The effect of different sugars in the medium on carbon dioxide production in *Saccharomyces cerevisiae*

Jason Angustia, Maggie Chan, Deirdre Dinneen, Shamim Hortamani, Diane Mutabaruka

Abstract

Carbon dioxide is the by-product of many metabolic processes in *Saccharomyces cerevisiae* including fermentation and oxidative phosphorylation. The objective of this experiment was to examine the effect of two different monosaccharides, glucose and fructose, as well as two different disaccharides, sucrose and maltose, on the rate of carbon dioxide production in *Saccharomyces cerevisiae,* when the sugars were metabolized separately. We measured the amount of CO_2 produced by *Saccharomyces cerevisiae* in media with the different sugars within an hour, at 5-minute intervals. The volume of carbon dioxide produced was measured using respirometers. We found that sucrose resulted in a faster rate of carbon dioxide production than maltose; while as a whole, monosaccharides produced more carbon dioxide compared to disaccharides. Glucose and fructose, however, produced carbon dioxide at the same rate. Our results can largely be explained by an understanding of the different transport mechanisms used by the sugars to enter the cell before they are metabolized (Lagunas 1993).

Introduction

Saccharomyces cerevisiae is commonly known as Baker's and Brewer's yeast (Fugelsang 2007). It is a globular shaped unicellular organism with its size ranging between 5-10 µm. Yeast was the first eukaryote with a complete genome sequence discovered in 1996 (Oliver 1997). This allowed researchers to study the genomes of more complex eukaryotes.

S. cerevisiae plays an important role for biological research and brewing industries, however this paper focuses on the production of carbon dioxide by yeast in different sugar media. Carbon dioxide is the by-product of many metabolic processes including fermentation and oxidative phosphorylation. However, these processes will depend on the presence or absence of oxygen. In aerobic conditions, pyruvate will breakdown into carbon dioxide and water to produce adenosine triphosphate (ATP) energy. This is known as oxidative phosphorylation (Pronk *et al.* 1996). In anaerobic

conditions, the process observed is fermentation, the breakdown of sugars into carbon dioxide and ethanol known as the Gay-Lussac equation (Fugelsang 2007). This can be observed in the following equation: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 3CO_2$ (Fugelsang 2007). Ultimately, yeast cells undergo oxidative phosphorylation or fermentation depending on the conditions in their environment (Pronk et al. 1996).

Within our study, we were unable to distinguish the carbon dioxide produced between oxidative phosphorylation and fermentation. In order to measure the rate of fermentation by measuring the carbon dioxide, we would have to raise the concentrations of our sugar media to 1.0M to achieve the Crabtree effect. This is when conditions are anaerobic so fermentation occurs rather than oxidative phosphorylation. Although the Crabtree conditions were not met, our experiment will alter the reactant, the sugar in the medium, to observe the optimal carbon dioxide produced by *S. cerevisiae.*

Previous studies have observed that when two different monosaccharides, glucose and fructose, were added together in the same medium, glucose was utilized at a faster rate than fructose (Cason and Reid 1987). Similarly, among the two disaccharides sucrose and maltose, yeasts utilize maltose more quickly due to its double glucose composition, as opposed to sucrose composed of glucose and fructose (De La Fuente and Sols 1962). Evidently, in the presence of all these sugars, glucose has the fastest rate of carbon dioxide production, followed by fructose, maltose and sucrose (Lagunas 1993).

The objectives of the experiment were to test and observe the rate of carbon dioxide production of *Saccharomyces cerevisiae* by using different sugars, mainly monosaccharides: glucose and fructose, and disaccharides: sucrose and maltose, when they were added in separately. Many studies have shown that when monosaccharides and

disaccharides are placed together, monosaccharides are utilized more rapidly, and therefore produce a higher rate of carbon dioxide production (Lagunas 1993). However, very little is known about the rate of carbon dioxide when the sugars are utilized separately (Cason and Reid 1987). By varying the type of sugars used and separating them, we wanted to confirm whether monosaccharides or disaccharides produced a higher carbon dioxide production rate.

We had three sets of hypotheses to test. Our first null hypothesis was that the monosaccharide, fructose, decreases or has no effect on, the rate of carbon dioxide production in *S. cerevisiae* as compared to glucose. The alternate hypothesis was that the monosaccharide fructose increases the rate of carbon dioxide production in *S. cerevisiae* as compared to glucose. Our second null hypothesis was that the disaccharide sucrose decreases or has no effect on, the rate of carbon dioxide production in *S. cerevisiae* as compared to maltose. The alternate hypothesis was that the disaccharide sucrose increases the rate of carbon dioxide production in *S. cerevisiae* as compared to maltose. Finally, the third null hypothesis was that disaccharides decreases or have no effect on, the rate of carbon dioxide production in *Saccharomyces cerevisiae* as compared to monosaccharides. The alternate hypothesis was that disaccharides increase the rate of carbon dioxide production in *S. cerevisiae* as compared to monosaccharides.

Wider implications of our study include the obvious applications in industry: the sugar with the greatest rate of fermentation is likely to be the most efficient for brewery businesses. However, the implications also stretch further; studies of glucose transporters, for example, are another demonstration of the similarity between yeast and mammalian cells (Lagunas 1993). Yeast studies can therefore be of interest when studying

mammalian pancreatic α and β cells (Jiang *et al.* 2000), with potential implications for diabetes research.

Methods

The yeast cells were kept in a flask at room temperature (approximately 25°C), sealed with aluminum foil to prevent contamination. We were also provided with four flasks containing 100 mL of media without cells at one of 0.1M glucose, 0.1M fructose, 0.1M sucrose or 0.1M maltose sugar concentrations. Our initial step was to perform a cell count of the wild type yeast cells using a haemocytometer (Figure 1a).
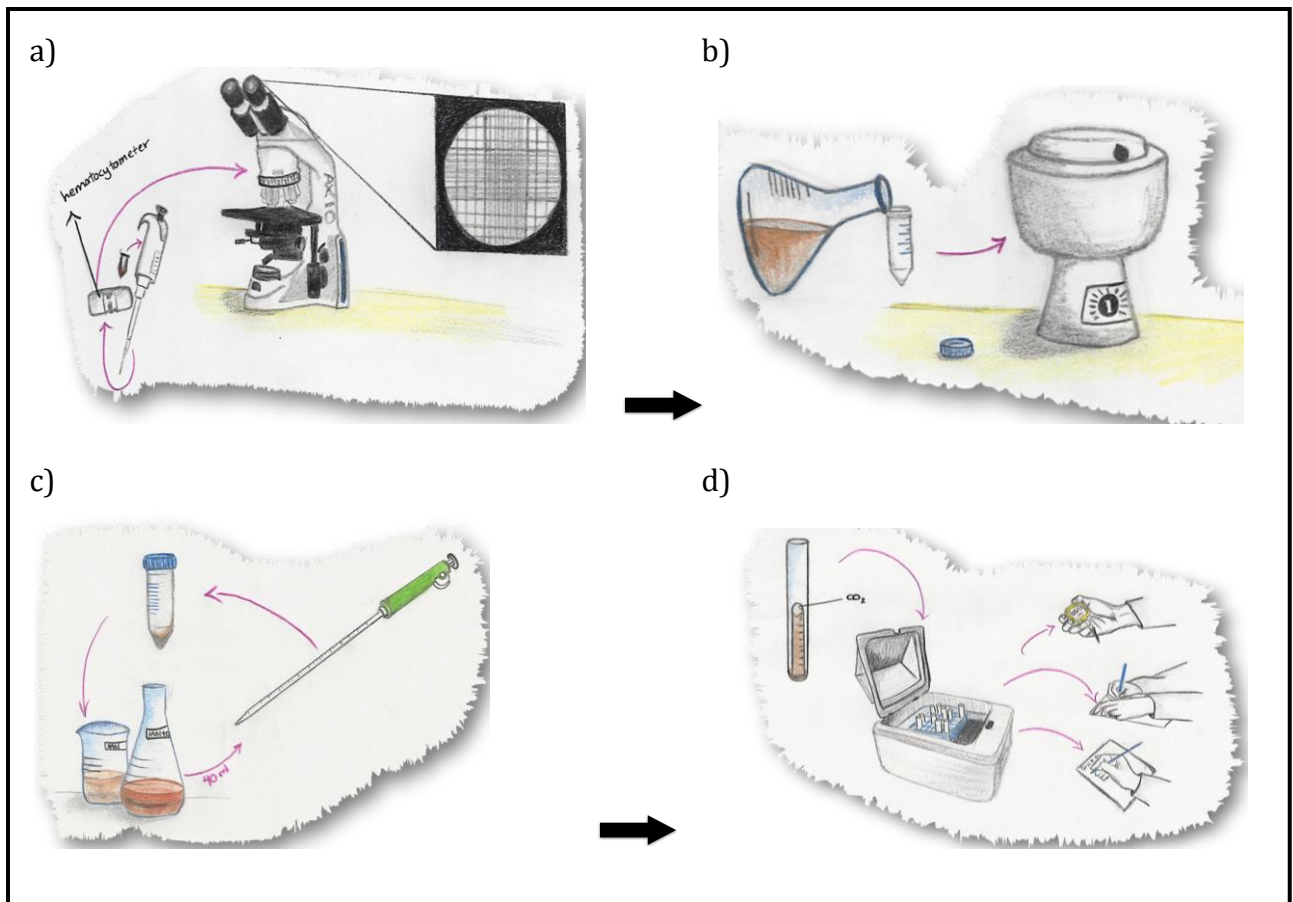

Figure 1. Experimental methods summary a) yeast cell count using haemocytometer, b) centrifuge yeast cells for 5 minutes at highest speed, c) preparing yeast cells in different sugar media(glucose, fructose, maltose and sucrose) d) Recording CO_2 volume using respirometers in 30°C water bath over the one-hour time frame.

Figure 2. Placing the 50 mL micro-centrifuge tubes for the centrifugation of wild-type yeast cells.

We centrifuged the cells (see Figure 2) to concentrate them by one order of magnitude- to ~10^9 cells/ mL- in order for the respirometer experiment to proceed at a reasonable rate (personal communication, Dr. C. Pollock).

We then re-suspended our pellets in the desired media; 40 mL of each media was added to separate pellets (Figure 1c) and the tubes were vortexed for approximately one minute in order to mix the cells and media well. Secondary cell counts were then performed. One individual performed all cell counts to reduce error. We did this for two reasons: firstly, to check that we had an optimal cell count for the respirometer and secondly, to check that each new suspension had the same number of cells. Cell density had to be a controlled variable in order for variation in CO_2 production to be solely due to the type of sugar used. Following the second cell count, we added more media to some of the suspensions where necessary, in order for all cell counts/mL to be equal. We used the equation $C_1V_1 = C_2V_2$ to calculate this volume.

Once we were confident our cell densities were equal, we filled the respirometer tubes with our different suspensions. We had previously marked our small, clean

respirometer tubes with 0.5 μL increments for ease of recording the volume of CO_2 produced (see Figure 3).

Figure 3. Example setup of respirometer tube. Figure shows CO_2 produced at top of small inner tube and yeast-sugar solution being pushed out of the bottom. Markings represent 0.5 μL increments.

The tubes were then placed in a water bath that was kept at a constant temperature of 30°C (Cason and Reid 1987) throughout the duration of the experiment. CO_2 measurements were recorded at 5-minute intervals for 30 minutes following entry to the water bath (Figures 1d and 3). There were three replicates for each sugar treatment. There were also procedural controls for each treatment (media and sugar, no cells), with three replicates each.

We calculated 95% confidence intervals for all our results and performed t-tests where appropriate.

Results

As illustrated in Figure 4, the calculated 95% confidence intervals (C.I) of the measured volume of produced CO_2 in glucose and fructose treatments, overlap at all times except for the 5 minutes (glucose C.I. = $0.8 \pm 0.14 \ \mu L^3$, fructose C.I = $0.167 \pm 0.288 \ \mu L^3$).
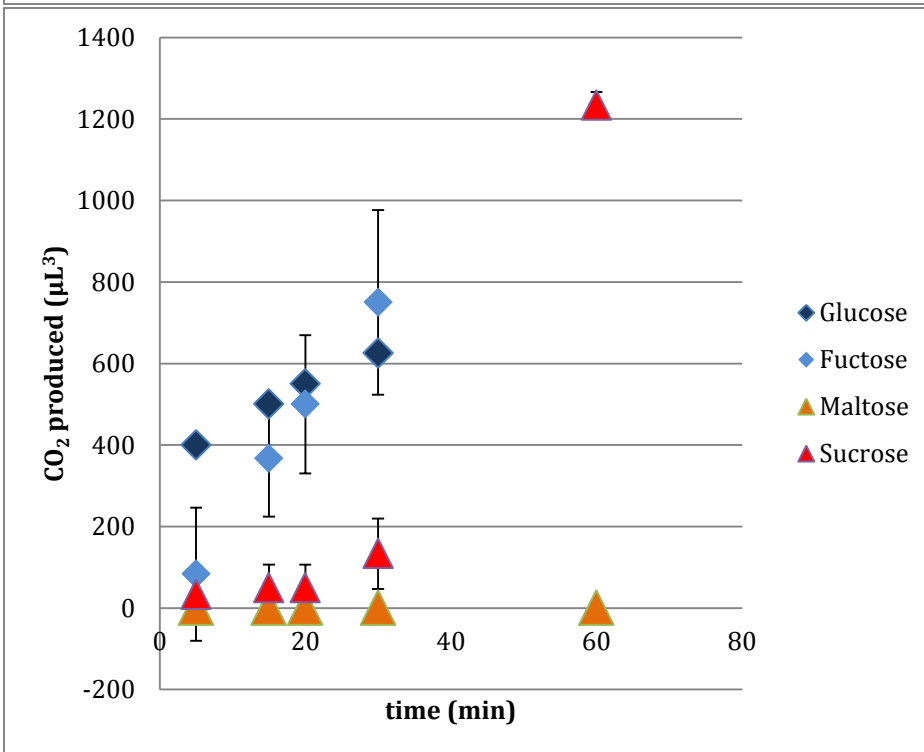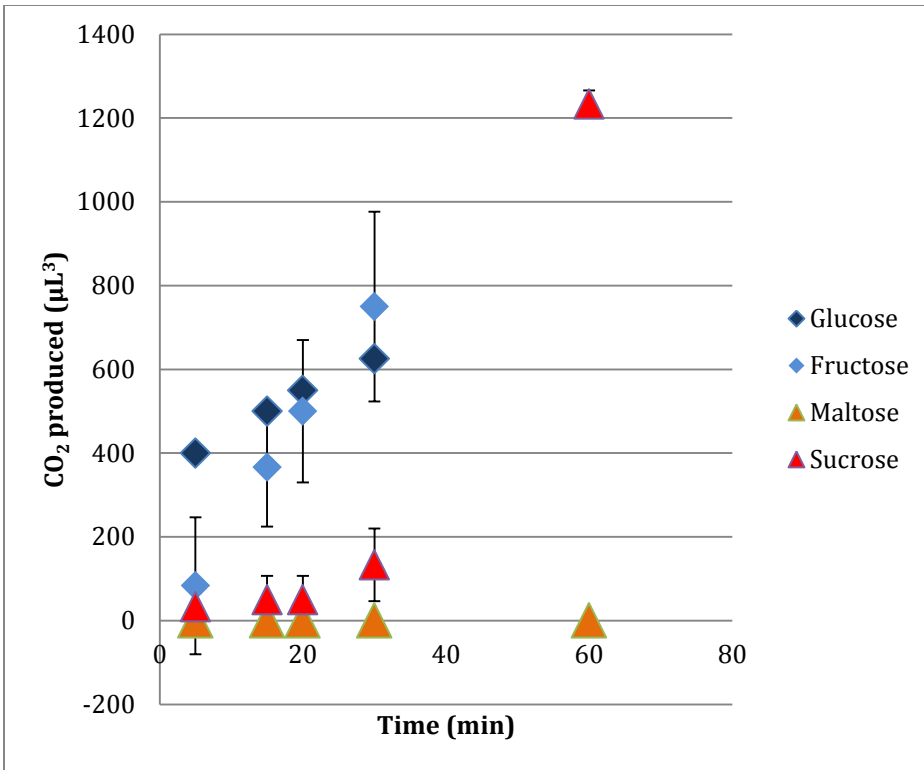
Figure 4. Mean change in CO_2 production rate of *Saccharomyces cerevisiae* at 5, 15, 20, 30 and 60 minutes following suspension in either monosaccharide (glucose and fructose) or disaccharide (maltose and sucrose) media. Bars represent 95% confidence intervals, n=3 for each treatment except for glucose treatment (n =2).

The results indicate that there is no significant difference between the metabolism rate of glucose and fructose between 15 and 30 minutes time points. However, applying a t-test on the fructose and glucose treatments at 5 minutes indicates that, since the calculated t-value for these data (2.781) is greater than the theoretical t-value (2.353), the calculated means of CO_2 volume are significantly different from each other (i.e. P-value = 0.05).

In Figure 4, the volume of CO_2 produced increased as time passed since after each measurement the CO_2 produced was not removed from the respirometer. Therefore, the minimum volume of CO_2 for all treatments was recorded at 5 minutes, while the highest volume of produced CO_2 was recorded at 30 minutes for glucose and fructose treatments and at 60 minutes for the sucrose treatment.

The same pattern can be obtained by comparing the rate of metabolism of the disaccharides, sucrose and maltose and their calculated confidence intervals (see Figure 4. However in the trial with disaccharides, maltose showed no signs of CO_2 production throughout the entire duration of the trial (even over the extended time period of 60 minutes). As this figure demonstrates, none of the 95% confidence intervals of sucrose and maltose overlap, which indicates that there are significant statistical differences among the mean CO_2 volumes produced. Since all four sugars are represented on the same graph it might seem that sucrose and maltose C.Is overlap however, the calculations shows that CO_2 values for sucrose at 5 minutes creates a range of 0.0013 μL^3 to 0.132 μL^3, which is very close to 0 μL^3 of CO_2).

Figure 4 also shows all four of the sugars to compare their CO_2 production rates as two distinct groups of monosaccharides and disaccharides. Except at the 5-minute time

at which all four sugars share confidence intervals, none of the calculated 95% confidence intervals of monosaccharides and disaccharides overlap. This is also relevant when performing a t-test for any of the data sets between 15 and 60 minutes.

Sample Calculations:

The following sample calculations are done for the CO_2 readings of fructose treatment at 15 minutes for CO_2 readings of replicate number 1, 2 and 3 respectively at 250 μL^3, 350 μL^3, and 500 μL^3.

- Using formula for mean change: mean $= \dfrac{\sum xi}{n}$

  Mean $= (250+350+500) / 3 = 366.67 \ \mu L^3$

- Using formula for standard deviation: $s = \sqrt{\dfrac{\sum(\bar{x} - xi)^2}{(3-1)}}$

  $s = \sqrt{\dfrac{(366.67 - 250)^2 + (366.67 - 350)^2 + (366.67 - 500)^2}{(3-1)}}$

  $s = 125.83 \ \mu L^3$

- Using formula for calculating 95% confidence intervals: C.I. $=$ mean $\pm (s / \sqrt{n})$

  C.I. $= 366.67 \pm 125.83/ \text{sqrt}(3)$

  C.I. $= 366.67 \pm 142.39 \ \mu L^3$

Discussion

We expected to reject our first null hypothesis and support the alternative that the rate of production of carbon dioxide by glucose is faster than that of fructose. This is because glucose is the primary input for glycolysis, the first step in fermentation and aerobic oxidation; whereas fructose has to be converted to a useable form before it enters the process as an intermediate. Our expectation was also based on considerable literature that suggests that yeast has a higher affinity for glucose than fructose (Cason and Reid

1987, D'Amore *et al.* 1989). Briefly, notice for Figure 4, that we used two replicates for glucose as opposed to three as stated in methods and used for all other analyses. This is because we ignored our results for the third glucose replicate as no CO_2 was produced at all over the entire observed time period. We considered two sources of error for this anomaly. Firstly, that there were no cells present in the tube, which is unlikely considering the other replicates most definitely had live cells present. Alternatively, we noticed while recording results that there was an unusual air bubble in the third replicate tube that looked to be preventing the movement of liquid out of the tube, thus artificially returning results of zero CO_2 production when in fact fermentation and oxidative phosphorylation were occurring.

As n=2 for glucose, we cannot perform statistical analysis on this data. Unfortunately therefore, we cannot say anything statistically significant about our glucose and fructose results. We can however recognize the very similar trend in carbon dioxide production between the two sugars that strongly indicates that we should fail to reject our first null hypothesis. After further investigation of sugar transport across yeast membranes, it became clear that this was in fact a reasonable result. Sugars do not freely permeate biological membranes (Lagunas 1993) therefore in order for sugars to enter the organism and be metabolized they must first cross the membrane via permeases, or transporters. Multiple studies report that fructose and glucose share the same membrane carrier (D'Amore *et al.* 1989, Lagunas 1993) thus when yeast are in media containing either glucose or fructose, as in the case of our comparative monosaccharide study, there should be no significant difference in rate of carbon dioxide production between the two as there is no competition for the carrier (D'Amore *et al.* 1989, Lagunas 1993). The

difference in affinity becomes important when glucose and fructose are in the same media, in which case the yeast preferentially uptake glucose (D'Amore *et al.* 1989).

If we were to repeat our experiments we would be able to eliminate a considerable source of error we experienced, thus adding increased confidence to our results. The source of error arose via the considerable amount of time that elapsed between re-suspending yeast cells in media after centrifugation and getting the mixtures into the water bath to record CO_2 produced. We recorded this to be a period of 23 minutes and 45 seconds. We performed secondary cell counts and equalized cell densities during this time, a necessary, but time consuming step. This contributed error as yeast cells will commence metabolic processes immediately after being re-suspended in media. Important trends could have been missed during this time, particularly as we do not know whether fermentation and oxidative phosphorylation in *Saccharomyces cerevisiae* occur at a linear rate.

As an extension of our first null hypothesis, we expected maltose, a disaccharide composed of two glucose residues, to produce carbon dioxide faster than sucrose, a disaccharide made up of one glucose and one fructose residue (De La Fuente and Sols 1962). Figure 4 clearly shows that this is not the case, thus we reject the second null hypothesis and support the alternative hypothesis that the rate of carbon dioxide production with sucrose is faster than the rate of carbon dioxide production with maltose in *Saccharomyces cerevisiae.* In our experiment, maltose did not result in any carbon dioxide production. This was surprising to us as there are multiple accounts of maltose metabolism in wild type *Saccharomyces* in the literature (Cason and Reid 1987, D'Amore *et al*. 1989, Lagunas 1993).

Further research presented us with two possible explanations. Firstly, there is evidence that glucose represses the expression of MAL genes for maltose permeases (Jiang *et al.* 2000, Lagunas 1993). This would result in no expression of maltose permeases and thus maltose could not be transported across the membrane to enter the yeast cells and be metabolized inside the cell. This explanation is not satisfactory however as our maltose medium lacked glucose. Contamination of the maltose media with glucose by poor experimental procedure- a potential source of error - is also unlikely as our results show that volume of CO_2 produced was consistently zero across the entire time frame of our experiment. If glucose contamination had occurred, we would expect some CO_2 to be produced as the yeast could now metabolize this glucose. This leads us to our second explanation that one hour was not long enough to observe utilisation of maltose and that a longer-term experiment is necessary. This is made more plausible by the fact that D'Amore *et al.* (1989) used a 168-hour time frame for their maltose experiments with observable decreases in maltose in the media being observed after approximately 24 hours (Figure 4, D'Amore *et al.* 1989).

Figure 4 clearly shows that we fail to reject our third null hypothesis that disaccharides decrease the rate of carbon dioxide production compared to monosaccharides. In the case of sucrose, this is because the disaccharide must first be broken down into glucose and fructose by the invertase enzyme prior to transport of the monosaccharides into the cell (Cason and Reid 1987, Lagunas 1993). *Saccharomyces cerevisiae* secrete the invertase enzyme into the periplasmic space where sucrose is hydrolyzed extracellularly (Lagunas 1993). Although the action of invertase is rapid (D'Amore *et al.* 1989), it is an extra step in the process, and therefore likely more time

consuming than simple monosaccharide transport. Moreover, as previously mentioned, fructose and glucose compete for the same membrane carrier; therefore twice the amount of monosaccharides does not mean twice the amount of carbon dioxide production. When measuring the rate of carbon dioxide production with sucrose, we noticed immediately that it was occurring at a slower rate, but that it was most definitely occurring; therefore we extended our recording time to one hour to see how much CO_2 could be produced within this extended time. This is consistent with the literature that the sucrose had to first be broken down before the monosaccharides could be transported and metabolized (Cason and Reid 1987, Lagunas 1993).

The case of maltose is slightly different, as it is not broken down extracellularly into two molecules of glucose (as we expected), but instead transported into the organism as a disaccharide (Lagunas 1993). While it is likely that monosaccharides are transported by simple facilitated diffusion, disaccharides such as maltose cross the membrane via active transport (D'Amore *et al.* 1989, Lagunas 1993). The difference in rate of carbon dioxide production is then likely due to differences in the affinities of enzymes within the cell that initiate intracellular metabolism (Lagunas 1993). A high affinity for a substrate means a faster rate of reaction, whereas a low affinity means that larger amounts of substrate are needed before the same rate of reaction can be observed. Kinases phosphorylate hexoses, such as fructose and glucose, and have a high affinity, whereas the glucosidases that hydrolyze disaccharides have a lower substrate affinity (Lagunas 1993). This means that the likely explanation for the slower rate of carbon dioxide production with disaccharides such as maltose is that it takes time before active transport accumulates enough maltose inside the cell for an appreciable level of enzyme activity to

occur (Lagunas 1993). We thought that extending the time frame to one hour would allow us to see some maltose metabolism, but as discussed above, this was not a long enough extension of the experiment.

Further sources of error include the difficulty of reading the volume of CO_2 produced in the respirometer as yeast mixtures are a murky, often opaque, beige solution. Markings on the respirometer tubes were often obstructed by liquid (see Figure 3); we had a system in place to pipette out excess fluid, but this may have led to incorrect reading of values regardless. Furthermore, each of our replicates was going into the water bath when it was ready, this meant they all went in at irregular times. Again, we had a system in place that allowed us to keep track of each tube and take readings from each tube at 5-minute intervals as planned, but there were up to 18 tubes in the water bath at one time and so this could potentially have led to incorrect recording of results. Finally, in the interest of time, we did not perform a third cell count on the mixtures once we attempted equalizing all the cell densities. We are confident in our equations, thus we likely arrived at correct cell densities, but if any error occurred in the pipetting of volumes into the mixtures, these were not picked up and could have led to results that were not down to the sugar type used alone, but to variety in the number of yeast cells present.

Potential sources of variation between our results and results from previous studies include the aforementioned shorter time frame we used compared to other studies (D'Amore *et al.* 1989). The literature is also often divided on whether or not yeast uses glucose faster than fructose in media where the sugars are present in isolation. It has been suggested that differences in results are down to different concentrations of sugars used

in different experiments (D'Amore *et al.* 1989; Cason and Reid, 1987). In order to be more confident in rejecting or failing to reject our first null hypothesis we would repeat the comparative monosaccharide experiment again, perhaps at a range of sugar concentrations. A final source of variation could be down to the factor that we chose to measure. We recorded volume of CO_2 however other studies chose to measure the changing concentration of sugars in media over time (D'Amore *et al.* 1989). This may have led to slight variation in results and consequent conclusions.

Conclusion

Our studies demonstrate that *Saccharomyces cerevisiae* produce carbon dioxide at the same rate using glucose and fructose when the sugars are present in isolation in the media. Moreover, within the one-hour time frame, sucrose was metabolized, while maltose was not. Finally, monosaccharides produced carbon dioxide at a significantly faster rate than disaccharides within the one-hour time frame of our study.

Acknowledgements

References

Cason, D. T., and Reid, G. C. 1987. On the differing rates of fructose and glucose utilization in *Saccharomyces cerevisiae*. Journal of the institute of brewing, **93** (1): 23-25.

D'Amore, T., Russell, I., and Stewart, G. 1989. Sugar utilization by yeast during fermentation. Journal of Industrial Microbiology, **4** (4): 315-324.

De La Fuente, G., and Sols, A. 1962. Transport of sugars in yeasts: II. Mechanisms of utilization of disaccharides and related glycosides. Biochimica et Biophysica Acta, **56**: 49-62.

Fugelsang, K. C. 2007. Wine microbiology: Practical applications and procedures. Chapter 8: Fermentation and post-fermentation processing. pp. 115-138. New York, NY: Springer.

Jiang, H., Medintz, I., Zhang, B., and Michels, C. A. 2000. Metabolic Signals Trigger Glucose-Induced Inactivation of Maltose Permease in *Saccharomyces.* Journal of Bacteriology, **182** (3): 647-654.

Lagunas, R. 1993. Sugar transport in *Saccharomyces cerevisiae*. FEMS Microbiology Letters, **104** (3): 229-242.

Oliver, S. G. 1997. Yeast as a navigational aid in genome analysis. Microbiology, **143**: 1483-1487.

Pronk, J., Steensmays, Y. and Van Dijkent, J. 1996. Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast, **12**: 1607-1633.