Effect of Sugars on Rate of Respiration of Baker's Yeast

Biology HL Internal Assessment

November 5, 2018

Design

Research Question: What is the effect of different types of sugars (maltose, sucrose, glucose, fructose) on the rate of anaerobic respiration of *Saccharomyces cerevisiae* (Baker's yeast) obtained by the amount of carbon dioxide (ppm) produced?

Personal Engagement

My interest in this topic was sparked when I helped my mother bake *naan*, a type of Indian flatbread. I was fascinated to see how the yeast helped the *naan* to rise and was curious as to the scientific explanation behind it. When I learnt more about respiration and its applicability to real-life (for breadmaking and alcoholic drinks), I was curious about how different sugars may affect how fast yeast respired.

Background Research

Cellular respiration is the conversion of carbohydrates into usable energy, in the form of ATP, which is used for metabolism, cell function and maintenance, and growth (Perry & Burggren, 2007). Respiration is important to study because it is universal; almost every organism respires (Perry & Burggren, 2007). The formula for cellular respiration is:

$$C_6H_{12}O_6 \text{ (glucose)} + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{ATP (energy)}$$

This lab, however, will not be focusing on aerobic respiration which requires oxygen. The lab investigates the rate of respiration in *Saccharomyces Cerevisiae*, commonly known as Baker's yeast. When it has run out of oxygen, yeast uses anaerobic respiration to break down glucose molecules and convert them to ATP. After, it starts producing ethanol--this process is called fermentation. It is important to note that glucose isn't yeast's only source of energy, but the preferred source (Rodrigues, Ludoviko, & Leão, 2006). The first step of anaerobic respiration is glycolysis. In glycolysis, the starting glucose molecule is rearranged into fructose-6-phosphate by enzymes, where two phosphate groups attach to it, making it unstable and causing the molecule to split (Bear & Rintoul, 2013). This uses 2 ATP molecules. The resulting 3-carbon sugars are converted to pyruvates through several reactions, each 3-carbon sugar producing 2 ATP and 1 NADH (electron carrier) molecule (Bear & Rintoul, 2013). Thus, glycolysis results in the net production of 2 ATP and 2 molecules of NADH. The second step of anaerobic respiration is fermentation. The purpose of fermentation is to oxidise NADH so it can be reused as an electron carrier; fermentation produces ethanol (Bear & Rintoul, 2013). The equation for fermentation is:

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2CH_3CH_2OH \text{ (ethanol)} + 2CO_2$$

The metabolism of disaccharides is a bit different from that of monosaccharides. In yeast, an enzyme called invertase hydrolyses sucrose into fructose and glucose **outside** of the cell (Godoy et al., 2014). Maltose is transported **into** the cell (via active transport) and metabolised by an enzyme called glucosidases (Godoy et al., 2014). Then the monosaccharides go through glycolysis. CO_2 is one of the products of respiration. Therefore, the volume of CO_2 produced will be measured to calculate the rate of respiration. Yeast requires five minutes in warm water to be activated to carry out respiration (Jaworski). This step will thus be added in the method.

Aim

The yeast *S. cerevisiae* was used for the experiment because it grows quickly and efficiently in anaerobic conditions (Rodrigues, Ludoviko, & Leão, 2006). At first glance, the choice of sugars may seem random. Glucose and fructose are monosaccharides whereas maltose and sucrose are disaccharides. However, the aim of this lab is to investigate whether sugars are

metabolised faster as disaccharides or monosaccharides. Glucose molecules are the monomers of maltose, whereas fructose and glucose are monomers of sucrose (Rodriguez et al.).

Hypotheses

Null: IF the rate of respiration of different sugars (maltose, sucrose, glucose, fructose) is calculated by measuring the amount of carbon dioxide produced, **THEN** there will be no difference in the rate of respiration **BECAUSE** the type of sugar doesn't affect the rate of respiration.

Alternate: IF the rate of respiration of different sugars (maltose, sucrose, glucose, fructose) is calculated by measuring the amount of carbon dioxide produced, **THEN** fructose will have the fastest rate of respiration followed by glucose, sucrose, and maltose **BECAUSE** during glycolysis, fructose will not have to be converted to fructose-6-phosphate as it is already in that form. In addition, the monosaccharides will have a faster rate of reaction than disaccharides because they don't have to be broken down into monomers first. Sucrose will have a faster rate of reaction than maltose because it is metabolised outside of the cell (Godoy et al., 2014), thus saving that much amount of time. Furthermore, sucrose is made of fructose and glucose, and as stated previously, fructose will be metabolised faster as it won't need to be converted into an isomer.

Table 1. Independent and Dependent Variables

Variable	Manipulation	Units & Uncertainties
Independent (x) -- type of sugar	Maltose, sucrose, glucose, and fructose will be used. There will be a controlled trial where no sugar is put into water to ensure that the type of sugar affects respiration.	g +/- 0.01
Dependent (y) -- rate of respiration	The volume of carbon dioxide (ppm) produced will be measured and used to calculate the rate of respiration.	ppm min-1 +/- 0.1 ppm min^{-1}

Table 2. Controlled Variables

Controlled Variables	Manipulation	Units & Uncertainties
Controlled fermentation group	There will be trials done where there is no sugar added in the solution.	
Amount of sugar	The sugar solutions will have a concentration of 10g/ml	ml +/- 0.05
Amount of yeast	5mL of the yeast solution will be used per trial for all sugars.	ml +/- 0.05
Carbon dioxide probe	The same probe will be used for all trials.	ppm +/- 10% (source: Vernier)
Time period for	The yeast will have ten minutes to respire.	+/- 00:01s

respiration		
Type of water	Distilled water will be used for all trials.	
Type of yeast	The yeast, S. cerevisiae, will be used for all trials.	Supplied by school.
Lab environment	All trials will be carried out in the same laboratory at 25ºC.	
Weighing scale	The same weighing scale will be used for all trials to take all measurements.	+/- 0.01g
Yeast activation	The yeast will have five minutes to activate by being placed in the water bath.	

Table 3. Materials

Item	**Quantity**	**Specifics**
Dried yeast	200g +/- 0.01g	*S. cerevisiae* species
Distilled water	100ml +/- 0.05	For each solution
Sugars	50g per sugar +/- 0.01g	The sugars used are maltose, sucrose, glucose, and fructose
Weighing scale	1	+/- 0.01
Beakers	4 +/- 25g	Not used for measurement.
Graduated cylinder	1	+/- 0.5 ml
Stirring rod	4	
Water bath	1	Set at 40ºC
Test tube	5	
Timer	1	+/- 0.01s
Vernier CO_2 gas sensor	1	+/- 10% ppm (source: Vernier)

Method

1. Prepare a water bath. It should be 40ºC at all times.
2. Fill a beaker with 50 ml of water and put 3g of dried *S. cerevisiae* in it. Use a graduated cylinder to measure the water (for more accuracy).
3. Prepare five sugar solutions (one for each sugar and one control).

   a. Put 10g of each sugar in a separate beaker using a weighing scale to measure the exact amount.
   b. Pour 100 ml of distilled water into each of the beakers using a graduated cylinder to measure the water.
   c. Stir the solutions until the sugar has dissolved completely.
4. Pour 5mL of the yeast solution into a test tube and put it in the water bath for five minutes to activate the yeast.
5. Pour the yeast solution (5mL) and one of the sugar solutions (100mL) into the carbon dioxide probe bottle and cap it (with the probe). Make sure to start the timer.
6. Record the amount of CO_2 produced in 1-minute intervals for ten minutes. Take qualitative observations.
7. Repeat steps 5-6 with the all the sugars (maltose, sucrose, glucose and fructose) and no sugar added. Do five trials for each sugar.

Note: If the sugar solution or yeast solution are all used up, repeat Steps 2 and 3 to make more as needed keeping the concentration the same.

Table 4. Safety

Safety Categories	**Description**	**How To Avoid**
Physical	1. Glassware can break and cause injuries. 2. Solutions may spill onto clothes or into eyes.	1. Handle glassware carefully. If it breaks, call an adult immediately. 2. Wear lab coat and goggles.
Ethical	1. Wasting sugar by using it for a lab and then throwing it away may be seen as unethical.	1. Use the minimum amount of sugar for the experiment.
Environmental	1. Dumping the yeast solution in the sink will most likely go down the drain and end up in the river and pollute it.	1. Dispose of yeast solution in a separate container.

Data Processing

Table 5. Raw Data

Time (min)	0	1	2	3	4	5	6	7	8	9	10
Control (no sugar) -- Volume of CO_2 (ppm +/- 10%)											
Trial 1	0	0	0	0	0	0	0	0	0	0	0
Trial 2	0	0	0	0	0	0	0	0	0	0	0
Trial 3	0	0	0	0	0	0	0	0	0	0	0
Trial 4	0	0	0	0	0	0	0	0	0	0	0
Trial 5	0	0	0	0	0	0	0	0	0	0	0
Maltose -- Volume of CO_2 (ppm +/- 10%)											
Trial 1	0	16	44	60	110	119	132	144	144	166	169
Trial 2	0	109	178	203	206	222	272	306	337	362	384
Trial 3	0	162	212	266	331	365	415	440	478	484	512
Trial 4	0	25	34	69	75	75	81	112	125	131	162
Trial 5	0	243	296	334	390	393	424	455	480	496	502
Sucrose -- Volume of CO_2 (ppm +/- 10%)											
Trial 1	0	50	71	100	109	112	131	137	140	146	156
Trial 2	0	3	4	10	23	29	49	51	57	62	64
Trial 3	0	112	172	216	244	272	287	297	315	328	331
Trial 4	0	32	78	113	141	169	191	206	228	235	250
Trial 5	0	13	101	141	176	191	223	251	272	294	310
Glucose -- Volume of CO_2 (ppm +/- 10%)											
Trial 1	0	130	146	180	186	196	202	269	387	405	492
Trial 2	0	200	259	309	350	365	378	400	419	428	440
Trial 3	0	191	316	384	425	444	459	469	487	500	512
Trial 4	0	57	78	100	106	122	135	153	163	181	191

Trial 5	0	94	150	188	209	231	244	287	303	325	341
Fructose -- Volume of CO_2 (ppm +/- 10%)											
Trial 1	0	56	79	98	109	118	149	171	203	214	234
Trial 2	0	59	65	68	75	78	84	84	87	93	100
Trial 3	0	4	35	57	66	78	91	100	113	119	125
Trial 4	0	57	78	100	106	122	135	153	163	181	191
Trial 5	0	94	150	188	209	231	244	287	303	325	341

To obtain accurate data, at minute 0, 0 ppm of CO_2 was assumed. The rest of the values were subtracted from the starting measurement. For example, if there was 210 ppm at 0 minutes, and 243 ppm at 1 minute, the measurement for 1 minute would be 243-210 = 33 ppm.

Table 6. Qualitative Observations

Sugar	**Photograph**	**Observations**
Control		• There is no reaction at all.
Maltose		• The solution is much hazier than that of glucose. • Beaded particles of what I think is maltose appeared on the sides of the bottle.
Sucrose		• The solution is lighter in colour than maltose and glucose. • A lot of the sucrose particles have sank to the bottom even though in the sugar solution, the sucrose had been dissolved.

Glucose		• The solution is a very pale colour and seemed to get paler over the trial. • It smelled a bit like rotten eggs. • The yeast had sort of dissolved in the sugar solution. • The solution is very hazy/foggy. • During one trial, one bubble appeared.
Fructose		• It was harder to dissolve the fructose into water. • The solution was yellow-y in colour.

How Data Will Be Processed

The RQ aims to investigate the rate of reaction. Hence, the rate will be calculated by finding the mean average CO_2 produced and dividing it by time (10 minutes). This is to facilitate comparing the different rates of reactions. If an instantaneous rate was obtained, it would be difficult to come to a conclusion as to which sugar is metabolised faster.

Mean average: The mean average CO_2 produced will be calculated using the following formula to be used for identifying a pattern between rate and amount of CO_2 produced.

$$mean\ average = \frac{total\ carbon\ dioxide\ produced\ in\ all\ trials\ (ppm)}{number\ of\ trials}$$

Example calculation for glucose:

$$mean\ average = \frac{492+440+512+191+341}{5} = 395.2\ ppm$$

Rate of reaction: Then, the rate of reaction will be calculated using the following formula so that the fastest sugar which ferments can be identified.

$$rate\ of\ reaction = \frac{mean\ average\ amount\ of\ carbon\ dioxide\ produced\ (ppm)}{time\ (minutes)}$$

Example calculation for glucose:

$$rate\ of\ reaction = \frac{395.2\ ppm}{10\ min} = 39.52\ ppm\ min^{-1}$$

Standard deviation: Will be done to measure the distribution of data relative to the central tendency using the following formula.

$$\sigma = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n}}$$

σ = standard deviation, Σ = sum, x = value in data set, $\bar{x}$ = mean value, n = # of data sets

Table 7. Example calculation for glucose

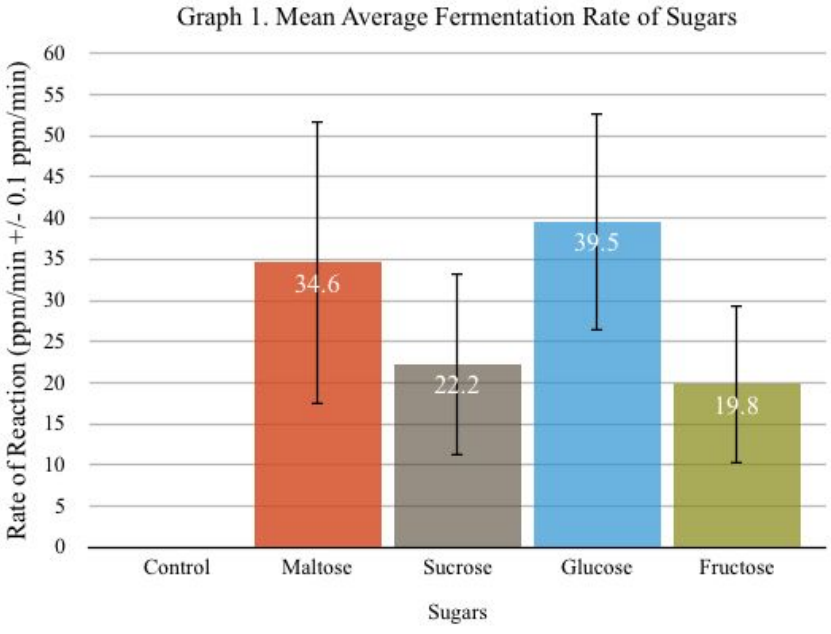
CO_2 produced (x)	**Mean Average ($\bar{x}$)**	**$x-\bar{x} = d$**	**d^2**	**Mean of d^2 = v**	**SD = $\sqrt{v}$**
492	395.2	96.8	9370.2	13930.9	118.0
440		44.8	2007.0		
512		116.8	13642.2		
191		-204.2	41697.6		
341		-54.2	2937.6		

Table 8. Processed Data: Mean Average CO_2 produced**,** Rate of Reaction and Standard Deviation

Sugar	**Mean Average (ppm +/- 0.1 ppm)**	**Rate of Reaction (ppm min^{-1} +/- 0.1 ppm min^{-1})**	**Standard Deviation**
Control	0	0	0
Maltose	345.8	34.6	172.1
Sucrose	222.2	22.2	111.5
Glucose	395.2	39.5	132.0
Fructose	198.2	19.8	95.8

Glucose produced the highest volume of CO_2 and also had the fast rate of reaction. The standard deviations of all the sugars is very high suggesting that the data is not precise.

Graph



A bar graph was chosen to represent the mean average rate of reaction of each sugar mixture because it clearly shows the difference in rate. The data for each sugar is also independent. The error bars were created by finding the standard deviation of the average rates of reactions for all the trials of each sugar. Glucose seems to have the fastest mean average rate. However, the rates of other sugars are pretty close in value. The error bars are overlapping which suggests that the difference in rates isn't significantly different.

Discussion

The experiment aimed to investigate the effect of different types of sugars (maltose, sucrose, glucose, fructose) on the rate of anaerobic respiration of Saccharomyces cerevisiae (Baker's yeast). The alternative hypothesis argued that fructose would have the fastest rate of respiration followed by glucose, sucrose, and maltose because fructose didn't have to be converted for respiration to occur, and monosaccharides would ferment faster than disaccharides. The findings (see **Table 7**) showed that glucose had the highest rate of reaction, 39.5 ppm min^{-1}. This was followed by maltose (34.6 ppm min^{-1}), sucrose (22.2 ppm min^{-1}) and fructose (19.8 ppm min^{-1}). The controlled solution with no sugar produced no CO_2. The control was used to ensure that fermentation by yeast only occurred when sugar was present. Since no CO_2 was produced, it is safe to assume that this is indeed the case.

The qualitative observations, to a certain extent, supports the quantitative data. After fermentation, the glucose solution was the most odorous and the solution seemed to get paler almost immediately after yeast was added. The fructose solution was the most difficult to dissolve in the water. One trend in the raw data (**Table 4**) is that the reactions for most trials of maltose, sucrose and fructose started fast and then gradually slowed down. For glucose, however, the rate of reaction starts fast, starts slowing down after around five minutes, and speeds up again. This may be a possible evaluation point. However, it also supports that *S. Cerevisiae* is more efficient in fermenting glucose.

The results of this experiment are in accordance with Hopkins (1928) who did a study investigating the difference in fermentation rates of fructose and glucose by *S. Cerevisiae*. and found that glucose fermented faster than fructose. Hopkins (1928) also found that glucose doesn't convert into fructose while being fermented by *S. Cerevisiae.* This is important because it counters my alternative hypothesis. However, Hopkins (1928) also pointed out that other species of yeast such as Sauterne yeasts ferment fructose faster than glucose. Therefore, the high fermentation rate of glucose is attributed to enzymes of *S. Cerevisiae* being more efficient. This brings up an evaluation point of this experiment which is that there are many factors which show the rate of fermentation, not just the production of CO_2. The enzymes used, as pointed out, are a better indication of fermentation rate. This experiment is reductionist in that sense.

The rate of fermentation of sucrose was slower than maltose contrary to the alternative hypothesis. Based on the Hopkins (1928) study, this makes sense because maltose is made of two glucose molecules whereas sucrose--of fructose and glucose. It has been shown that glucose ferments much faster than fructose as stated above. Furthermore, according to Batista et al., the metabolic pathway (of fermentation) of sucrose is actually inefficient because it is metabolised outside the cell which is a bit counter-intuitive because monosaccharides would be easier to transport into the cell than disaccharides.

Although the fermentation rates of the sugars were different, it is important to know whether there is a statistically significant difference. Unfortunately, an ANOVA test cannot be done

because the data was continuous. Furthermore, the mean average rates of reaction couldn't be used because they were enough data points. Therefore, the study must conclude that there is a difference between fermentation rates in different sugars by *S. Cerevisiae* but it isn't clear whether the difference is statistically significant or not.

Relating this back to what inspired me to carry out this experiment, my mother uses sucrose, refined white sugar ("Types of Carbohydrates"), to make *naan*. My mother and I take two hours to make *naan*. However, this experiment suggests that using glucose would be better because the rate of respiration is faster. Thus, we would take a lesser amount of time to make *naan*.

Evaluation

Although the data shows that there is a difference in fermentation rates of sugars, there are several improvements that can be made to this experiment. First is the need for more trials. There were twenty-five trials in total resulting in twenty-four degrees of freedom. More trials will lead to better statistical results. Second, the length taken for fermentation was only ten minutes and as can be seen from **Table 4**, a plateau wasn't reached which suggests that complete fermentation hadn't occurred. This is a concern because the data doesn't represent the full reaction that takes place and the average rate of fermentation isn't accurate. An improvement would be to allow the yeast to respire for a longer period of time. Another improvement concerning time is the increments in which data was collected. Shorter increments would have led to better data because the trends and patterns would have been easier to see.

There is also a question of whether the range of independent variables was sufficient or not. The aim of the experiment was to compare the rate of metabolism of disaccharides to that of monosaccharides. Considering this aim, the range of independent variables was sufficient because glucose and fructose are monosaccharides whereas maltose and sucrose are disaccharides made up of glucose, and glucose and fructose respectively. Thus, monosaccharides are effectively being compared to disaccharides. Below is a table of more errors and improvements. A wider variety of sugars could also have been tested to determine the best sugar for respiration by Baker's yeast.

Table 9. Sources of Errors

Source of error	Significance	Improvement
There were some clusters of yeast at the bottom of the CO_2 probe's bottle.	**Moderate:** The yeast may have needed more time to be activated. It was only kept in the water bath for five minutes prior to each trial. No measure was taken of whether the yeast was fully activated or not. This would have affected the rate of fermentation.	Put the yeast in the water bath for at least ten minutes.
The data wasn't collected on the same day at the same time.	**Low:** The surrounding environment, although done in the same lab, would have	All data should be collected on the same day under highly controlled conditions.

	different conditions such as temperature of the room, humidity etc. This may have affected the fermentation of the yeast.	
The equipment used all had large uncertainties. For instance, the CO_2 probe had an uncertainty of 10%.	**High:** The data obtained isn't as accurate as it could have been.	Better and more accurate instruments with lower uncertainties should be used.
There was a short gap of time (of about two or three seconds) after the yeast and sugar solution had been poured into the CO_2 sensor's bottle and the probe's recording had started.	**Low:** The yeast may have started fermenting the sugar immediately after being poured into the sugar solution. There would have been a delay in the data collected. Thus, the data collected isn't as accurate as it could have been.	If the same equipment (Vernier's CO_2 probe is being used), it is difficult to eliminate the problem. However, the delay can become a controlled variable instead. CO_2 production can be recorded after exactly five seconds so that it's the same for all trials.

The standard deviation was also calculated for all sugars. With the exception of the controlled, which had a standard deviation of 0, the data deviated a lot from the mean average. This indicates that the results were not precise nor accurate. The standard deviations ranged from 95.8 to 172.1. There was indeed a lot of variation in the data. This also affected the mean average rate of fermentation to become very skewed. By replicating this experiment and following the improvements listed above, such a high standard deviation can be avoided.

Next testable question: What is the effect of different types of sugars (maltose, sucrose, glucose, fructose) on the rate of reaction by zymase?

According to Alba-Lois and Segal-Kischinevzky, zymase is an enzyme used by yeast to ferment sugars. It was mentioned that a limitation of this experiment was it used too broad of an approach--the production of CO_2--to measure fermentation rate. By focusing on the work of a specific enzyme in yeast, we can get a better understanding of which sugars are fermented faster.

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