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Investigating the Effects of Disaccharides and Monosaccharides on the Rate of Respiration in *Saccharomyces cerevisiae* (*S. cerevisiae*)

Questioning and Predicting

Research question:

What are the effects of different types of disaccharides (maltose, sucrose) and monosaccharides (fructose, glucose) on the rates of respiration in *S. cerevisiae* (yeast), as measured by the concentration of carbon dioxide gas produced during the experiment?

Hypothesis:

It is predicted that allowing *S. cerevisiae* to respire in the presence of monosaccharides will yield higher concentrations of carbon dioxide gas produced, which would also result in higher rates of respiration compared to respiration in the presence of disaccharides. This is because monosaccharides comprise only a single monomer, so they will be absorbed into and utilised in *S. cerevisiae* cells for respiration at a much faster rate than disaccharides, which consist of two monomers linked together by a glycosidic bond that has to be broken down via hydrolysis before its constituent monomers can be metabolised during respiration (Cason and Reid, 1987; Lagunas, 1993). Therefore, it is hypothesised that respiring in the presence of a monosaccharide, rather than a disaccharide, will result in a higher rate of respiration and concentration of carbon dioxide gas produced in the 5-minute duration of the experimental trials.

Planning and Conducting

Background information:

Monosaccharides are single sugar units, or monomers, that can be linked together through condensation reactions in order to form carbohydrates (Allott and Mindorff, 2014). Monosaccharides include sugars like fructose and glucose. Disaccharides comprise two monosaccharides linked together with a glycosidic bond through condensation reactions. These are anabolic reactions that require energy from adenosine triphosphate (ATP), and that involve the loss of a -H group from one monosaccharide and an -OH group from another monosaccharide, forming water as a by-product, along with the disaccharide produced (Allott and Mindorff, 2014). Disaccharides comprise sugars such as maltose and sucrose.

Saccharomyces cerevisiae, a species of yeast more commonly known as "baker's yeast" (JoVE, 2020), is a eukaryotic unicellular fungus that lives in habitats in which there is a supply of sugar present. It has a preference for consuming disaccharides and monosaccharides relative to any other carbon source (Lagunas, 1993). It is able to respire either aerobically or anaerobically, releasing energy in the form of ATP in the process. During aerobic respiration, *S. cerevisiae* undergoes the three stages of glycolysis (an anaerobic process), the Krebs cycle and oxidative phosphorylation to convert a sugar such as glucose and oxygen into carbon dioxide and water. Meanwhile, during anaerobic respiration, *S. cerevisiae* undergoes glycolysis and fermentation to convert a sugar and oxygen into carbon dioxide and ethanol (Allott and Mindorff, 2014). For example, in the presence of glucose ($C_6H_{12}O_6$), the equation for aerobic respiration in *S. cerevisiae* is:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP,$$

While the equation for anaerobic respiration in *S. cerevisiae* in the presence of glucose is:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_5H_5OH + ATP.$$

Anaerobic respiration, or alcoholic fermentation, in *S. cerevisiae* is integral in the manufacture of various types of biofuel, drinks and food, and one of its significant uses is in the production of bread. This is because as *S. cerevisiae* in bread respires anaerobically, bubbles of carbon dioxide gas are produced and swells bread dough, causing it to rise (Allott and Mindorff, 2014). This develops a light texture that is sought after when baking bread. As an avid baker, with a wide variety of recipes commonly utilised in my household involving bread, I have always wanted to compose a personal recipe for homemade bread, especially as I would be able to ensure its high nutritional content myself. To do this, I would like to identify the sugar that maximises *S. cerevisiae* respiration within a certain period of time, which will be highly advantageous in developing a light texture in the bread. Although *S. cerevisiae* is able to respire in the presence of any type of sugar, various studies have demonstrated that when *S. cerevisiae* is placed in the presence of both disaccharides and monosaccharides together, the latter is used up more rapidly in *S. cerevisiae* respiration and thereby produces a higher rate of carbon dioxide than the former in the short-term (Lagunas, 1993). This is in line with the fact that monosaccharides comprise solely single monomers, and hence necessitates less energy to break them down into constituent molecules than disaccharides, which are more complex since they comprise two monomers and thus do not provide energy that is as readily available as provided by monosaccharides (Barakat and Abd El-Wahab, 1951).

In order to test this, four different types of sugar are investigated: the disaccharides are maltose and sucrose, both of which possess the molecular formula of $C_{12}H_{22}O_{11}$, and the monosaccharides tested include fructose and glucose, both of which have the molecular formula of $C_6H_{12}O_6$. Fructose is naturally found in fruits, honey and vegetables (Park and Yetley, 1993), while glucose can be found in most dietary carbohydrates and honey. Meanwhile, maltose is composed of two units of the monomer glucose joined with an α1,4-glycosidic bond and is found in abundance in starch products like acid-thinned starch, corn syrup and maltodextrin, all of which have been partially hydrolysed (Furia, 1973); and sucrose, which comprises one fructose monomer linked to one glucose monomer, exists in many plants such as sugar beet and sugarcane, which is where sucrose is mainly extracted from in order to create table sugar (Pakpahan and Supriono, 2005).

The concentration of carbon dioxide gas produced by *S. cerevisiae* respiration in the presence of each type of sugar will be measured by a Vernier carbon dioxide gas sensor. This method of data collection was chosen as carbon dioxide is a major product of respiration in *S.*

cerevisiae, whether aerobic and anaerobic, and is much easier to measure in an effective and reasonably accurate manner in comparison with its other waste products like ethanol and water. Additionally, there are minimal risks associated with this method of experimentation, yet the results gained would still be accurate and precise to a relatively high extent. The measured carbon dioxide concentrations will then be used to calculate the average respiration rates, and determining the type of sugar that yields the highest respiration rate will result in the identification of the optimal sugar for *S. cerevisiae* respiration These results can then be applied to bread baking in the real world.

Aim:
The aim of this experiment is to investigate the impacts of utilising different types of disaccharides (maltose and sucrose) and monosaccharides (fructose and glucose) on the rates of respiration of *S. cerevisiae* through measuring the concentration of carbon dioxide gas produced, a product of *S. cerevisiae* respiration, for the 5-minute duration of each trial.

Independent variable:
The type of sugar utilised for respiration of *S. cerevisiae*, whether disaccharide (maltose, sucrose) or monosaccharide (fructose, glucose). The experiment was initially planned to include five variations of the independent variable, the fifth variation being the disaccharide lactose; however, due to laboratory constraints, this type of sugar was unable to be provided by the school laboratory and was hence not tested in this experiment. Nevertheless, the current four variations of the independent variable are sufficient to test the aim and hypothesis of the experiment, since they comprise two disaccharides and two monosaccharides.

Dependent variable:
The concentration of carbon dioxide gas produced from *S. cerevisiae* respiration in parts per million during 5 minutes, as measured by the Vernier carbon dioxide gas probe (±10% of measurement).

Controlled variables to ensure that experiment is a 'fair test':

Controlled Variable	Method of Control	Effects on the Experiment
Mass of different types of disaccharides and monosaccharides	All disaccharide and monosaccharide samples have been weighed by utilising an electronic balance (±0.001g).	Adding more sugar than necessitated would affect *S. cerevisiae* respiration rates, as there is more sugar available to utilise in respiration.
Mass of *S. cerevisiae*	All *S. cerevisiae* samples have been weighed by using an electronic balance (±0.001g).	The amount of *S. cerevisiae* in the solution along with distilled water and a type of sugar would impact respiration rates, because a larger amount of *S. cerevisiae* would engender more respiration happening at once.
Strain/type of *S. cerevisiae*	All *S. cerevisiae* samples are of the same strain and type.	Since different strains/types of *S. cerevisiae* may vary in their preferred types of sugar to use in respiration, this would influence the *S. cerevisiae* respiration rates in the experiment.
Temperature in which *S. cerevisiae* respires	The mixtures of *S. cerevisiae*, sugar and water were kept in a bottle soaked in a water bath set at 35 °C, which is the ideal temperature for *S. cerevisiae* respiration (*Janssens et al., 2016*).	As the optimum temperature for *S. cerevisiae* respiration is 35 °C, decreasing/increasing the temperature would affect the *S. cerevisiae* respiration rates.
Time taken to measure *S. cerevisiae* respiration rates	The duration of the trials, each taking 5 minutes, was controlled by making use of a stopwatch.	A longer/shorter duration of experimental trials would result in higher/lower *S. cerevisiae* respiration rates compared to the rates at which they would respire in the span of exactly 5 minutes.
Volume of distilled water in which *S. cerevisiae* respires	The volume of water was measured using a 10cm^3 measuring cylinder (±0.5cm^3) to be 10cm^3 for each trial.	Since the sugars are water-soluble, varying the volume of distilled water in which the *S. cerevisiae* respires would impact their respiration rates as some sugars would become more accessible than others.

Uncontrolled variables:
Although all the *S. cerevisiae* utilised in the experiment was ordered at the same time, the ages at which they are tested in the experiment were not controlled because of time constraints that only enabled the experimental trials to be completed over a period of approximately

two weeks of class time. This may have influenced their respiration rates, as they may vary with age. Nevertheless, as 5 experimental trials were conducted for each variation of the independent variable, the effects of this uncontrolled variable were minimised.

Procedure:

There was no assistance received during the performance of this experiment, other than the provision of fructose, glucose, maltose, sucrose, *S. cerevisiae*, the 250ml gas sampling Nalgene plastic bottle and the Vernier carbon dioxide gas sensor (±10% of measurement) and its USB cable by the school's laboratory technician prior to the commencement of the 1st experimental trial.

1. Set a Serrata water bath (±0.1°C) to 35 degrees Celsius and connect the Vernier carbon dioxide gas probe (±10% of measurement) to a laptop by utilising a USB cable, as shown in Figure 1.
2. Measure out 10cm^3 of distilled water into the 10cm^3 measuring cylinder (±0.5cm^3).
3. Weigh 1g of *S. cerevisiae* and 2g of fructose on an electronic balance (±0.001g) separately.
4. When the water bath has reached exactly 35 degrees Celsius, as indicated by the water bath's temperature indicator (±0.1°C) and confirmed by a glass thermometer (±0.05°C) measurement, pour 10cm^3 of distilled water into the 250ml gas sampling Nalgene plastic bottle.
5. By utilising a glass thermometer (±0.05°C), check that the temperature of the distilled water in the plastic bottle has reached 35 degrees Celsius as well.
6. Once it is exactly 35 degrees Celsius, add 1g of *S. cerevisiae* and 2g of fructose into the plastic bottle and stir with a glass stirring rod until mixed, for approximately 30 seconds.
7. Insert the carbon dioxide gas probe (±10% of measurement) into the bottle, as demonstrated in Figure 2.
8. Simultaneously, begin data collection and start the stopwatch.
9. End data collection after 5 minutes and repeat steps 2-8 for the other types of sugar (glucose, maltose and sucrose), until 5 trials have been performed for each type of disaccharide and monosaccharide.

Figure 1 – Setup of the Experiment

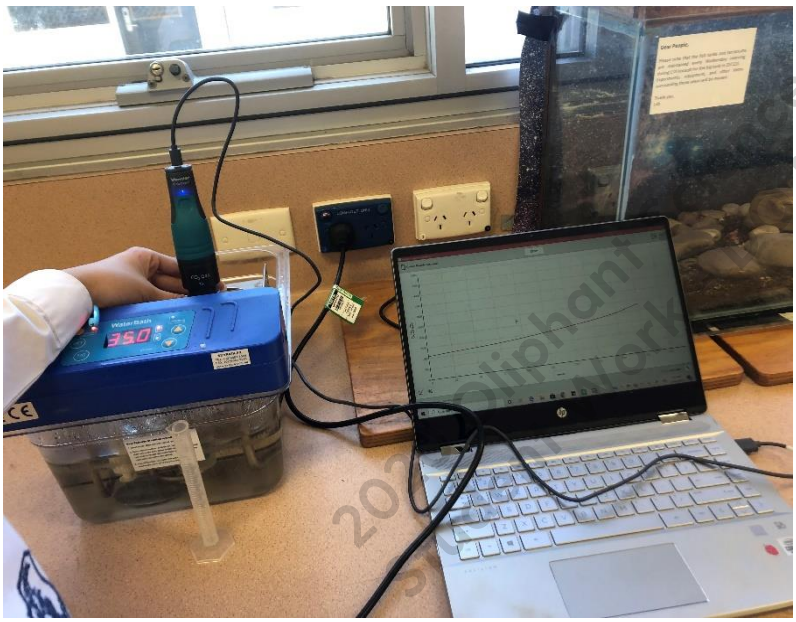


Figure 2 – Positioning of the Vernier carbon dioxide gas probe inside the experimental bottle

Equipment and Materials

- 10cm^3 plastic measuring cylinder (±0.5cm^3)
- 2 x watch glasses
- 25 x 10cm^3 of distilled water
- 25 x 1g of *S. cerevisiae*
- 250ml gas sampling Nalgene plastic bottle
- 5 x 2g of fructose
- 5 x 2g of glucose
- 5 x 2g of maltose
- 5 x 2g of sucrose
- Glass stirring rod
- Glass thermometer (±0.05°C)
- OHAUS® Pioneer® Precision digital balance (±0.01g)
- Serrata water bath (±0.1°C)
- Stopwatch (±0.01s)
- Teat pipette

- Vernier carbon dioxide gas sensor (±10% of measurement) connected to a laptop through a USB cable

Risk Assessment:

Environmental considerations:

There were no significant environmental risks associated with the materials made use of in this experiment. The solutions used in the experiment, containing different types of sugars, distilled water and *S. cerevisiae*, are non-toxic and posed no hazards to the environment, so it is safe to dispose of them down the laboratory sinks.

Ethical considerations:

The *S. cerevisiae* used in the experiment was the type of dry yeast utilised in baking that remains dormant until mixed with lukewarm water; hence, there are no associated ethical considerations with its use during the experimental trials. There were no other live organisms made use of in the experiment.

Safety precautions:

During the experiment, the water bath was checked for electrical safety before utilising it for each trial. Additionally, as the water bath's electrical components are possible ignition sources, it was kept away from any potential electrical and flammable hazards so as to prevent the risk of electrocution. The electronic balance was kept back from the edge of the laboratory bench on which it was placed, which was also kept dry. Any spilled substances were removed from the electronic balance immediately in order to keep it clean and tidy, and its wiring was checked for damage each time before using it for each trial. Furthermore, standard laboratory safety measures of apparel, i.e. wearing an apron, gloves and goggles, were adhered to. Contact between eyes and the sugar, water and *S. cerevisiae* mixture was also avoided, as well as contact between eyes and tongue and *S. cerevisiae*. It was also ensured that (distilled) water used was kept away from all electrical sources. In addition, as the glass stirring rod, thermometer and watch glasses may break, they were inspected for any chipped edges and/or other damage before each use. If broken, glass pieces should be swept up with a brush and a dustpan and should not be touched with fingers. There were no harmful chemicals utilised in the experiment and the carbon dioxide gas produced is harmless in its relatively small experimental quantities.

Processing and Analysing Data and Information

Raw data:

The uncertainty for the periods of time recorded are ±1 second, as the data was taken from a continuous line graph of time (in seconds) plotted against carbon dioxide concentration (in parts per million) produced by a data logger connected to the Vernier carbon dioxide gas sensor.

The uncertainty for the concentration of carbon dioxide gas produced is ±10% of its reading, as stated by the Vernier carbon dioxide gas sensor manual (Vernier, 2016).

Table 1 – Concentration of Carbon Dioxide Gas Produced During *S. cerevisiae* Respiration in the Presence of Monosaccharide Fructose

	Concentration of Carbon Dioxide Gas Produced in ppm (±10% of reading)									
Trial	**30s**	**60s**	**90s**	**120s**	**150s**	**180s**	**210s**	**240s**	**270s**	**300s**
1	84	161	270	412	581	763	1046	1484	2003	2657
2	195	348	480	671	854	1098	1365	1699	3346	3951
3	402	615	778	926	1074	1281	1535	1969	2607	3344
4	120	255	401	560	761	1008	1349	1869	2482	3142
5	110	251	421	618	846	1126	1594	2114	2710	3354

Table 2 – Concentration of Carbon Dioxide Gas Produced During *S. cerevisiae* Respiration in the Presence of Monosaccharide Glucose

	Carbon Dioxide Gas Concentration in ppm (±10% of reading)									
Trial	**30s**	**60s**	**90s**	**120s**	**150s**	**180s**	**210s**	**240s**	**270s**	**300s**
1	4	12	37	78	157	276	416	580	789	1166
2	-14	35	150	293	443	621	810	1009	1353	1844
3	71	180	289	398	519	647	792	961	1729	2188
4	192	335	443	540	638	744	864	1000	1159	1489
5	179	358	491	617	751	923	1145	1486	1913	2376

Table 3 – Concentration of Carbon Dioxide Gas Produced During *S. cerevisiae* Respiration in the Presence of Disaccharide Maltose

Concentration of Carbon Dioxide Gas Produced in ppm (±10% of reading)										
Trial	**30s**	**60s**	**90s**	**120s**	**150s**	**180s**	**210s**	**240s**	**270s**	**300s**
1	145	255	343	425	520	658	800	1002	1266	1573
2	62	132	199	268	349	441	534	655	810	1100
3	51	158	260	375	526	688	869	1062	1368	1761
4	103	227	359	485	631	799	984	1225	1597	2007
5	79	168	270	423	608	833	1082	1373	1810	2295

Table 4 – Concentration of Carbon Dioxide Gas Produced During *S. cerevisiae* Respiration in the Presence of Disaccharide Sucrose

Concentration of Carbon Dioxide Gas Produced in ppm (±10% of reading)										
Trial	**30s**	**60s**	**90s**	**120s**	**150s**	**180s**	**210s**	**240s**	**270s**	**300s**
1	155	265	342	413	479	554	640	738	884	1298
2	236	438	581	717	852	1001	1240	1522	1976	2605
3	13	80	176	291	418	567	738	927	1232	1798
4	66	178	299	421	549	689	852	1043	1314	1757
5	58	150	263	401	610	1900	2526	3060	3561	4163

Note: As yeast cannot respire without the presence of sugar as a source of nutrition, there were no trials in the control condition, i.e. trials that involved no sugar at all.

Qualitative Observations:

- In every single trial, the solution containing disaccharides/monosaccharides, distilled water and *S. cerevisiae*. turned into a pale brown colour as its constituent ingredients were mixed together.
- A thin layer of light brown froth was produced during each trial of the experiment and increased as the duration of the trials increased.

Sample calculations:

All calculations were done on a CASIO FX-CG50 AU graphics calculator, unless specified otherwise.

Calculating the rate of carbon dioxide gas production from *S. cerevisiae* respiration in 5 minutes:

$$\frac{Total\ carbon\ dioxide\ gas\ produced\ (concentration\ of\ carbon\ dioxide\ gas\ at\ 300\ seconds)}{300\ seconds} \quad (1)$$

Thus, the rate of carbon dioxide gas production from *S. cerevisiae* respiration in the presence of fructose in 5 minutes for the first experimental trial can be calculated through the aforementioned formula:

$$\frac{2657\ ppm}{300\ seconds} = 8.87 ppm/s, (\pm 0.887\ \text{ppm/s})$$

Calculating the average concentration of carbon dioxide gas produced at each 5-second interval and the average rates of *S. cerevisiae* respiration in 5 minutes:

The average, or mean value, of a set of data can be defined by:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}, i = 1 \cdots n \quad (2)$$

where x_i represents one particular data value at ith trial and n is the total number of data values. In order to calculate the average concentration of carbon dioxide gas produced at each 5-second interval during the experiment, x_i represents the concentration of carbon dioxide gas produced in each trial for a particular type of sugar and n is equivalent to the number of experimental trials, which is 5. When calculating the average rates of *S. cerevisiae* respiration in the presence of each different type of sugar, x_i represents the *S. cerevisiae* respiration rate for each trial in the presence of a specific type of sugar, while n is equivalent to the number of experimental trials, which is 5.

Therefore, the average concentration of carbon dioxide gas produced in 30 seconds of *S. cerevisiae* respiration, in the presence of fructose, can be calculated through the aforementioned formula:

$$\frac{84\ ppm + 195\ ppm + 402\ ppm + 120\ ppm + 110\ ppm}{5\ trials} = 182.2\ \text{ppm}\ (\pm 18.22\ \text{ppm})$$

The average rate of *S. cerevisiae* respiration in the present of fructose, can also be calculated through the aforementioned formula:

$$\frac{8.87ppms^{-1} + 13.17ppms^{-1} + 11.15ppms^{-1} + 10.47ppms^{-1} + 11.18ppms^{-1}}{5} = 10.97ppms^{-1} (\pm 1.097ppms^{-1})$$

Calculating the standard deviation of the concentration of carbon dioxide gas produced at each 5-second interval and of the average rate of carbon dioxide gas production from *S. cerevisiae* respiration in 5 minutes:

The standard deviation of a set of values, σ, can be defined by =

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n}}, i = 1 \cdots n \quad (3)$$

where x_i represents each data value, $\bar{x}$ represents the mean of the data values (as calculated by equation (2)) and n represents the total number of data values.

The standard deviation values of the concentration of carbon dioxide gas produced at each 5-second interval and of the average rates of *S. cerevisiae* respiration in the presence of each type of sugar were calculated on Microsoft Excel, a computer software belonging to Microsoft Office Home and Student 2019.

Processed data:

The data conveyed in Tables 1-4 have been processed in order to obtain the average concentration, based on equation (2), and standard deviation values of carbon dioxide gas, based on equation (3), in ppm, as demonstrated in Table 5. The total concentrations of carbon dioxide gas produced, at 300 seconds, were subsequently utilised to calculate *S. cerevisiae* respiration rates according to equation (1), as indicated in Table 6. Equation (2) was then used to calculate the average *S. cerevisiae* respiration rates in the presence of each type of sugar for the 5-minute durations of the experiment and their standard deviation values were calculated by making use of equation (3).

Table 5 – Average Concentrations and Standard Deviations of Carbon Dioxide Gas Produced During *S. cerevisiae* Respiration in the Presence of Different Types of Disaccharides and Monosaccharides

Average Concentration and Standard Deviation of Carbon Dioxide Gas Produced in ppm (±10% of reading)											
Type of Sugar	**Processed Data Type**	**30s**	**60s**	**90s**	**120s**	**150s**	**180s**	**210s**	**240s**	**270s**	**300s**
Fructose	**Average**	182.2	326	470	673.4	823.2	1055.2	1377.8	1827	2629.6	3289.6
	Standard Deviation	129.6	48.5	24.5	25.1	30.5	38.1	87.8	76.9	474.1	48.1
Glucose	**Average**	86.4	184	282	385.2	501.6	642.2	805.4	1007.2	1388.6	1812.6
	Standard Deviation	96.0	69.3	42.0	38.9	28.3	32.4	41.4	81.0	240.9	67.6
Maltose	**Average**	88	188	286.2	395.2	526.8	683.8	853.8	1063.4	1370.2	1747.2
	Standard Deviation	37.4	20.9	23.7	33.9	42.8	48.4	57.7	62.8	107.3	79.8
Sucrose	**Average**	105.6	222.2	332.2	448.6	581.6	942.2	1199.2	1458	1793.4	2324.2
	Standard Deviation	89.2	51.0	25.0	27.1	50.8	520.5	213.3	167.0	143.6	96.6

Table 6 – Rates of Carbon Dioxide Gas Production from *S. cerevisiae* Respiration in the Presence of Different Types of Disaccharides and Monosaccharides in 5 Minutes

Type of Sugar	**Rate of Carbon Dioxide Gas Produced in ppm/s (±10% of rate)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
Fructose	8.87	13.17	11.15	10.47	11.18
Glucose	3.87	6.15	7.29	4.96	7.92
Maltose	5.24	3.67	5.87	6.69	7.65
Sucrose	4.33	8.68	5.99	5.87	13.88

Table 7 – Average Rates of Carbon Dioxide Gas Production from *S. cerevisiae* Respiration in the Presence of Different Types of Disaccharides and Monosaccharides in 5 Minutes

Type of Sugar	**Average Rate of Carbon Dioxide Gas Production in ppm/s (±10% of rate)**	**Standard Deviation**
Fructose	10.97	1.55
Glucose	6.04	1.65
Maltose	5.82	1.51
Sucrose	7.75	3.77

Figure 3 – Graph of Average Concentration of Carbon Dioxide Gas Produced from *S. cerevisiae* Respiration in the Presence of Different Types of Disaccharides and Monosaccharides in 5 minutes

The error bars represent the standard deviation of the data points and are different for each data value according to Table 5 of the Processed Data section.

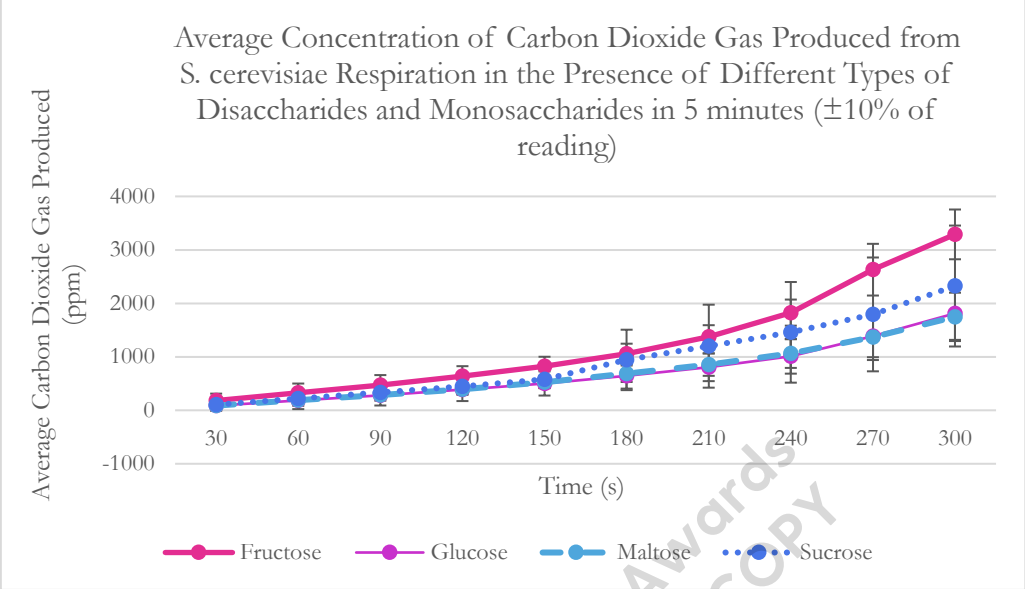
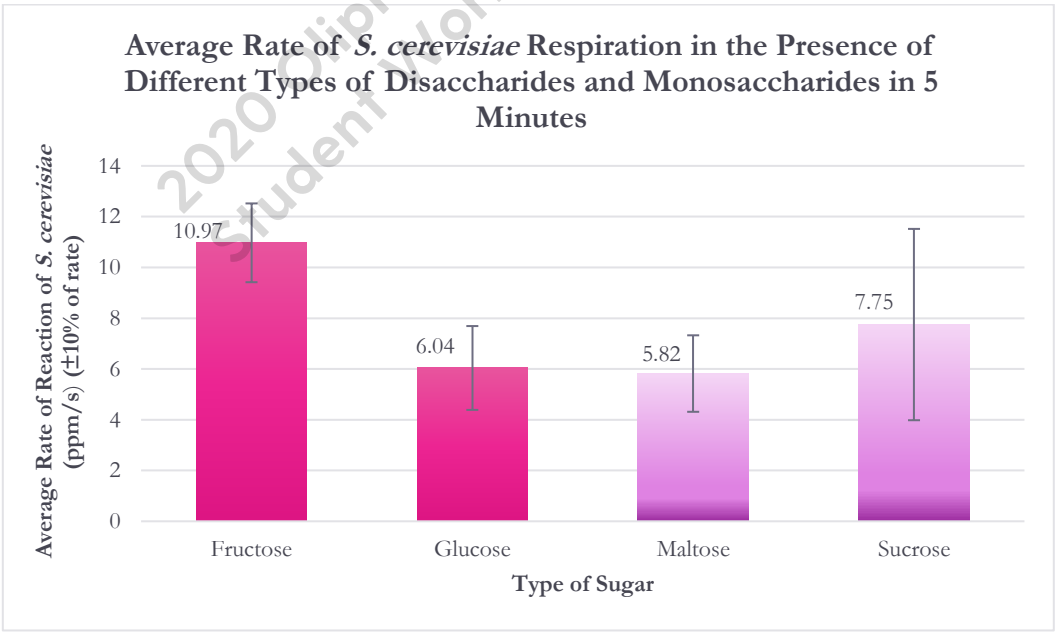


Figure 4 – Graph of Average Rate of Carbon Dioxide Production from *S. cerevisiae* Respiration in the Presence of Different Types of Disaccharides and Monosaccharides in 5 Minutes

In the graph below, the pink bars demonstrate data values for monosaccharides, whereas the purple bars present data values of disaccharides. The error bars represent the standard deviation of the average rates and are different for each type of sugar according to Table 7 of the Processed Data section.



Analysis of Variance (One-Way ANOVA):

An Analysis of Variance, or one-way ANOVA, has been utilised in order to analyse whether the results of this experiment possess statistical significance. There was no control condition in this experiment; however, there were four variations of the independent variable of the type of sugar utilised in *S. cerevisiae* respiration, all of which are independent in their measurements because the results of one

variable does not impact those of another. Hence, instead of performing multiple T-tests, a one-way ANOVA was undertaken to compare the means of the results produced by the four variations of the independent variable by determining whether there is a significant difference between those mean values, or whether two or more of them are equal.

Because data was taken every 30 seconds during each experimental trial, only the total concentrations of carbon dioxide gas produced (300 seconds) for each type of sugar in all 5 trials were taken into consideration, so as to clarify and simplify the results of the ANOVA because they were the data values utilised to calculate respiration rates. In this statistical analysis, the null hypothesis is that there is no significant difference between the different types of sugar utilised for *S. cerevisiae* respiration, and any observed differences may have simply occurred due to chance and/or sampling error. On the other hand, the alternative hypothesis states that there is a significant difference between the different types of sugar utilised for *S. cerevisiae* respiration, and that these observed differences are most likely not due to chance and/or sampling error.

An ANOVA of the experiment's results was carried out based on the summarised data shown in Table 8, all of which were calculated through Microsoft Excel, a constituent software of Microsoft Office Home and Student 2019. The results are shown in Table 9.

Table 8 – Intermediate Steps in Analysis of Variance

Type of Sugar	Count	Sum	Average	Variance
Fructose	5	16448	3289.6	216631.3
Glucose	5	9063	1812.6	245534.8
Maltose	5	8736	1747.2	204246.2
Sucrose	5	11621	2324.2	1277931

Table 9 – Analysis of Variance Results

Source of Variation	Sum of Square	Degrees of Freedom	Mean Squared	F-value	P-value	Critical F-value
Between Groups	7614331	3	2538110	5.221528	0.01052	3.238872
Within Groups	7777372	16	486085.8			
Total	15391703	19				

As the F-value is greater than the critical F-value, this statistical analysis demonstrates that the null hypothesis can be rejected, because at least one of the mean values among the four different types of sugar is significantly different. Additionally, the P-value is 0.01052, which is less than 0.05 (the selected alpha value for ANOVA). This further confirms the rejection of the null hypothesis, which means that the alternative hypothesis can be accepted since the data acquired are most likely not due to chance and/or sampling error. Therefore, it can be concluded that the results of this experiment are statistically valid.

Analysis and discussion:

Figure 3 indicates that the type of sugar that yielded the highest average concentration of carbon dioxide gas produced is the monosaccharide fructose, with Table 5 conveying that 3298.6ppm of carbon dioxide gas has been produced throughout the 5-minute duration of the experiment in the presence of fructose. This is followed by sucrose, that has produced the average carbon dioxide concentration of 2324.2ppm in its presence. *S. cerevisiae* respiration in the presence of glucose engendered the third highest average concentration of carbon dioxide gas produced, which is 1812.6ppm, while the least average concentration of carbon dioxide gas was produced in the presence of maltose, at a value of only 1747.2ppm. These results are not entirely consistent with the hypothesis of the experiment, as although the sugar that engendered the highest concentration of carbon dioxide gas produced and the highest average *S. cerevisiae* respiration rate is the monosaccharide fructose, which is expected because it requires less energy to break down fructose than disaccharides, it is not followed by glucose, the other monosaccharide in this experiment. Instead, fructose is followed by the disaccharide sucrose, which is unexpected because it would theoretically take more time to break sucrose's glycosidic bond that links its two constituent monomers of fructose and glucose together, thereby suggesting that sucrose would be a more efficient sugar to be utilised in long-term respiration rather than respiration in a short period of time like in this experiment. Nevertheless, as sucrose comprises one fructose monomer linked to one glucose monomer and is broken down to those constituent parts when used in respiration, these results suggest that the *S. cerevisiae* sample utilised in this experiment may have a particular affinity to using fructose in respiration compared to other types of sugars.

Table 5 signifies that the standard deviation of the concentration of carbon dioxide gas produced from *S. cerevisiae* respiration in the presence of fructose are relatively low values, hence increasing the precision of the data, except for the higher standard deviation values at 30 and 270 seconds, which are 129.6 and 474.1, respectively. Two outliers have been identified that may have contributed to these abnormally high standard deviation values, as Table 1 shows that 402ppm of carbon dioxide have been produced at 30 seconds in the

third trial of fructose, while there is a large 1647ppm increase in concentration of carbon dioxide gas produced from 240 to 270 seconds. Additionally, Table 5 demonstrates that the standard deviation values of the concentration of carbon dioxide gas produced from *S. cerevisiae* respiration in the presence of glucose are relatively low as well, except for another abnormally high value of 240.9 at 270 seconds. This pattern of consistently low standard deviation values throughout the data, with the exception of an abnormally high standard deviation value at 270 seconds, recurs in maltose and sucrose, with standard deviation values of 107.3 and 143.6 seconds respectively. However, in sucrose, there are also extreme standard deviation values of 520.5 and 213.3 at 180 and 210 seconds, which are much higher than its other standard deviation values. These abnormal standard deviations in sucrose are caused by outlier data acquired from the fifth trial, as the concentration of carbon dioxide gas produced begins to increase rapidly from 150 seconds onwards, going from an initial value of 1303ppm to 2593ppm by 180 seconds and 4163ppm by 300 seconds, as signified by Table 4. These values would have contributed to the second highest average concentration of carbon dioxide gas produced from *S. cerevisiae* respiration at 2324.2 in the presence of sucrose; although sucrose would still yield the second highest average final concentration of carbon dioxide gas produced at 1864.5ppm even if the outlier data was excluded in calculations of average values. These extreme standard deviation values impact the precision and reliability of the results, as they demonstrate an abnormally large spread at certain times in the experimental trials. Furthermore, it is significant that there are particularly high standard deviation values for all sugars at 270 seconds; this conveys that there may be a significant step/transition stage in *S. cerevisiae* respiration that occurs from approximately 240-270 seconds after mixed with the sugars, such as possible transitions between aerobic respiration and anaerobic respiration as a result of low remaining concentrations of the sugars and/or low oxygen concentration in the laboratory in which the experiment took place (Otterstedt et al., 2004). Furthermore, the standard deviation values of the average rates for each type of sugar, as shown in Table 7, are of considerable amount and range from 1.50 to 3.77. In the case of sucrose, the standard deviation is particularly large at a value of 3.77, which is almost half of the average rate of *S. cerevisiae* respiration in the presence of sucrose during 5 minutes at 7.75ppm/s. The outlier data value that has caused this abnormally large standard deviation value has been identified as the rate of *S. cerevisiae* respiration in the presence of sucrose during the 5th trial, which is considerably high at 13.88ppm/s, as indicated by Table 6. These standard deviation values decrease the precision of the data values acquired during the experiment, hence impacting the reliability of the conclusions derived from this experiment and suggesting that further improvements to the performance of the experiment should be made to augment its results' precision, such as by increasing the amount of experimental trials so that more precise and reliable data values can be obtained.

Additionally, the monosaccharide fructose has also yielded the highest average *S. cerevisiae* respiration rate at 10.97ppm of carbon dioxide per second, as indicated by Figure 4. This is expected as the duration of the experimental trials is the same for each type of sugar, so the higher the amount of carbon dioxide gas produced, the higher average rate of *S. cerevisiae* respiration will be achieved. The type of sugar that yielded the second highest average *S. cerevisiae* respiration rate at 7.75ppm of carbon dioxide per second is sucrose, which is yet again unexpected, since it is a disaccharide and would have taken a longer time to be utilised in *S. cerevisiae* respiration as it would have to be broken down into its constituent fructose and glucose monomers that can be used directly in respiration. This is followed by the monosaccharide glucose, with an average *S. cerevisiae* respiration rate of 6.04ppm of carbon dioxide per second, and lastly, the disaccharide maltose, with an average *S. cerevisiae* respiration rate of 5.82ppm of carbon dioxide per second. The fact that glucose has the third highest respiration rate while fructose possesses the highest respiration rate in this experiment is unusual, as existing literature either portrays *S. cerevisiae* as using fructose more slowly than glucose during respiration when they are metabolised separately (Caron and Reid, 1987), or that fructose and glucose are made use of at similar rates during *S. cerevisiae* respiration when they are metabolised separately, but that *S. cerevisiae* has a greater affinity for glucose than fructose in general (D'amore et al., 1987). This can be explained by the fact that the experimental trials comprised only 5 minutes, which is too short of a time to determine the long-term effects of utilising different types of sugars on *S. cerevisiae* respiration rates in comparison with the aforementioned experiments that went on for hours. As the type of respiration that the *S. cerevisiae* underwent during the experiment was not controlled, i.e. whether it was aerobic or anaerobic, another possible explanation to this discrepancy is that the preferred type of sugar for the two types of respiration are different. Moreover, *S. cerevisiae* and the different types of sugar were mixed with distilled water in this experiment to form a solution, and because fructose is more soluble than other types of sugar in water (Hanover and White, 1993), this may have contributed to the high concentration of carbon dioxide gas produced from *S. cerevisiae* in the presence of fructose.

A significant factor that influences the reliability of the results is the extent of uncertainty associated with the apparatus that were made use of in order to obtain the data values. Both the raw and processed data tables convey the message that the uncertainty of all data values acquired from the experiment is ±10%, which is a systematic uncertainty inherent in the Vernier carbon dioxide gas probe used to gain the data values. This uncertainty percentage would significantly influence the accuracy of the data values acquired in the experiment. In addition, even though this considerably large uncertainty percentage would not significantly affect the positions of fructose and sucrose as the types of sugar that yielded the highest and second-highest *S. cerevisiae* respiration rates respectively, this would pose a major impact on the accuracy of the data values concerning the average concentration of carbon dioxide gas produced during *S. cerevisiae* respiration in the presence of glucose (1812.6ppm) and maltose (1747.2ppm) during 5 minutes, between which there is only a relatively small difference. This alludes to the fact that the data gained may not be a true representation of the real values of the concentration of carbon dioxide gas produced at particular points in the experiment, thereby decreasing the accuracy of the processed data, results and subsequent conclusions that can be derived from this experiment. Furthermore, even though the *S. cerevisiae* and the various types of sugars utilised for the experiment were weighed to be 1g and 2g respectively, the electronic balance used to weigh them possessed the inherent uncertainty of ±0.001g, hence impacting the precision of the materials made use of in the experiment in random directions to a small degree. This would affect the reliability of the experiment's results in the sense that there may have been the masses of *S. cerevisiae* and/or sugar may have varied between different trials, which would affect the data gained regarding the carbon dioxide gas

produced and the *S. cerevisiae* respiration rates during the 5-minute experimental trials, even though the uncertainty of ±0.001g is relatively minuscule. Moreover, the effects of the aforementioned uncertainty concerning the masses of materials involved in the experiment would have been minimised by the presence of 5 experimental trials for each type of sugar.

Evaluating

Strengths:

There are several strengths of this experiment, the most prominent being its fairly simple procedure which is not time-consuming and presents minimal risks to the experimenter. The extraneous variables of amounts of disaccharides, monosaccharides and *S. cerevisiae*; strain/type of *S. cerevisiae*; temperature and volume of distilled water in which *S. cerevisiae* respired and the amount of time taken to measure *S. cerevisiae* respiration were all controlled for in this experiment, thereby allowing for stronger correlations between the dependent variable (concentration of carbon dioxide gas produced) and independent variable (type of disaccharide/monosaccharide) utilised for *S. cerevisiae* respiration to be established. Additionally, most of the apparatus used in the experiment enabled a relatively high degree of accuracy and precision of data to be achieved. For example, the concentration of carbon dioxide produced in each trial was measured by utilising a sensor that developed a line graph of time in seconds (±0.5s) against carbon dioxide concentration in ppm (±10% of measurement) over the course of the experiment, enabling data values to be taken at the precise 30-second intervals. Thus, the confounding variable of time was controlled, increasing the reliability of the data acquired. Meanwhile, the digital balance used was calibrated before use, allowing relatively accurate data to be acquired. Furthermore, the bottle in which the solution of distilled water, *S. cerevisiae* and the different types of sugar was placed was specifically designed to fit the Vernier carbon dioxide gas probe perfectly in its neck, hence minimising the amount of carbon dioxide gas produced during the experiment that may have escaped through the opening of the bottle and affected the results of the experiment. The distilled water utilised in each experimental trial was also read correctly every time, at the level of the meniscus in the $10cm^3$ plastic measuring cylinder ($\pm 0.5cm^3$) used to measure its volume, thereby increasing the precision of the materials involved in the experiment. Finally, another strength of this experiment is the considerable number of trials performed, as 5 experimental trials for each type of sugar engendered the acquisition of 20 data sets that enables statistical analysis and validity and reduced the effects of random errors on the data.

Limitations:

Nevertheless, this experiment possesses some limitations. As one of the motivations behind the development of this investigation was to relate its results to the process of baking bread, the samples of *S. cerevisiae* used in the experiment may have been too small compared to the amounts of baker's yeast that are commonly made use of to bake bread. Furthermore, the concentration of carbon dioxide gas produced from *S. cerevisiae* respiration was only observed for 5 minutes because of time constraints and this may not have provided a true reflection of the optimal disaccharide or monosaccharide to utilise for *S. cerevisiae* respiration in a longer period of time, especially as bread baking takes a much longer amount of time than the 5-minute duration of each trial in this experiment. Hence, these decrease the applicability of this experiment's results towards real-life circumstances, because the results acquired from the study may have only represented a small portion of the respiration process. Since there were no significant mistakes committed during the experiment, the rest of its limitations can be divided into random and systematic errors as described below. Moreover, the study did not control for the type of *S. cerevisiae* respiration, i.e. whether it respired aerobically or anaerobically, so the rates of respiration in the presence of different types of disaccharides and monosaccharides may have varied because of the different types of respiration involved, as one type of respiration may inherently produce a higher concentration of carbon dioxide gas produced than the other, increasing the randomness of the results' distribution.

Random errors

The most significant source of possible random errors produced during the undertaking of the experiment are the random fluctuations of readings of mass and temperature on the digital balance and in the water bath respectively, as those measured values often decreased and increased unpredictably. The random fluctuations in the readings of mass on the digital balance decreases the precision of the mass of the different types of sugars and/or the *S. cerevisiae* utilised in the experiment, because they may have weighed significantly heavier/lighter in reality than as stated by the digital balance. This decreases the extent to which extraneous variables are controlled in the experiment, hence reducing its characteristic of being a "fair test". Meanwhile, the random fluctuations of temperature in the water bath means that the *S. cerevisiae* may not have respired at a constant temperature throughout the numerous trials, which is a confounding variable that may have affected the results acquired, since temperature plays a part in respiration rates. Additionally, although the *S. cerevisiae* and the various types of sugars utilised for the experiment were weighed to be 1g and 2g respectively, the electronic balance used to weigh them possessed the inherent uncertainty of ±0.001g, hence impacting the precision of the materials made use of in the experiment in random directions. Lastly, due to time constraints, the experimental trials were not performed on the same day, but throughout a period of approximately 2 weeks. This allows for random variables such as differing oxygen concentrations on different days to have possibly influenced *S. cerevisiae* rates, in particular, during aerobic respiration as it involves oxygen, and hence decreased the precision of the experiment. These random errors would have negatively affected the precision of the results, because they would have added variability to the distribution of the data and decrease its reliability.

Systematic errors

The systematic errors in this experiment were primarily caused by the inherent uncertainty of the apparatus utilised, because the Vernier carbon dioxide gas sensor utilised for data collection has quite a large uncertainty percentage at ±10%, so the data acquired may not be a true representation of the real values of the concentration of carbon dioxide gas produced at particular points in the experiment, hence decreasing the accuracy of the results. In addition, there is no option to calibrate the Vernier carbon dioxide gas sensor used for data collection, so this may have decreased the accuracy of data values concerning the concentration of carbon dioxide gas produced during *S. cerevisiae* respiration by skewing the results in one particular direction, whether through making it higher or lower than its true value. It was also assumed that there was no increase in carbon dioxide gas concentration at 0 seconds into the experiment; however, as the solutions containing distilled water, *S. cerevisiae* and sugar were mixed for 30 seconds prior to the beginning of data collection, in order to ensure that the solution is mixed evenly and that the sugar utilised in respiration is spread throughout the solution, some of the *S. cerevisiae* may have already respired during that time. This means that the data obtained, of the concentration of carbon dioxide gas produced at 30-second intervals in 5 minutes, does not represent the immediate products of *S. cerevisiae* respiration, as some time may have already passed between the beginning of respiration and data collection. Additionally, although the temperature of the water bath at 35°C was confirmed by measuring the temperature with a glass thermometer, it is possible that both of them may have been inaccurate, as the temperature-measuring device inherent in the water bath could have been erroneous and an equally inaccurate glass thermometer would not have been able to detect these errors. Last of all, the *S. cerevisiae* utilised in this experiment may have been too old and this may have affected their respiration rates and affinity towards certain types of sugar in a particular direction. These systematic errors contribute to the decrease in accuracy of this experiment's results, since they may have shifted the central tendency of the data's distribution.

Improvements:

In order to improve the methodology of the study so as to increase its empirical data's accuracy and precision, several improvements can be made, such as by ensuring that the effects of random errors on the acquired results are minimised. First of all, random fluctuations of readings of mass on the digital balance be minimised by using a more precise electronic balance that is less sensitive to environmental circumstances, such as air currents passing through the laboratory, that may cause the readings of mass to fluctuate randomly. Fluctuations of temperature in the water bath can also be minimised by utilising a water bath that enables more precise control of the temperature of water that it contains, so that the *S. cerevisiae* can respire at the exact same temperature for each trial. Lastly, a more general method of decreasing the influences of random errors on the data is by increasing the number of trials. Although 5 trials for each type of sugar enable statistical validity, they can still be increased to a higher number so that the extent to which random errors affect the results can be significantly minimised.

Improvements can also be made to reduce systematic errors during the experiment by making use of equipment with a lower amount of inherent uncertainty. Hence, the Vernier carbon dioxide gas probe can be replaced with a different carbon dioxide gas probe that measures the concentration of carbon dioxide gas in the air more accurately and that can be easily calibrated, in order to ensure the collection of accurate data throughout the experiment so that the distribution of the data acquired is not skewed in a particular direction. The glass thermometer and water bath made use of in the experiment could also be replaced with a more accurate thermometer and water bath respectively, such as ones with even lower inherent uncertainty values; concerning the water bath, specifically, it could be replaced by one that possesses an internal temperature-measuring device that is less prone to making errors in measurement. In addition, instead of mixing the solution containing distilled water, *S. cerevisiae* and the different types of sugars for 30 seconds prior to the experiment, they can be centrifuged for an extended period of time so as to further ensure that the sugars are spread evenly throughout the solution. It should also be ensured that the *S. cerevisiae* used in this experiment are not too old that their age affects their affinity to metabolise certain types of sugar, so as to improve the accuracy of the results acquired.

Extension:

A significant extension to this experiment could be varying the strain and/or type of *S. cerevisiae* used, which will allow the determination of the optimal type of sugar for respiration in different strains and/or types of *S. cerevisiae*. Additionally, the type of respiration that the *S. cerevisiae* undergoes throughout the experiment can be controlled in order to investigate whether the effects of different types of sugars vary depending on whether the *S. cerevisiae* is respiring aerobically or anaerobically. The duration of each experimental trial can also be increased to longer than 5 minutes, so as to increase the applicability of the experiment's results to the real-life action of bread baking, which takes a significantly longer period of time than 5 minutes. This would also ensure that respiration would be completed and all sugars have been used up. Another extension to this experiment can be achieved by varying the concentrations of the different types of disaccharides and monosaccharides tested, as doing so would enable the discovery of the optimal concentration of sugar to utilise in *S. cerevisiae* respiration, thereby allowing ingredients for baking bread in the real world to be used in a more economic and efficient way.

Conclusion

As indicated by the results, it can be inferred that within the 5-minute durations of the experimental trials, the monosaccharide fructose yielded the highest average concentration of carbon dioxide gas produced and the highest average rate of *S. cerevisiae* respiration. This is followed by the disaccharide sucrose, the monosaccharide glucose and the disaccharide maltose. The results partially support the hypothesis which predicted that allowing *S. cerevisiae* to respire in the presence of monosaccharides will yield higher concentrations of

carbon dioxide gas produced, which would also result in higher rates of respiration compared to respiration in the presence of disaccharides. This is because, although *S. cerevisiae* respiration in the presence of fructose, a monosaccharide, caused the highest average concentration of carbon dioxide gas produced, it was followed by a disaccharide, sucrose, instead of the other monosaccharide, glucose. Having said that, the answer to the research question is that fructose will engender a maximal level of *S. cerevisiae* respiration in comparison with the other sugars tested, which are glucose, maltose and sucrose, thereby rendering it the most optimal sugar to be utilised in baking bread. Thus, not only would the conclusions of this experiment be beneficial in developing my homemade bread recipe, but the results may also be extended to other applications of *S. cerevisiae* in industries, such as in maximising its respiration activity to manufacture other compounds like biofuel, drinks and food products in an efficient and optimal manner. However, because of several random and systematic errors inherent in the experiment, it is recommended that the experiment be repeated in the future so as to be able to derive more accurate and precise conclusions about the relationship between the dependent and independent variables. Nevertheless, the data obtained during the experiment was still sufficiently accurate and precise enough to draw valid conclusions from, as it is significant and possesses a P-value of under 0.05.

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