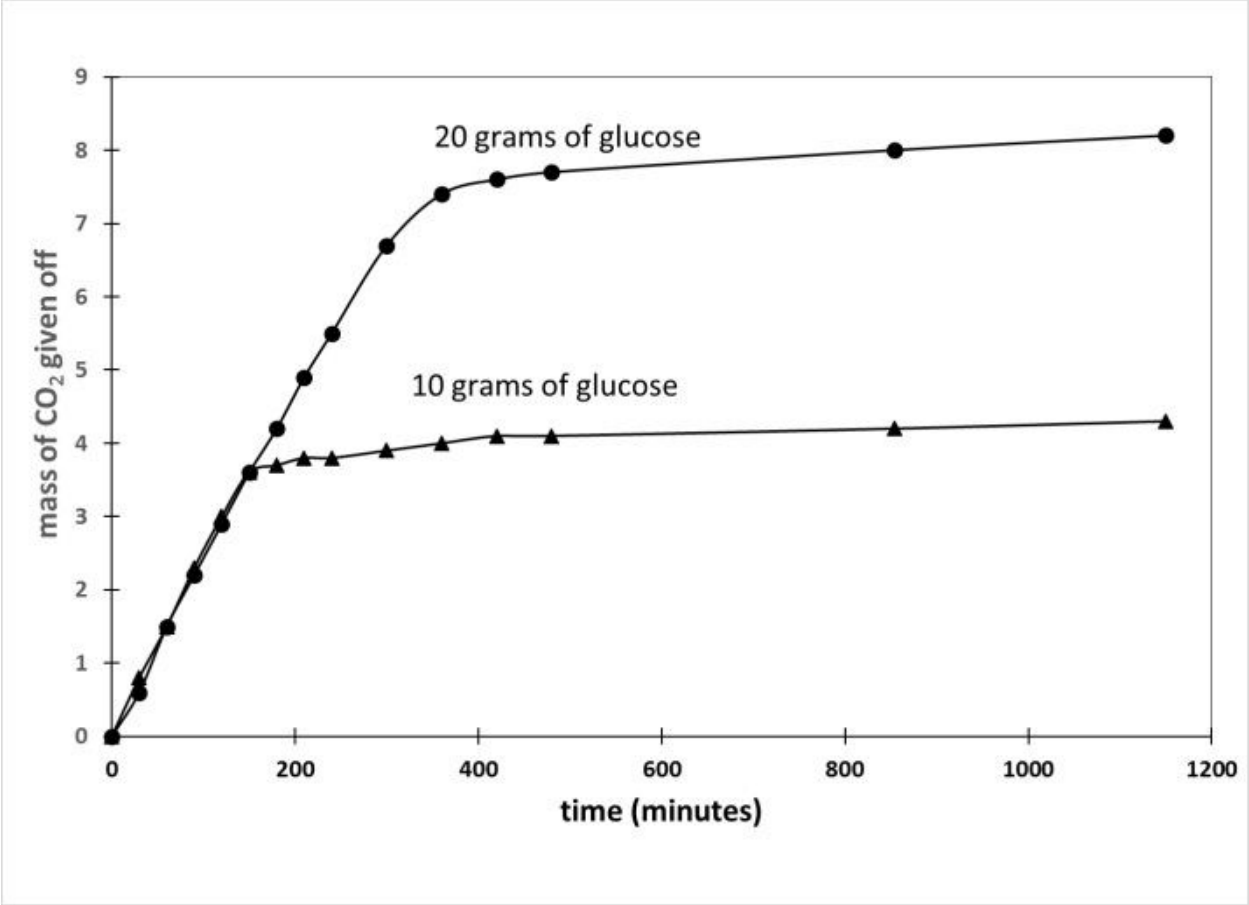
Results and Analysis

Table 1: Table showing the average volume of CO_2 produced in each trial after 32 minutes. The average and total volume of all trials is also presented.

Glucose concentration (M)	Volume of CO_2 produced after 32 minutes (ml) ±0.05						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	Total
1	50.0	45.2	34.6	39.2	40.7	41.9	209.7
1.5	36.9	39.1	31.5	28.9	37.7	34.8	174.1
2	22.0	31.5	22.5	35.0	27.5	27.7	138.5
2.5	23.5	28.0	24.3	27.0	20.0	24.6	122.8
3	13.4	18.9	9.3	20.0	19.7	16.3	81.3

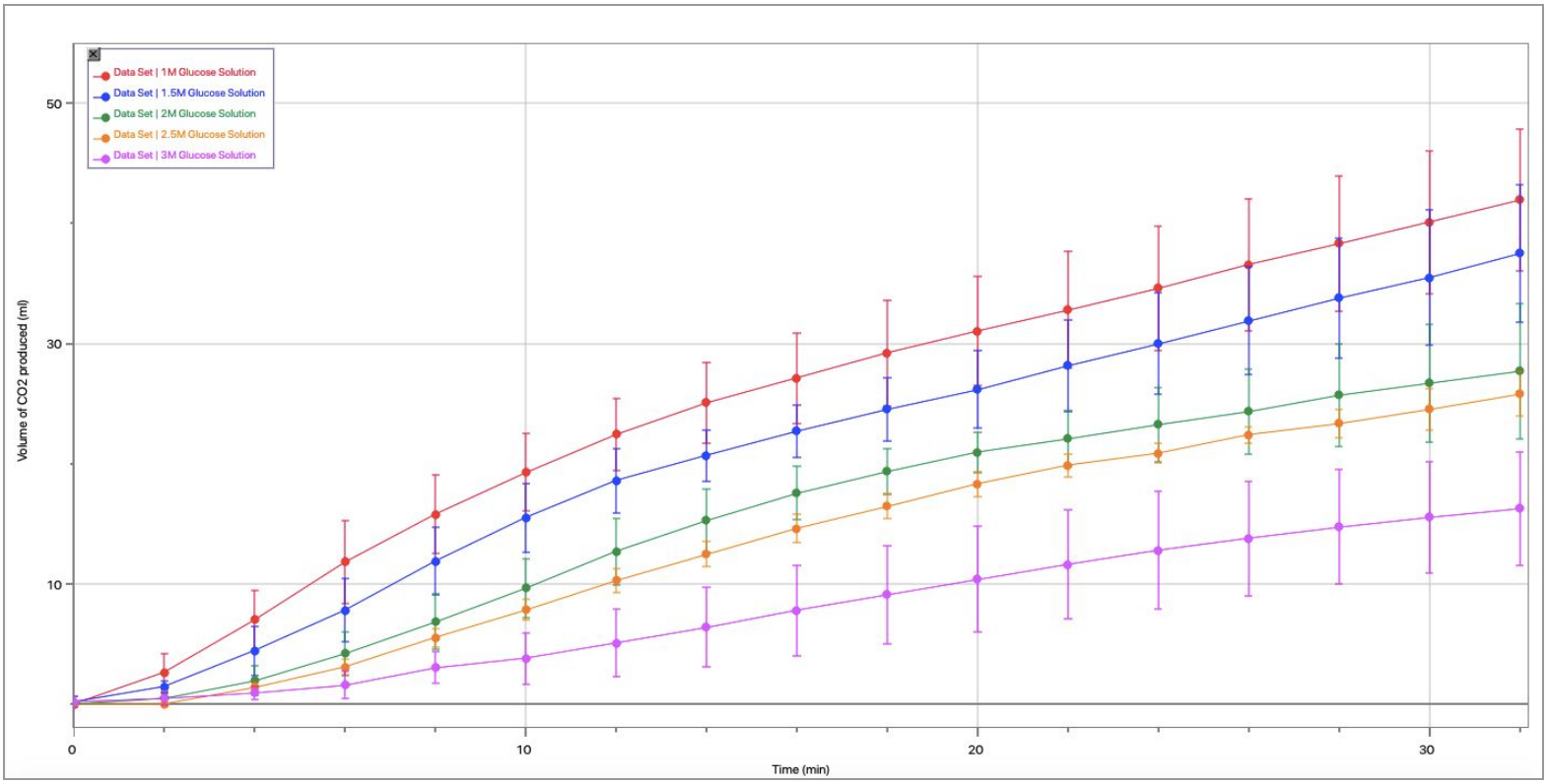
The results presented in table 1 show a definite distinction between the volume of CO_2 produced by yeast in the different glucose concentrated solutions. The difference between the average volume of CO_2 of all trials produced in the 1 M glucose solution and the 3 M glucose solution is 25.6 ml, a 61.2% decrease from the original average value in the 1 M solution which is 41.9 ml. This clearly demonstrates a correlation of decreasing production of CO_2 as the glucose concentration increases, suggesting that the yeast was highly affected by the osmotic stress that it was exposed to, leading to a complication in the fermentation process (Ishmayana et al., 2011).

Fig. 1: Graph displaying the mass of CO_2 given off in a fermentation experiment using 20 g and 10 g of glucose (Pepin, 2015)



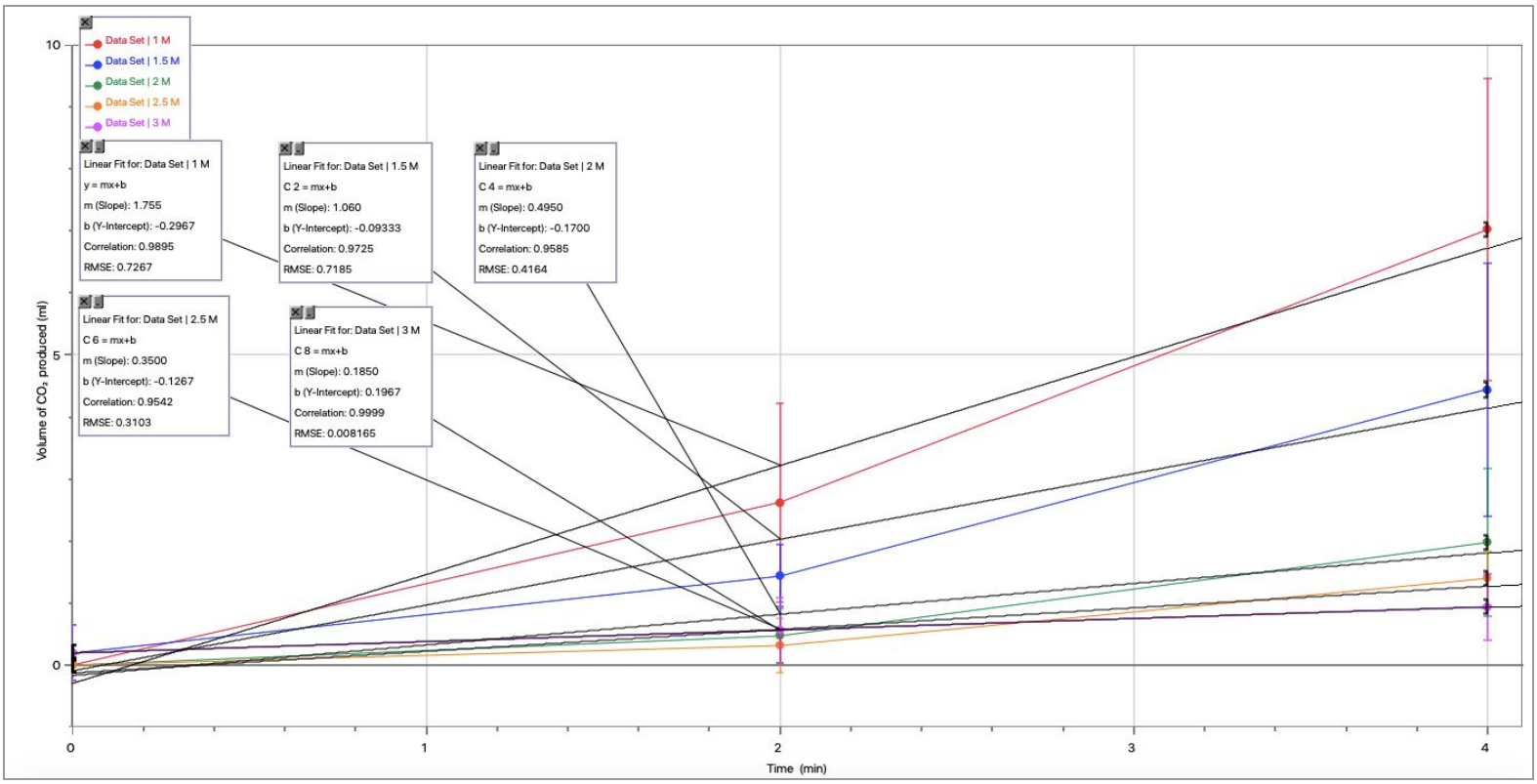
This graph demonstrates a fermentation experiment where different amounts of glucose were tested. The glucose amounts were dissolved into 100 ml of water with 7 g of yeast added to the solutions (Pepin, 2015). As seen in this graph, in both solutions the same mass of CO_2 is given off during the 200 minutes of the experiment. The solution with more sugar then continues to produce more CO_2 while the solution with less sugar does not. However, at 400 minutes the solution with 20 g glucose shows the same pattern, suggesting that the yeast exhibits alcoholic fermentation until the glucose is used up from the medium. This indicates that in relatively low glucose concentrations, the production of CO_2 is greater when the glucose concentration is higher.

Fig. 2: Graph showing the average volume of CO_2 produced in each glucose concentration solution over the course of 32 minutes. The standard deviation is also shown.



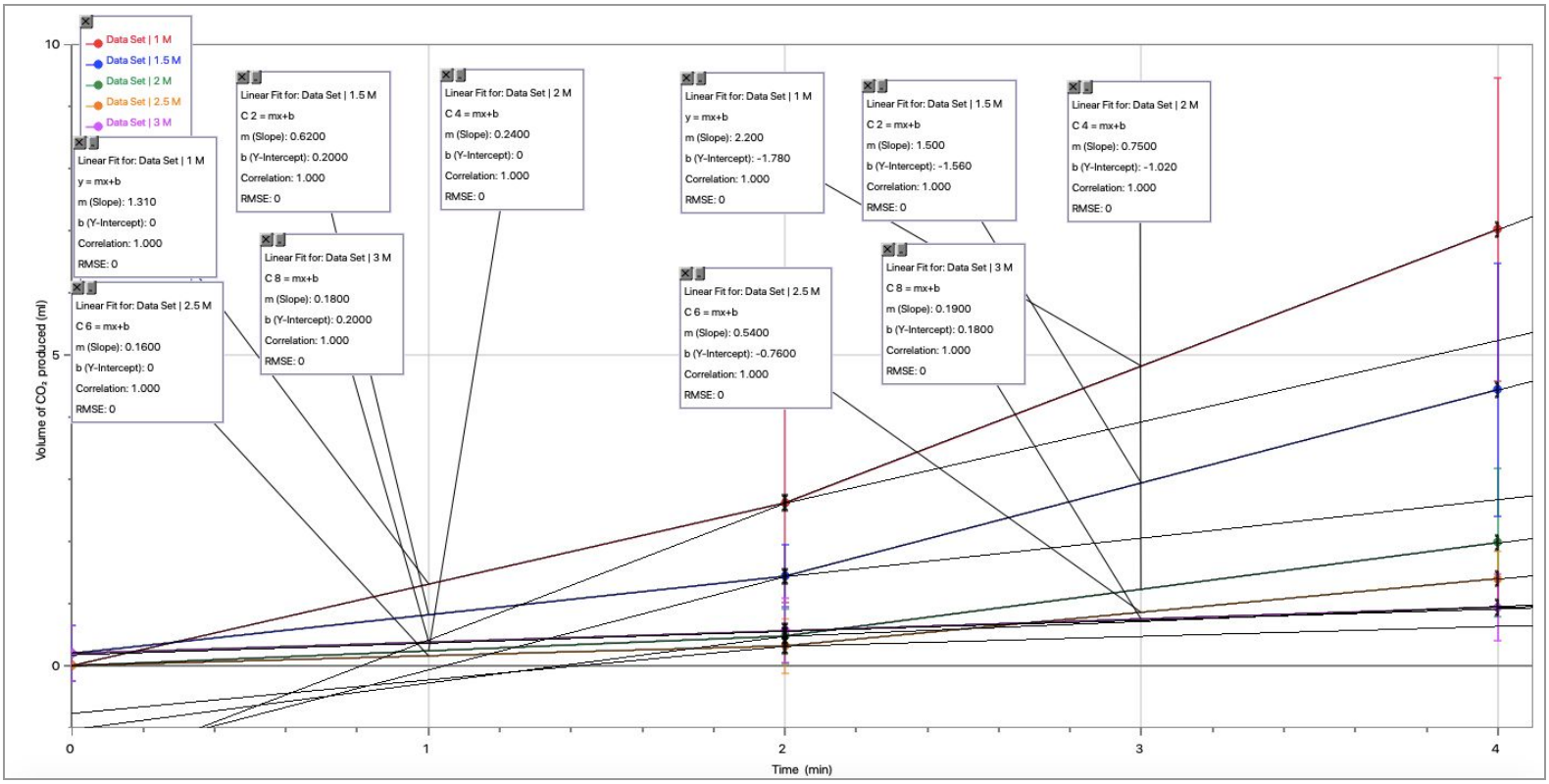
This graph demonstrates the results of the fermentation experiment. A trend of increasing CO_2 production over 32 minutes can be observed. It can be seen that, on average, the yeast in the 1 M glucose solution performed the best, followed by the 1.5 M solution, the 2 M solution, the 2.5 M solution, and lastly the 3 M solution. Although the 1 M solution seems to show the best performance, the standard deviation shows a significant overlap of values between the 1 M and the 1.5 M solution, especially from 24 minutes until the end of the experiment. To further analyze this, the overall rate of CO_2 production was divided into three separate rates.

Fig 3: Graph showing the average volume of CO_2 produced between zero and four minutes. The standard deviation is also shown.



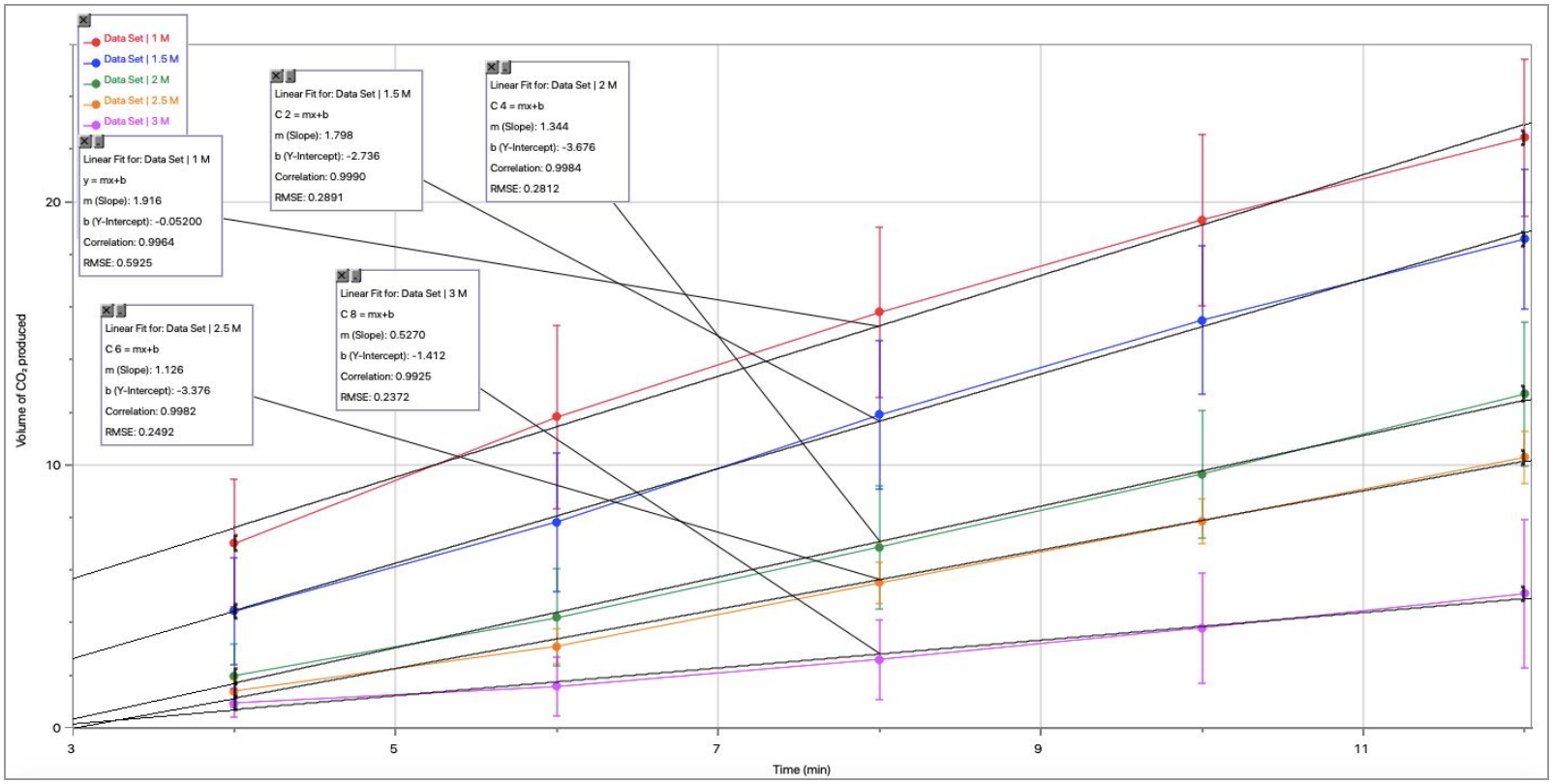
It can be seen from this graph that in the first two minutes the yeast in the 1 M and 1.5 M solution already produces significantly more CO_2 than in the other three solutions. A reason for this could be that the cells adjusted to the solution quicker when the concentration was fairly low and were able to start the fermentation process before the cells in the other solutions. At two minutes, the 2.5 M solution shows the worst performance, while the 3 M and the 2 M solution perform slightly better. At four minutes, however, the 2 M solution produces more CO_2 than the other two.

Fig 4: Graph showing the average volume of CO_2 produced between zero and four minutes. The line of regression for each solution in the first two minutes and also between two and four minutes.



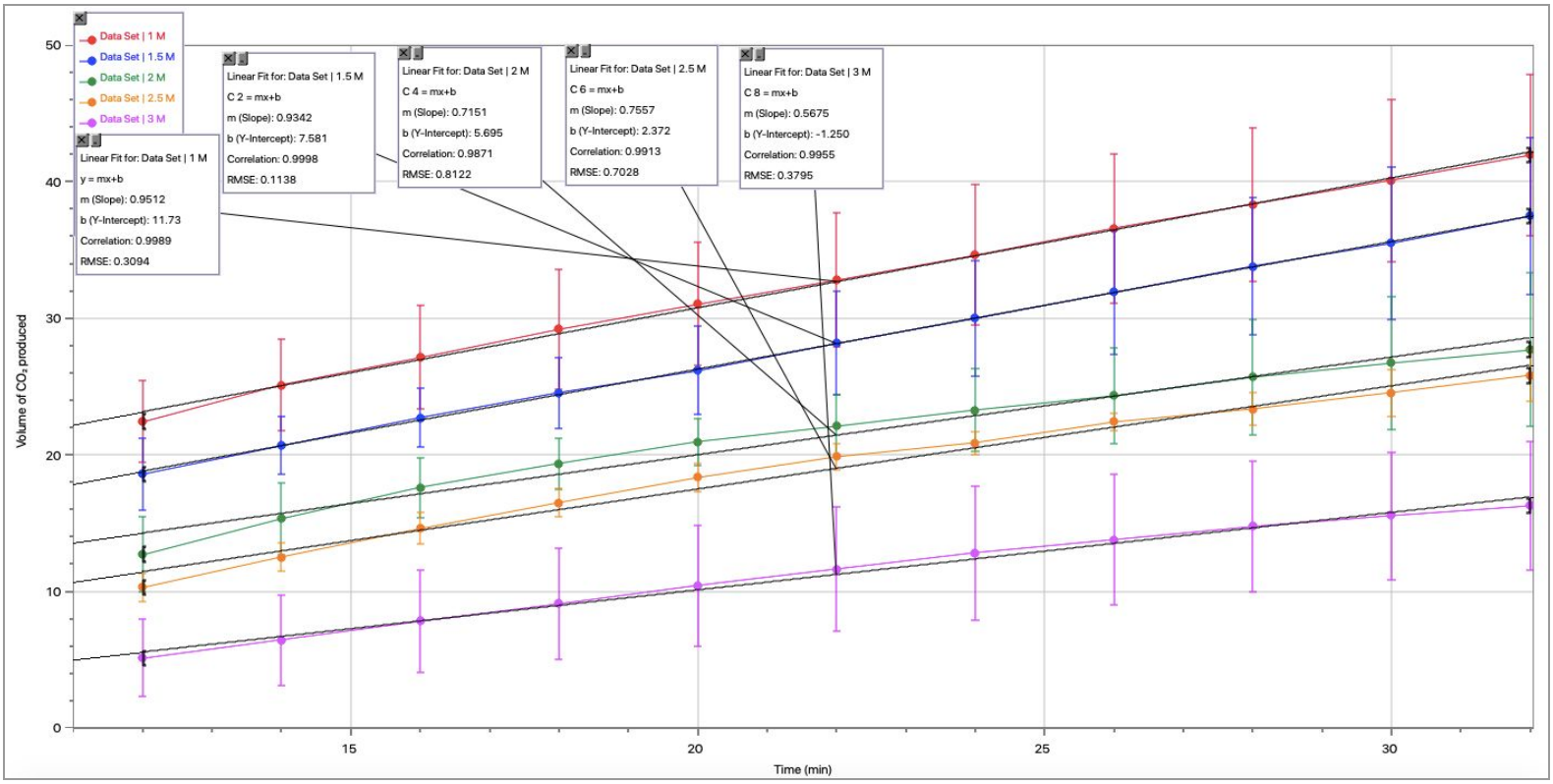
The slope of the 3 M solution in the first two minutes is 0.1800 and showing a slight increase to 0.1900 between two and four minutes. The 2.5 M glucose solution starts off with a slope of 0.1600 and increasing to 0.540 in the next two minutes. Simultaneously, the 2 M solution begins with a slope of 0.240 and increases to 0.750. This shows that although the 2 M started out as producing the least CO_2, between two and four minutes it experienced the greatest slope increase out of the three solutions which could have been due to a disrupture of the fermentation process caused by difficulties in rehydration of the cells.

Fig. 5: Graph showing the average volume of CO_2 produced between four and twelve minutes. The standard deviation and the lines of regression are shown.



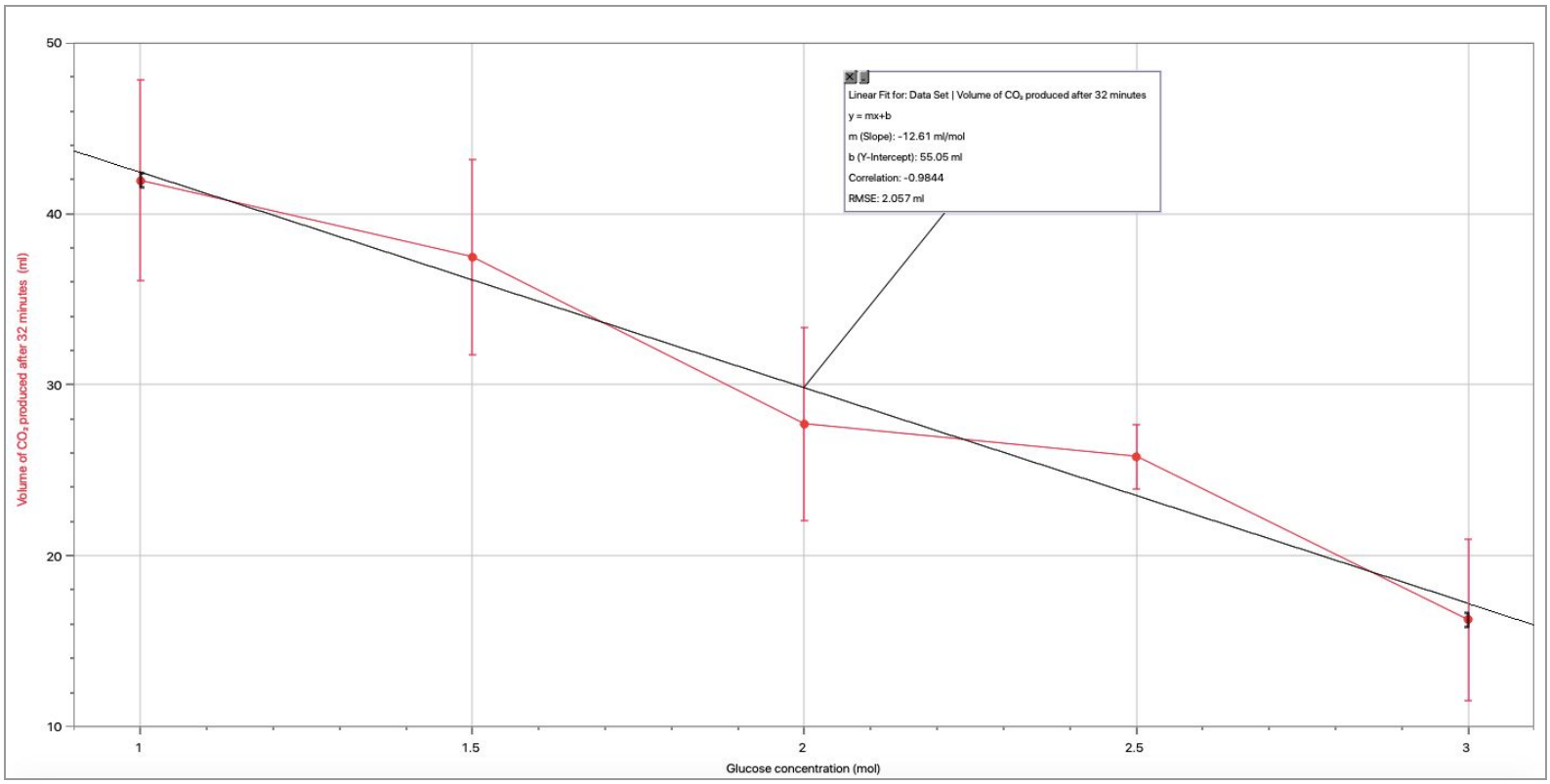
Between four and twelve minutes the same order of CO_2 production in the different glucose solutions can be observed. It has to be noticed, that the standard deviation of the 1 M and the 1.5 M solution are quite high and an overlap of values can be seen. This would demonstrate that the difference between CO_2 production in both solutions is minimal. The same can be seen in the 2 M and 2.5 M solution, showing that yeast performed nearly the same in these two concentrations, as well as in the 1 M and 1.5 M solutions.

Fig.6: Graph showing the average volume of CO_2 produced between twelve and thirty-two minutes. The standard deviation and the lines of regression for each concentration are also shown.



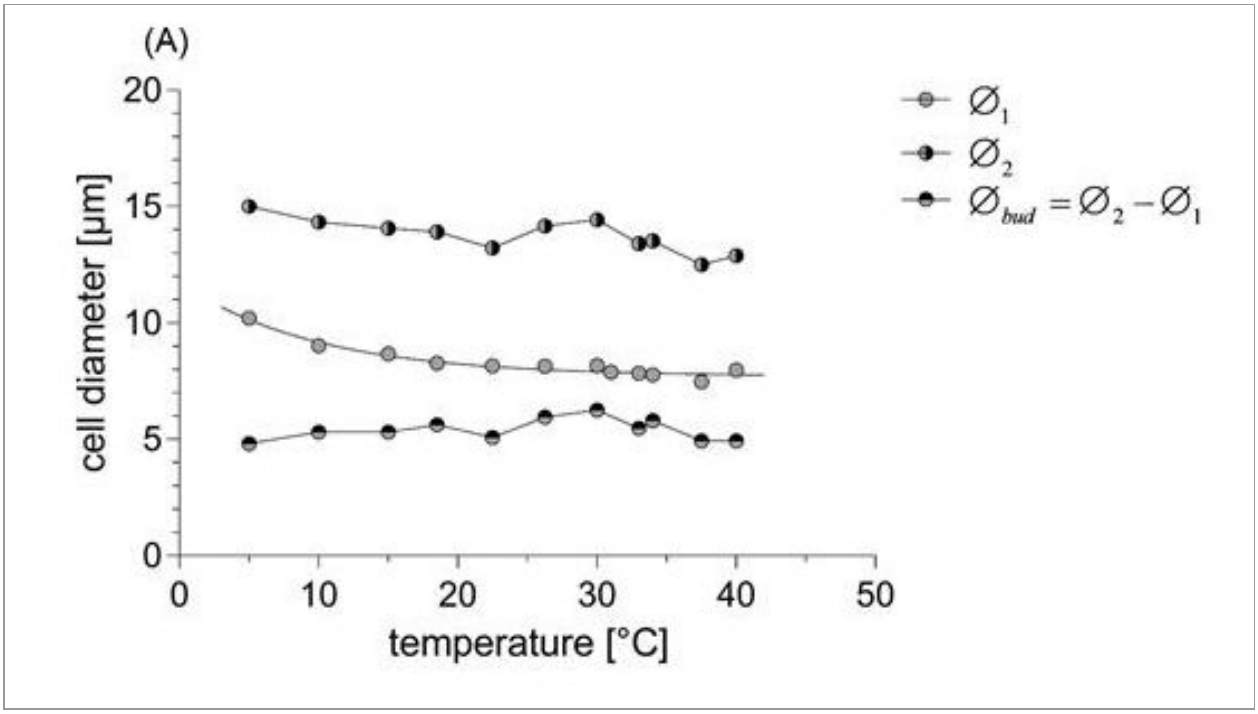
Between twelve and thirty-two minutes of the experiment, a slight decrease in the slope of each line can be observed. This would indicate a slowing down in the fermentation process due to the glucose starting to be used up. If data had been recorded for longer, a plateau for each line would have probably formed as can be seen in Figure 1.

Fig. 7: Graph showing the average volume of CO_2 produced after 32 minutes in each glucose concentration solution. The standard deviation, as well as the line of regression, are shown.



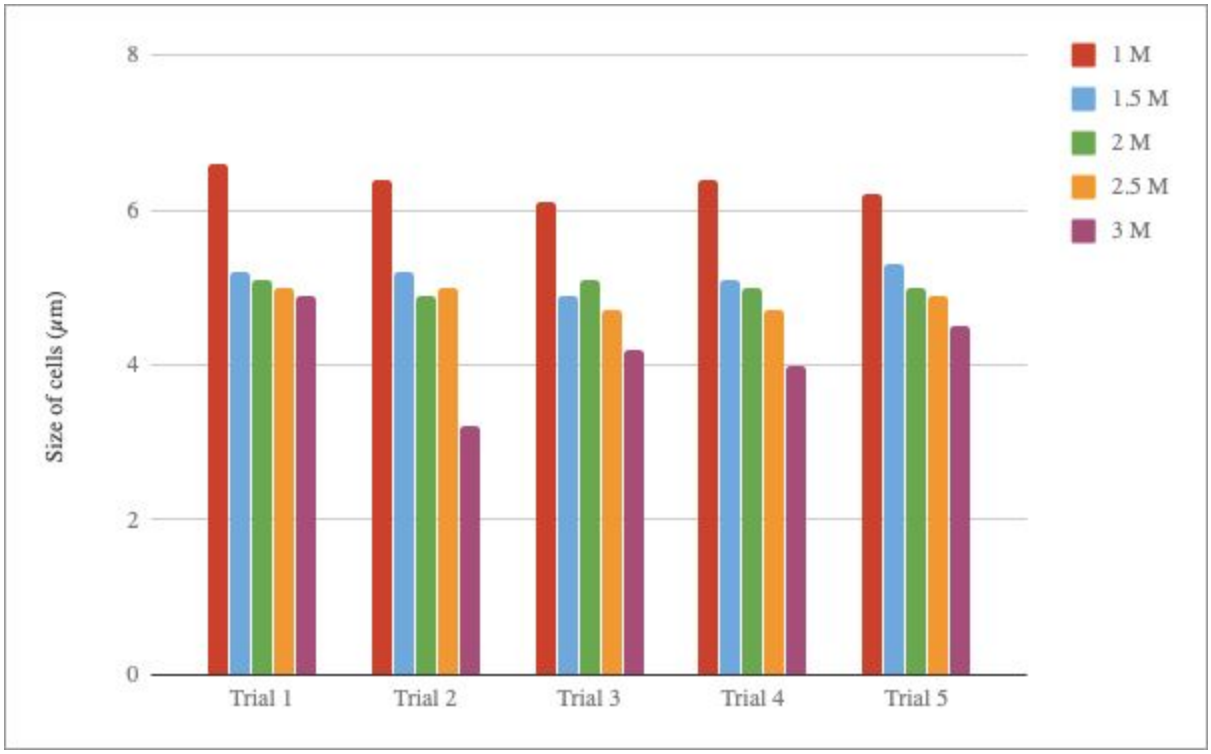
The correlation coefficient of the volume of CO_2 after 32 minutes as the glucose concentration increases is -0.984 which illustrates a strong negative correlation between the two values. This shows a clear connection between the decreasing fermentation performance and glucose concentration suggesting causation.

Fig 8: Graph showing the cell diameter of a mother cell (⌀1), a budding mother cell (⌀2), and the bud (⌀bud) as temperature increases (Zakhartsev and Reuss).



During cell division in yeast cells, the mother cell forms a bud which after a while detaches itself from the mother cells and grows into an identical replication of it. In this experiment, the cell diameter of *Saccharomyces cerevisiae* yeast cells was measured when in a substrate unlimited anaerobic growth batch under different temperatures. The diameter was calculated for mother cells, budding mother cells and the buds (Zakhartsev and Reuss). When the room temperature is taken to be 20 to 25 degrees Celsius, the size that a yeast cell at any stage in the cell cycle can range from is 5 to 13 µm (Zakhartsev and Reuss).

Fig. 9: Graph showing the average size of yeast cells of each trial in each glucose concentration solution



The averages of each trial are shown in this graph. It should be noted that the average cell size in trial 2 in the 3 M solution is unusually low which could be due to a poor quality batch of yeast perhaps caused by damage to the cells in packaging, transport or use. The calculations to determine whether it is a mathematical outlier are as follows.

Table 2: Table showing the calculations for the outlier

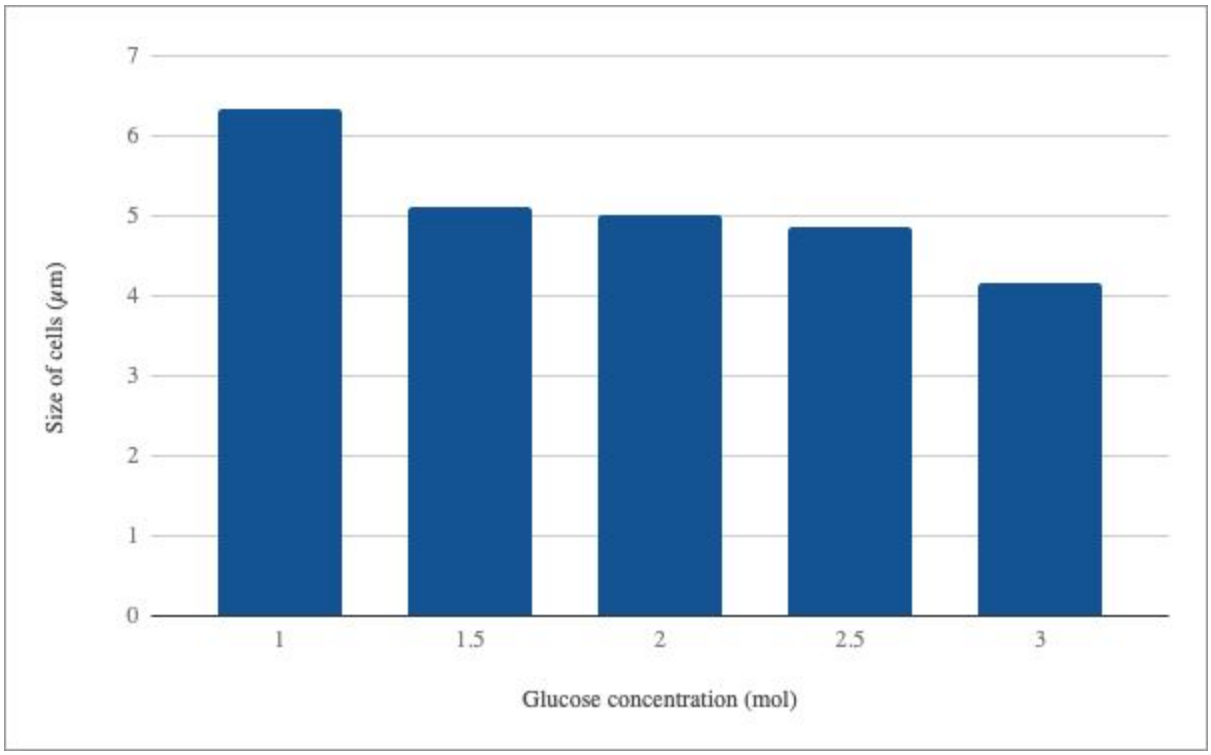
Quartile	Value	Interquartile Range (IQR)
First Quartile (Q_1)	3.6	$Q_3 - Q_1 = IQR$
Third Quartile (Q_3)	4.7	4.7 - 3.6 = 1.1
A value is considered an outlier if it is less than $Q_1 - (1.5 \times IQR)$ or greater than $Q_3 + (1.5 \times IQR)$ $Q_1 - (1.5 \times IQR)$ 3.6 - (1.5 × 1.1)		

= 1.95

3.2 ≮ 1.95 ∴ not an outlier

If that average of trial 2 is taken out, the total average size of cells in the 3 M solution would be 5.45% greater which, when measuring in micrometers, is a significant amount. Therefore, this anomaly should be considered in further analysis. However, the value was not taken out of total average.

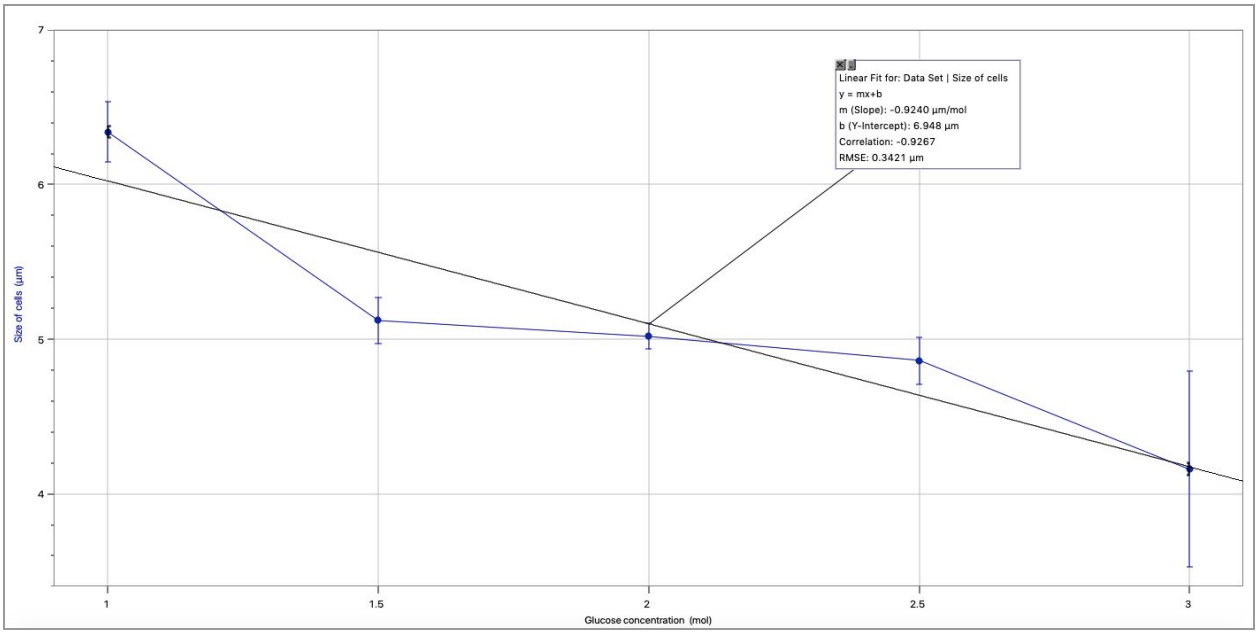
Fig. 10: Graph showing the average size of yeasts cells in each glucose concentration solution



This graph displays the processed results of the plasmolysis experiment. A decrease in cell size as glucose concentration increases can be observed. The biggest cells could be measured in the 1 M glucose solution, indicating that the cells were likely not affected by the glucose and were

able to function to their full ability. A control however was not used in the experiment thus the size of cells when in a neutral solution wasn't recorded. This makes it difficult to state that the cells weren't influenced by the 1 M glucose solution at all. A drastic decrease in size was detected in the 1.5 M solution which definitely indicates a shrinkage most likely plasmolysis due to a more concentrated solution. The smallest cells were found in the 3 M solution with a size decrease of 65.6%.

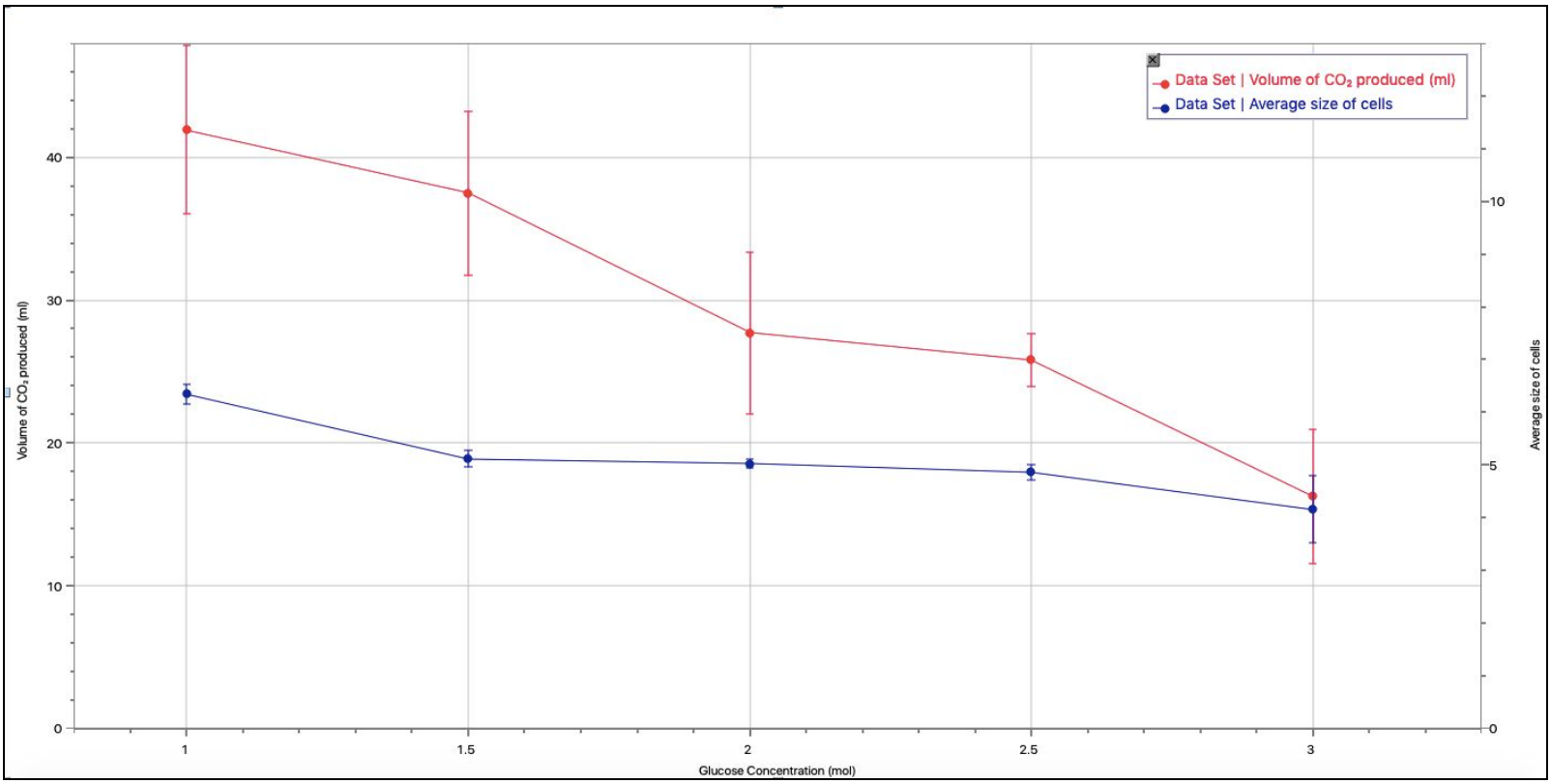
Fig. 11: Graph showing the average size of yeasts cells in each glucose concentration solution. The standard deviation as well as the line of regression are shown.



The correlation coefficient was calculated for the size of cells as the glucose concentration and is -0.927 which indicates a strong negative correlation between the two values. Even though a correlation doesn't necessarily imply that there is causation, in this case the cell shrinkage can be assumed to be at least to some extent caused by the increase in glucose concentration.

Discussion

Fig. 12: Graph showing the average volume of CO_2 produced after 32 minutes and the average size of cells as the glucose concentration increases. The standard deviation is shown as well.



The line of regression can be presented on a graph and can assist in estimating the x or y value when the other one is known. When looking at the graph we can see if there was no CO_2 produced during fermentation, the size of the cells would be approximately 3 μm. However, this regression line doesn't take into account the change in glucose concentration which caused the decrease in the volume of CO_2 that was produced, which means that the average cell size will only be approximately 3 μm if the glucose concentration increases at the same rate for the cells to continuously produce less CO_2 until they don't produce any anymore. This would imply that

the cells have died due to the excessive amount of glucose, making them shrivel up and lose the ability to carry out the fermentation process. Another limitation of this regression line is that it is never-ending and of course there is a limit to how much CO_2 the yeast cells can produce and also to how big the cells can get.

As mentioned before, the correlation coefficient of these two values demonstrates a strong, positive correlation, which means that the more CO_2 the yeast cells produce, the bigger they are.

The line of regression was calculated with a Texas Instruments TI-84 Plus for the average volume of CO_2 after 30 minutes of each trial and the average size of the yeast cells of each trial as follows.

Table 3: Showing the calculations of the line of regression

L1	L2
41.9	6.34
37.5	5.12
27.7	5.02
25.8	4.86
16.3	4.16

$y = ax+b$

$a = 0.070868091$

$b = 2.985579638$

$r^2 = 0.8286229296$

$r = 0.9102872786$

$\therefore$ line of regression

$y = 0.070868091x + 2.985579638$

The calculated line of regression helps to illustrate the relationship between both values. The r-value, also called the correlation coefficient that is calculated simultaneously, measures the strength and direction of the linear relationship between the two variables. A correlation coefficient of 0.910 indicates that there is a strong, positive correlation between the volume of CO_2 produced and the size of yeast cells when exposed to different glucose concentrations. However, just because there is a correlation between the two does not mean that there is necessarily causation, meaning that the volume of CO_2 produced in fermentation in a particular concentration of glucose might not be related in any way to the size of the cells in the same glucose concentration solution. Nevertheless, there is evidence that this is the case. For example research paper written by Vassilis Bitsikas et al. in 2010 talks about the behavior of fungi, particularly *S. cerevisiae*, when exposed to hypertonic media. They noticed that the exposure to this hypertonic media lead to plasmolysis, growth arrest and the blockage of endocytosis (Bitsikas et al., 2010). Deplasmolysis can occur within minutes after the hypertonic media is washed out but depending on the tonicity strength, a full reversal could take up to 14 hours (Bitsikas et al., 2010). Deplasmolysis was not looked at and analyzed in this experiment, however, it is worth mentioning as it is an interesting addition and extension to the topic of plasmolysis which is discussed in this essay. The researchers came to the conclusion that yeast cells experience water loss, cell shrinkage and growth arrest when exposed to hypertonic media. This could also be observed in this investigation when the yeast cells decreased significantly in size when exposed to a more concentrated glucose concentration. The smallest yeast cells, hence the ones in the 3M glucose concentration, performed worst in the fermentation experiment,

indicating a decreased ability to convert the excessive amount of glucose to carbon dioxide and ethanol. Agnès Miermont also mentions cell volume decrease as a function of osmotic stress in her research paper “Severe osmotic compression of the yeast Saccharomyces cerevisiae”. She measured the yeast cells before and after the stress and concluded that the volume decreased with osmotic stress. The final cell volume reached a minimum at approximately 2 M sorbitol corresponding to approximately 40% of the initial cell volume. Miermont points out that other authors have calculated a similar minimum cell volume which is about 50% of the initial cell volume (Miermont). Both of these research papers support the findings of my experiment, indicating that there is a correlation as well as causation to a certain extent.

Evaluation

An evaluation of the experimental procedures undertaken in the experiments of this essay is worth carrying out. One of the main limitations of this experiment was the measuring of the yeast cells in the different glucose concentrations only after 30 minutes. This lead to a limited comparison between the fermentation and the plasmolysis experiment because only the values of the volume of CO_2 produced after 30 minutes and the cell sizes after 30 minutes could be linked and analyzed together. This was due to the magnification of the oil immersion lens that was used. If the magnification was higher, smaller changes in the size of the cells could have been detected and the cells could have been measured multiple times over the course of 30 minutes which furthermore would have enabled a deeper and more detailed analysis of all of the data.

Another limitation of this experiment could have been the choice of glucose concentrations. A fermentation trial experiment was conducted where the volume of CO_2 produced in the 3M glucose solution was less than 10 ml which compared to the average volume produced in the 5 trials of the actual experiment, which was 16.3 ml, is relatively low. If more trial experiments with varying glucose concentrations would have been carried out, a more suitable variety and range of glucose concentrations could have been chosen in order to identify when the cells die due to a lack of CO_2 production. This would have additionally helped to determine the maximum shrinkage a yeast cell can experience when exposed to a very concentrated glucose solution.

Additionally, access to more advanced experimental equipment and materials would have enriched the depth and detail of the collected data. For instance the length of the gas collection tubes limited the collection of CO_2 gas to 50 ml which didn't allow for a point of plateau where the cells stop producing any carbon dioxide which was to be expected. This furthermore led to incomplete and different results in the fermentation experiment which affected the general analysis of not just this process but also the linkage to plasmolysis.

Conclusion

As predicted within the hypothesis, there was in fact a relationship between the process of fermentation and the process of plasmolysis in yeast cells when exposed to varying glucose concentrations. Based on the raw and the processed data, the following trend was found: The less

CO_2 that was produced by the yeast cells, the smaller the cells were when measured under a microscope. The increasing solute concentration around the cell due to the added glucose lead the cells to experience osmotic stress in which water flows out of the cell because the water potential is lower outside of them. This caused the increasing shrinkage in the cells. When the cells start to shrink, the process of fermentation gets disrupted and less of it is carried out, meaning that less CO_2 is produced. The correlation coefficient that was calculated for both of these values indicates a strong correlation which shows that there is a relationship between fermentation and plasmolysis in yeast cells.

Although there seems to be a correlation between the two processes, one process causing the other is a rather bold statement to make. It is a lot more likely that both processes are interconnected and are part of a system, meaning that if one of them changes, the other one will be affected and, as a result, change or adapt. This would explain

To fully accept and recognize these two processes as being linked together, more advanced equipment as well as more tests and experiment would be required.

This investigation supports previous experiments and research in this subject area which all seem to put forward evidence separately for both the process of fermentation and the process of plasmolysis in cells of *Saccharomyces cerevisiae*. Studies have been done to find the effect of temperature and mass of yeast in fermentation as well as identifying the cell size in different temperatures and in exposure to different concentrated sugar solutions. The two processes of fermentation and plasmolysis analyzed together, however, has not been studied extensively, thus the results of this investigation can be acknowledged but not fully recognized as the correct explanation of these extraordinary processes interacting together.

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