Internal assessment

Biology

Determining the fermentation rate of different monosaccharides glucose, fructose, mannose, galactose and arabinose by *Saccharomyces cerevisiae* (Baker's yeast)

Session number: 000898-0026

Introduction

Background information

Carbohydrates are one of the major carbon sources for yeasts. When yeasts respire, carbohydrates are broken down to produce ATP, the source of energy. Respiration can occur in both oxygen abundant condition and oxygen deficient condition. When respiration is processed in an oxygen deficient environment, an anaerobic respiration called fermentation will take place. Fermentation uses monosaccharide glucose, to produce two CO_2 molecules, two ethanol molecules and two ATP molecules. A monosaccharide is a form of sugar that cannot be further broken down into a simpler sugar molecule.

The overall reaction of alcoholic fermentation is as follows:

$C_6H_{12}O_{6\,(aq)} \rightarrow 2CO_{2\,(g)} + 2CH_3CH_2OH_{\,(aq)} + 2ATP$

The fermenter in this experiment will be the fungus *Saccharomyces cerevisiae* – a species of yeast. This particular species was chosen because *S. cerevisiae* are one of the few yeast species that can undergo fast metabolism under anaerobic conditions, hence a very viable species for experiments (Rodrigues, Ludovico & Leão, 2006). In addition, *S. cerevisiae* is widely used for baking, wine production, and beer production thus have a broad application in the world. It is therefore a species worth of investigation as it is prevalent in modern day.

Within alcoholic fermentation, monosaccharide only plays role during the first step of fermentation called glycolysis. This is when the six-carbon chain splits into two three-carbon chains called pyruvate. The product of glycolysis is always pyruvate. This means that fermentation rate of different monosaccharides is dependent on the reaction rate of glycolysis.

There are three intermediate phosphorylated monosaccharides before glucose splits into two three-carbon chains.

Glucose → Glucose-6-phosphate → Fructose-6-phosphate → Fructose-1,6-diphosphate

This means that for monosaccharides other than glucose, they must somehow enter somewhere into this step. Then they are split into two three-carbon chains and proceeds rest of the fermentation just like glucose (Rodrigues, Ludovico & Leão, 2006). Thus more specifically, the rate of conversion step that is required to enter the series above is likely to be the determinant of the fermentation rate.

A study suggested that yeasts in general ferment only glucose, fructose and mannose (Gottschalk, 1947). This is an indication that these three monosaccharides have a relatively fast conversion rate from a monosaccharide into one of the intermediate molecules, and therefore ferment faster than other monosaccharides. Among glucose and fructose, a study showed that yeasts seemed to ferment glucose faster than fructose when given in equal amounts (Hopkins, 1928). Nevertheless, another study showed that certain yeasts can ferment galactose as well (Wilkinson, 1949). In addition, a study has shown that arabinose increases fermentation rate in presence of glucose (Borzani & Aquarone, 1958), but it seems that not

many investigations have been conducted to determine the fermentation rate of arabinose itself or the relative fermentation rates of various monosaccharides other than glucose and fructose.

This had led to the investigation of relative rates of CO_2 release for different monosaccharides – glucose, fructose, mannose, galactose and arabinose at 0.2M – by *S. cerevisiae.*

Aim

The primary aim is to explore monosaccharide preference for *S. cerevisiae* by observing fermentation rate.

The secondary aim is to determine whether *S. cerevisiae* have the ability to ferment galactose and arabinose.

Hypothesis

It is predicted that monosaccharides other than glucose will have to be converted into either glucose or other intermediate phosphorylated monosaccharide before proceeding with glycolysis. Therefore, the hypothesis is as follows.

- Glucose will have the highest fermentation rate.
- The second and third fastest is going to be either between fructose and mannose.
- The fourth fastest is going to be galactose due to literature study suggesting that yeasts ferment glucose, fructose and mannose.
- Slowest fermentation rate is predicted to be arabinose. Arabinose is a pentose (five-carbon chain) and lacks one carbon while remaining four monosaccharides are hexoses (six-carbon chain). Therefore it will take longer time because one carbon has to be added.
- A control group with no sugar will also be tested. This is predicted to have no fermentation rate because there is no monosaccharide for the yeast to break down.

Methodology

Materials and apparatus

Materials	Apparatus
• α-D(+)-Glucose, 5.40 g • D(+)-Fructose, 5.40 g • D(+)-Mannose, 5.40 g • D(+)-Galactose, 5.40 g • D(+)-Arabinose, 4.50 g • Tap water, 1500 ml • Minimum 15 g of dried *S. cerevisiae*	• 3 conical flasks, 150 ml (± 25 ml) • Graduated cylinder, 50 ml (± 0.5 ml) • 3 beakers, 100 ml (± 5 ml) • 3 syringes (± 0.5 ml) • Scale (± 0.01 g) • Timer (± 0.01 s) • Spatula • Water bath, 42°C

Variables

Dependent variable is the volume of CO_2 gas produced, in ml, per minute.
Independent variables are 0.2M solutions of the five sugar types; glucose, fructose, mannose, galactose and arabinose.
Control variable is the solution with no sugar added.

Constant variables are as follows.

Variable	Significance	Method of control	The controlled value
Concentration of sugar solution	Different concentrations of sugar will affect the amount of CO_2 released. When yeast has high availability of sugar, it will take faster for yeast to find a sugar molecule and then process fermentation. The opposite is true when yeast has low availability of sugar.	All monosaccharide solutions will have the same concentration.	0.2M
Concentration of yeast solution	When the concentration of fermenter differs, in this case *S. cerevisiae*, the rate of fermentation may change. When there are more fermenters, more fermentation will take place per time. Therefore, it will affect the CO_2 release.	All yeast concentrations were the same.	1.00 g of dried *S. cerevisiae* per 50 ml of water
Interval of	Having different intervals of time	Record all the trials	Data should

measuring time	might lead to uncertainty when extrapolating the rate of fermentation.	with the same time interval.	be recorded every 2 minutes, for 20 minutes.
Temperature	Temperatures have a significant effect on the fermentation rate because enzyme activity is temperature dependent.	The temperature will be kept constant in a water bath at the dried yeast's optimum temperature.	42°C
pH	The enzymes present in the reaction will be affected since they have an optimum pH. Varying pH will therefore affect the rate of reaction.	Use same source of water.	pH=6.5-7.0

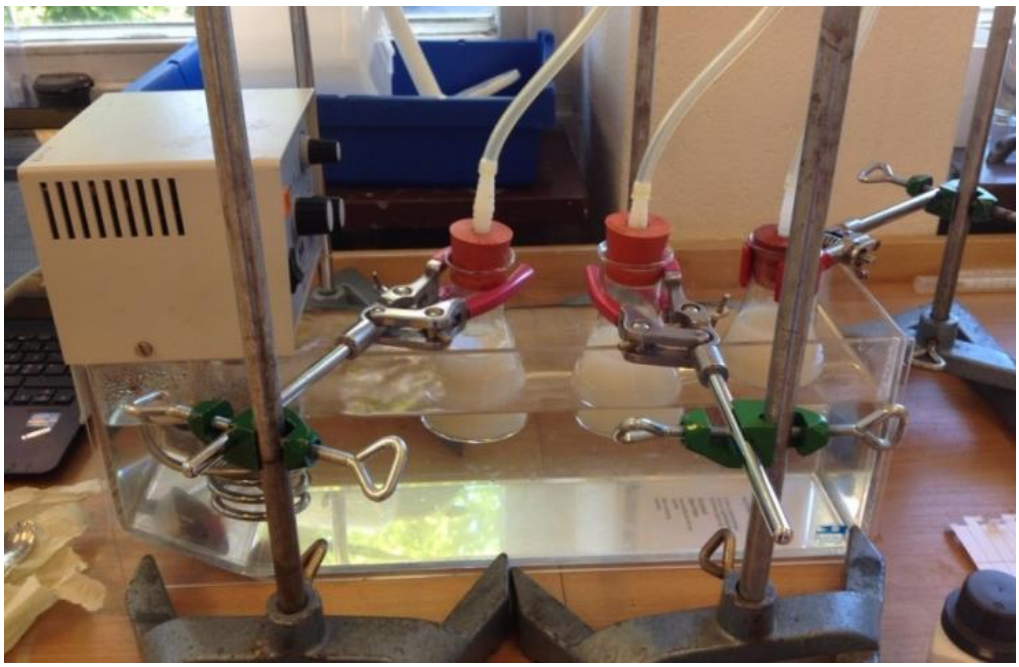
Precautions

Lab coat were worn when handling the materials and glass instruments. This experiment does not contain any hazardous chemicals, but the water bath was handled with extra caution as it has a heater.

Design

The conical flasks were half immersed in the water bath. The top was inserted with a rubber plug that was connected to a syringe. This will create an anaerobic environment and ensures minimal CO_2 gas escape. Pilot testing was carried out with glucose to assess the functionality of the design. The two things tested were firstly the movement of syringe, and secondly if 0.2M of sugar was abundant enough to initiate fermentation. The pilot test showed that there were constant movements in the syringe for well above 20 minutes. The design was therefore successful.

Figure 1 shows the set-up of the experiment.



Method

1. Set up three iron support stands and clamp holders as shown in the picture above.

2. Heat up water in the water bath to 42°C.

3. Prepare three 50 ml yeast solutions with 1.00 g of dried *S. cerevisiae* each in a conical flask. Use graduated cylinder to measure the water.

4. Prepare three 0.2M solutions of monosaccharide with volume 50 ml in a 100 ml beaker.
a) For glucose, fructose, mannose and galactose, add 1.80g in each beaker. For arabinose, add 1.50g in each beaker. For control, add no sugar.
b) Measure 50 ml of water using a graduated cylinder. Pour 50 ml in each beaker.
c) Swirl gently until no grains of sugar can be seen.

5. Place the yeast solutions in the water bath for 5 minutes. This will activate *S. cerevisiae*.

6. Add the sugar solution to the yeast solution. Then attach the plug connected to the syringe.

7. Start the timer.

8. Read the displacement of syringe every 2 minutes. Shake the conical flask gently before reading it to ensure that most CO_2 has been released.

9. Continue until 20 minutes has passed.

10. Repeat step 2-9 for fructose, galactose, mannose, arabinose and no sugar added.

Analysis

Raw data, quantitative

Table 1 shows the CO_2 released in ml measured every 120 seconds minutes for all six treatments.

	Control (no sugar added) Volume of CO_2 (ml ±0.5) per time (s ±5*)										
	0	120	240	360	480	600	720	840	960	1080	1200
T1	0	0	0	0	0	0	0	0	0	0	0
T2	0	0	0	0	0	0	0	0	0	0	0
T3	0	0	0	0	0	0	0	0	0	0	0
	Glucose Volume of CO_2 (ml ±0.5) per time (s ±5*)										
	0	120	240	360	480	600	720	840	960	1080	1200
T1	0	1	2	4	8	10	12	15	20	24	26
T2	0	1	2	5	8	11	14	17	19	25	31
T3	0	1	4	5	8	9	12	16	21	24	30
	Fructose Volume of CO_2 (ml ±0.5) per time (s ±5*)										
	0	120	240	360	480	600	720	840	960	1080	1200
T1	0	0	0	0	1	4	8	10	13	16	21
T2	0	0	0	2	6	9	12	15	20	24	29
T3	0	0	0	0	3	5	11	13	17	20	23
	Mannose Volume of CO_2 (ml ±0.5) per time (s ±5*)										
	0	120	240	360	480	600	720	840	960	1080	1200
T1	0	1	3	4	5	7	10	12	15	18	21
T2	0	1	2	3	5	7	8	11	15	17	20
T3	0	0.5	4	6	7	9	12	16	20	24	29
	Galactose Volume of CO_2 (ml ±0.5) per time (s ±5*)										
	0	120	240	360	480	600	720	840	960	1080	1200
T1	0	0	0	0	0.5	0.5	1	1	2	3	3
T2	0	0	0	1	1	1	1	2	3	3	3
T3	0	0	0	0	0.5	0.5	1	2	2	2	3
	Arabinose Volume of CO_2 (ml ±0.5) per time (s ±5*)										
	0	120	240	360	480	600	720	840	960	1080	1200
T1	0	0	0	0	0	0	0	0	0	0	0
T2	0	1	1	1	1	2	2	2	2	2	2
T3	0	0	0	1	1	1	1.5	1.5	1.5	1.5	1.5

* The uncertainty for time includes estimation of the time taken to read the values on the syringe and note them down. Therefore, the uncertainty in experiment is not the same as uncertainty of the time instrument.

Raw data, qualitative

Table 2 shows the qualitative changes observed.

Monosaccharide	Observations
Control	No changes could be seen.
Glucose	There were no immediate qualitative changes when sugar was added. However, small bubbles arising from the bottom could be observed.
Fructose	A circular array of foam could be observed after approximately 8 minutes. At around 15 minutes, clusters of *S. cerevisiae* were observed.
Mannose	Observations were very similar to glucose.
Galactose	No qualitative changes could be seen. There seemed to be very minimal bubbles in the solution.
Arabinose	Observations were very similar to galactose.

Calculation formulas

Percentage uncertainty

$$\%\ uncertainty = \frac{Uncertainty\ in\ syringe}{Mean\ total\ CO_2} * 100$$

Maximum and minimum CO_2 production per minute

$$Maximum = \frac{Trial\ with\ maximum\ value\ after\ 20\ min}{20}$$

$$Minimum = \frac{Trial\ with\ maximum\ value\ after\ 20\ min}{20}$$

Processed data

Table 3 shows the mean total CO_2 produced and percentage uncertainty.

Monosacharide	Mean total CO2 produced (± 0.5 ml)	Percentage uncertainty (%)	Maximum CO_2/time (ml/min)	Minimum CO_2/min (ml/min)
Control	0.0	N/A	0.0	0.0
Glucose	29.0	±1.72	1.55	1.30
Fructose	24.3	±2.06	1.45	1.05
Mannose	23.3	±2.15	1.45	1
Galactose	3.0	±16.7	0.15	0.15
Arabinose	1.2	±41.7	0.10	0.0

Figure 2 shows the average rate of fermentation from each monosaccharide with its maximum and minimum CO_2 production per minute.

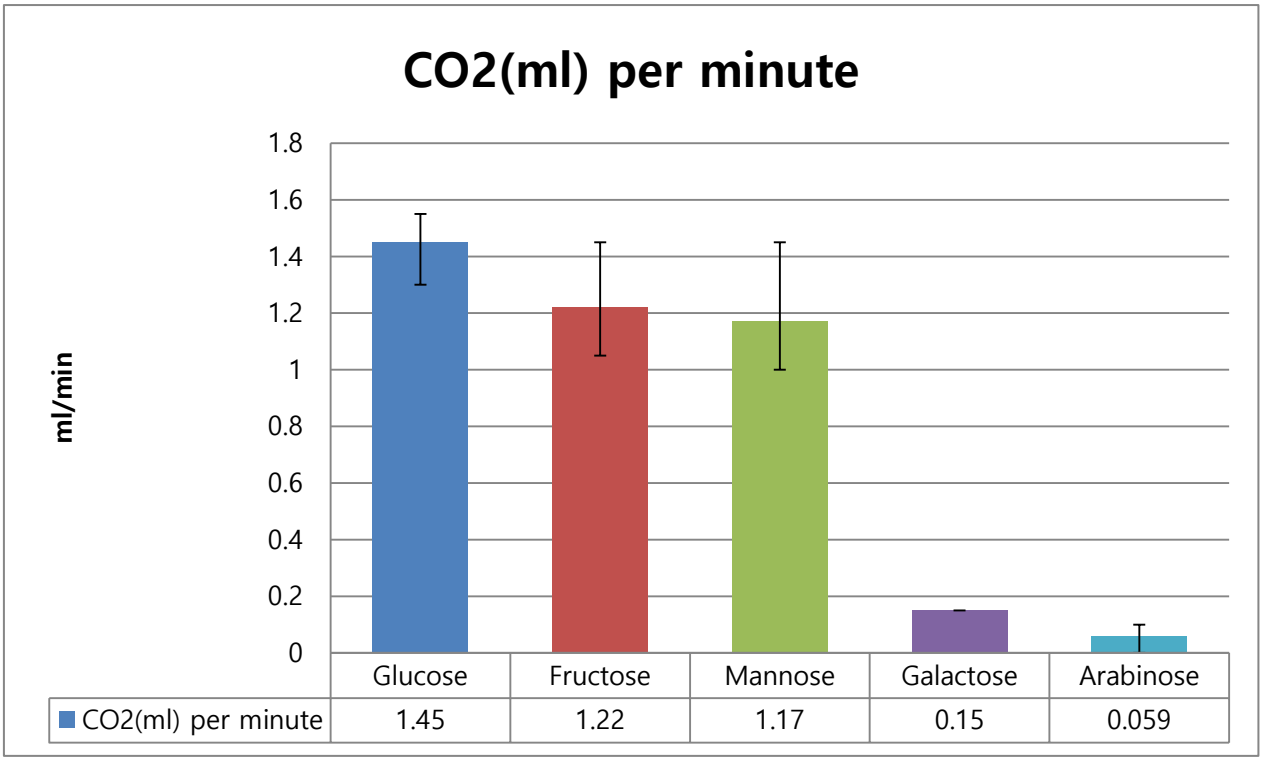
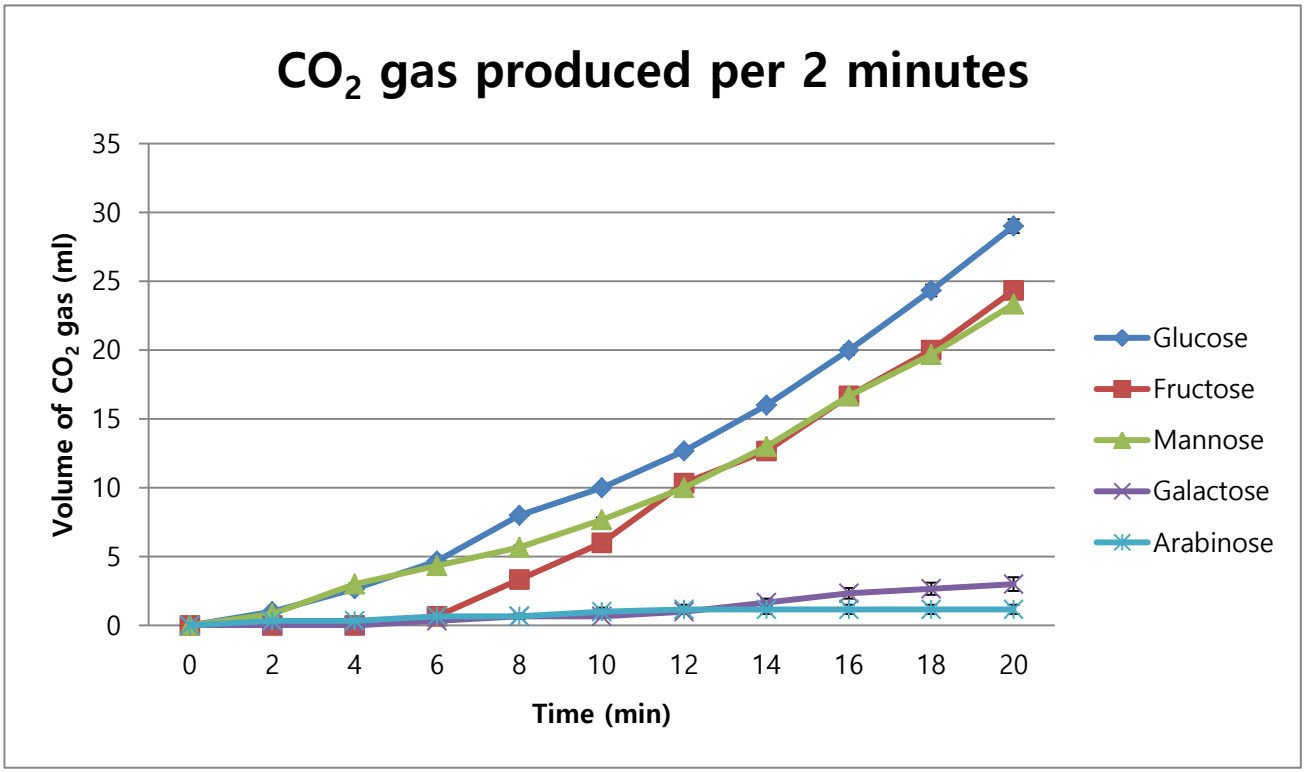


Figure 3 shows the average CO_2 release per time with their respective percentage uncertainty.



Conclusion

The primary aim of this experiment was to determine relative rate of fermentation for different monosaccharides by observing CO_2 release.

It can be concluded that the hypothesis was correct to the extent for control, glucose, galactose and arabinose. For the remaining monosaccharides, it can be concluded that fructose show a tendency to have second highest and mannose third highest fermentation rate.

The data from Table 3 suggests that during the same time interval, *S. cerevisiae* treated with glucose have the fastest metabolism rate because it produced the highest mean CO_2 at 29.0 ml. Therefore as predicted, glucose is a better source for fermentation than the four other monosaccharides. This supports the idea that a monosaccharide that does not have to be converted into an intermediate phosphorylated saccharide have fastest rate. Nevertheless, the fermentation rates for fructose and mannose is questionable in terms of uncertainty in syringe. The lower value for fructose is 23.8 ml and maximum value for mannose is 23.8 ml, thus there is a chance that fermentation rates for fructose and mannose are same.

Similarly, Figure 2 visually represents that glucose had indeed fastest rate of CO_2/min, followed by fructose, mannose, glucose and arabinose. However, the range of glucose, fructose and mannose shows an overlap thus one cannot say for certainty that glucose is the fastest fermenter solely based on the collected data. Nonetheless, mean values of collected data agrees with the study showing that *S. cerevisiae* "removes glucose more rapidly than fructose from a solution containing equal quantities of the two" (Hopkins, 1928).

Figure 3 shows that the average rate of glucose is consistently highest. This again supports that metabolic pathway from glucose to fructose-1,6-diphosphate is fastest. Out of fructose and mannose, fructose produced the more CO_2 in the end, but it had a later onset for fermentation than mannose. This is an indication of mannose having faster conversion rate than fructose. This is counterintuitive since it seems logical to say that conversion rate for fructose should be faster because it is already in the form fructose. On the other hand, fermentation rate after initiation of anaerobic respiration was faster for fructose than mannose. In either case, this suggests that different enzymes are used for fermentation of fructose and mannose. For galactose and arabinose, there are signs of CO_2 release. However, arabinose did not produce additional gas from 14 minutes to 20 minutes. Thus there is a chance that the movement in syringe was due to increase in pressure as the cork was plugged. The stall in fermentation for galactose and arabinose supports studies stating that yeasts selectively ferment glucose, mannose and fructose (Gottschalk, 1947), (Hopkins & Roberts, 1936).

An interesting finding is that the difference between glucose, fructose and mannose is not as large as the difference between them and galactose. Despite the same molecular formula, galactose ferments about ten times slower than glucose. A study suggested that this is because yeasts ferment monosaccharides that exist in special structures called "enol form", which galactose does not have (Hopkins, & Roberts, 1936). Another reason might be because *S. cerevisiae* does not have the enzymes to convert galactose into the intermediate phosphorylated monosaccharides. When it comes to fructose and glucose, certain enzymes

regulating fructose glycolysis been shown to be more sensitive to accumulation of alcohol than enzymes regulating glucose glycolysis (Zinnai, Venturi, Sanmartin, Quartacci, & Andrich, 2013). For most of the cases, it seems that enzymes are heavily involved in fermentation rate.

Evaluation

All trials were not conducted during the same day due to time constraint. This might be a problem because the dried yeast package was opened and preserved in a locker. Although the yeast is coated with dead cells, some yeast might have died and some survived due to damage or accidental rehydration which may activate them. Thus a new package should be opened when the experiment is on a new day. However, this would be a large waste of yeast and wastes should be minimized as much as possible for environmental reasons. Another way is to set up another water bath and have more fermentation reaction going on at the same time. This would reduce time constraint and at the same time have more trials to detect any outliers.

The time interval may have been too short for galactose and arabinose. This made it difficult to assess whether fermentation was really happening. There were some changes in the syringe, but not as significantly as glucose, fructose and mannose. Data should be collected over a longer time period. One hour may be more suitable. In addition, there was a problem of uncertainty with time. Although uncertainty on the timer was 0.01s, the uncertainty of time during experiment was not 0.01s but estimated at ±5s. Nevertheless, ±5s will not have adverse effect on the fermentation rate since the interval was 120 seconds.

Syringes were slightly differently lubricated and therefore had different resistance. This might have made some syringes displace more than others from the same pressure of CO_2 gas. By manually lubricating the syringes and making them all have as little resistance as possible will show fairer results. Another way to solve this problem is to use a data logging program that can sense CO_2 release. This would provide a more accurate data with a more continuous time interval.

There were arbitrary clusters of yeast on the bottom of the conical flask during fermentation. This may have reduced the fermentation rate as clusters lower the surface area, therefore expose less yeast to sugar. This could be improved by manually stirring the yeast solution well before adding sugar solution.

This experiment was a macroscopic research that measured the products CO_2. It seems by literature study that there are many different aspects to analyse monosaccharide metabolism, such as in structure of monosaccharides, enzymes involved in glycolysis and also how monosaccharides are transported into *S. cerevisiae* (Cirillo, 1968). Nevertheless, enzymes are most likely to have the largest impact on the rate of metabolism due to their catalysing ability. Thus in order to further investigate monosaccharide metabolism, it is recommended to focus on an enzyme that is known to be used in glycolysis such as “aldolase, triosephosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase and pyruvate kinase.” (Rodrigues, Ludovico & Leão, 2006) and see whether the enzyme poses an effect on the metabolism for different monosaccharides. This could specifically identify where in the glycolysis a particular monosaccharide enters.

References

1. Rodrigues, F., Ludovico, P., & Leão, C. (2006). Sugar metabolism in yeasts: an overview of aerobic and anaerobic glucose catabolism. In *Biodiversity and ecophysiology of yeasts* (pp. 101-121). Springer Berlin Heidelberg.

2. Gottschalk, A. (1947). The effect of temperature on the fermentation of D-mannose by yeast. *Biochemical Journal*, *41*(2), 276.

3. Hopkins, R. H. (1928). The selective fermentation of glucose and fructose by brewer's yeast. *Biochemical Journal*, *22*(4), 1145.

4. Wilkinson, J. F. (1949). The pathway of the adaptive fermentation of galactose by yeast. *Biochemical Journal*, *44*(4), 460.

5. Borzani, W., & Aquarone, E. (1958). Effect of Arabinose on Glucose Fermentation by Saccharomyces cerevisiae. *Applied microbiology*, *6*(4), 225.

6. Hopkins, R. H., & Roberts, R. H. (1936). The kinetics of alcoholic fermentation of sugars by brewer's yeast: Specificity. The rates of fermentation of α-and β-glucose. *Biochemical Journal*, *30*(1), 76.

7. Zinnai, A., Venturi, F., Sanmartin, C., Quartacci, M. F., & Andrich, G. (2013). Kinetics of D-glucose and D-fructose conversion during the alcoholic fermentation promoted by Saccharomyces cerevisiae. *Journal of bioscience and bioengineering*, *115*(1), 43-49.

8. Cirillo, V. P. (1968). Relationship between sugar structure and competition for the sugar transport system in bakers' yeast. *Journal of bacteriology*, *95*(2), 603-611.