Carbon dioxide production in wild type and *PDC1* mutant *Saccharomyces cerevisiae* undergoing the Crabtree effect

Mahsa Kabolizadeh, Brahmjot K. Parhar, Masoud Pourrahmat, Yusra Qaiser

Abstract

The production of carbon dioxide (CO_2) was measured under various glucose concentrations in mutant yeast, *Saccharomyces cerevisiae* (YLR044) and wild type yeast by the use of respirometers. We investigated the effect of high glucose concentrations on yeast cells—referred to as the Crabtree effect. Three different glucose treatments were tested; the concentration found in the growth medium (0.11 M), the optimal concentration for gas production (0.50 M) and the concentration at which the Crabtree effect takes place (1.00 M). We also determined how much CO_2 was produced by the wild type yeast compared to the mutant under high glucose concentrations ($\geq$ 0.50 M). The Crabtree effect was observed in the wild type yeast as 3.4 times less CO_2 was made per cell at 1.00 M glucose than at 0.11 M. This effect may be due to an evolutionary adaptation in yeast cells to allow competition with other microorganisms for food. The Crabtree effect was not observed in the mutant yeast even though 1.4 times less CO_2 was made per cell at 1.00 M glucose than at 0.11 M because this difference was not significant. The final CO_2 concentration for mutant and wild type yeast cells show that there was a greater production of CO_2 in wild type as compared to mutant yeast, which is presumed to be the result of the low pyruvate decarboxylase activity in the *PDC1* mutants.

Introduction

Saccharomyces cerevisiae, commonly known as yeast, are eukaryotic unicellular organisms that belong to the fungi kingdom. Yeast cells contain approximately 5773 protein coding genes and approximately 40 percent of these genes are found within the human genome (Sherman 2002). Normal haploid cells of yeast take approximately 90-140 minutes to divide by budding in a Yeast Extract Peptone Dextrose (YPD) medium (Sherman 2002). This medium is used for normal growth at an optimal temperature of 30ºC (Sherman 2002).

Yeast cells use glucose as a source of energy and metabolize the molecules to CO_2, water, and ethanol (Figure 1). Yeast cells can metabolize glucose by using two different pathways; aerobic and anaerobic (Pronk *et al.* 1996). The aerobic pathway uses oxidative phosphorylation to break down pyruvate, the by-product of glycolysis, into CO_2 and water in

order to produce energy in the form of adenosine triphosphate (ATP) (Pronk *et al.* 1996). The anaerobic pathway uses pyruvate decarboxylase to decarboxylate pyruvate molecules to acetaldehyde. Then, pyruvate dehydrogenase breaks down acetaldehyde into the products of anaerobic fermentation; ethanol and CO_2 (Pronk *et al.* 1996). Yeast cells use respiration or fermentation depending on the environment they are located in (Pronk *et al.* 1996).

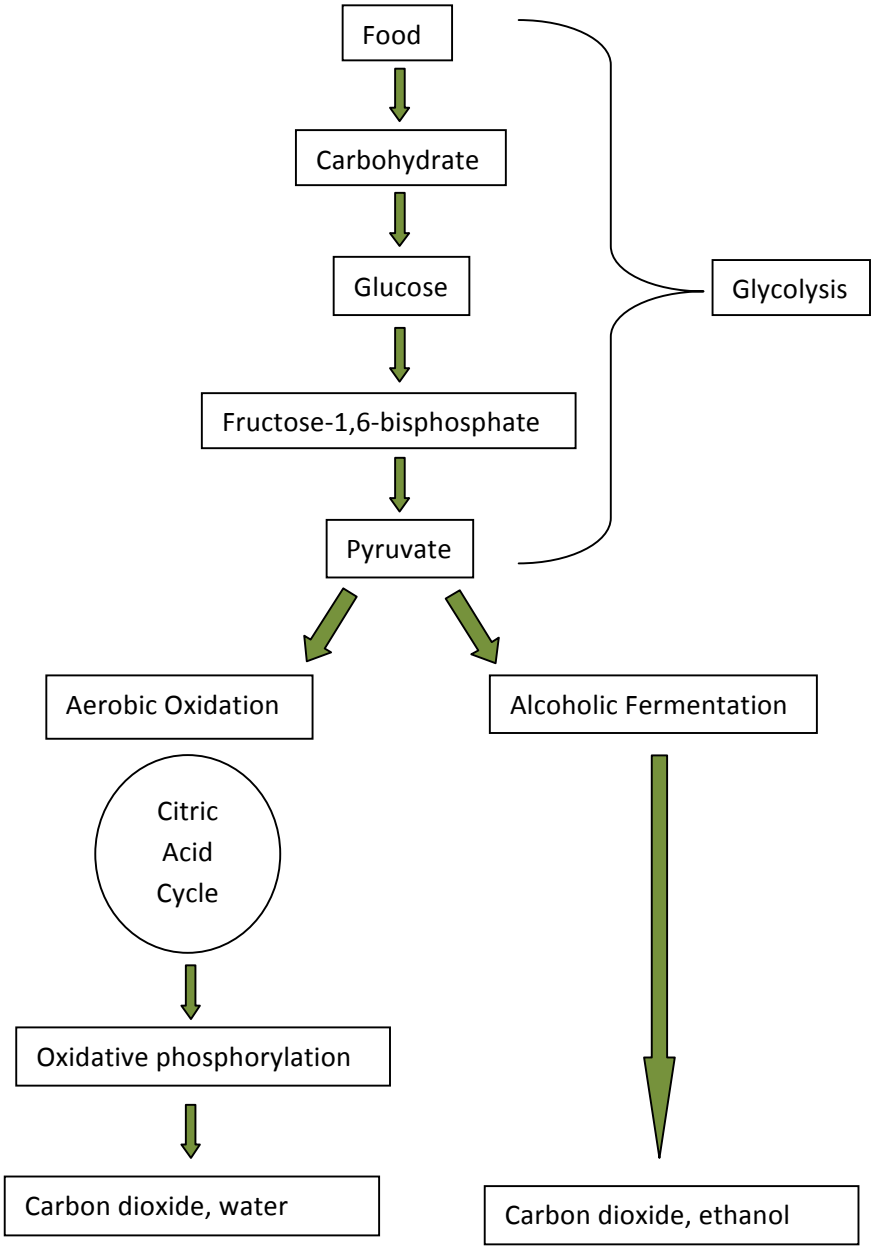

Figure 1. Glycolysis and the two metabolic pathways involved in pyruvate breakdown.

At low glucose concentrations (0.16 M and lower), the rate of respiration is increased and the rate of fermentation is decreased (Deken 1965). At concentrations above 0.16 M, there is a repression of respiratory enzymes inhibiting the rate of respiration and enabling glucose to be metabolized via fermentation (Deken 1965). This repression is referred to as the Crabtree effect (Deken 1965, Meijer *et al.* 1998). Glucose metabolism via fermentation in aerobic environments is a very slow process; however, the rate increases exponentially at high glucose concentrations (Deken 1965).

The mutant used in this experiment had an insertion mutation affecting the *PDC1* gene. *PDC1* is a key gene in the production of the pyruvate decarboxylase enzyme (Hohmann and Cederberg 1990). This enzyme is essential in the fermentation process as it decarboxylates pyruvate into acetaldehyde, which is then dehydrogenated to ethanol and CO_2 by pyruvate dehydrogenase. The *PDC1* mutation inhibits or slows down the rate of fermentation due to the decreased activity of the PDC1 enzyme (Hohmann and Cederberg 1990).

The objective of this experiment is to further investigate the Crabtree effect on mutated yeast cells by measuring the amount of CO_2 produced. Much research has been done regarding yeast cells and the Crabtree effect, however there is not much information regarding yeast cells with a mutated pyruvate decarboxylase gene. This experiment investigates the effects of the mutated *PDC1* gene in yeast with respect to the production of CO_2.

Our first alternate hypothesis is that according to the Crabtree effect, high glucose concentration ($\geq$ 0.5 M) will lead to a decrease in the production of CO_2 in the wild type *Saccharomyces cerevisiae*. Our null hypothesis is that high glucose concentration will lead to a greater production or no change of CO_2 production in the wild type *Saccharomyces cerevisiae*.

Our second alternate hypothesis is that according to the Crabtree effect, high glucose concentrations (≥ 0.5 M) will lead to a decrease in the production of CO_2 in *PDC1* mutant *Saccharomyces cerevisiae* (YLR044). Our null hypothesis is that high glucose concentration will lead to a greater production or no change in CO_2 production in the *PDC1* mutant *Saccharomyces cerevisiae.*

Although the *PDC1* gene that codes for the anaerobic pathway enzyme is mutated, there is still pyruvate decarboxylase activity due to the presence of PDC5. However this enzyme is 20 percent less active than PDC1. Thus a small amount of CO_2 should be produced at high glucose concentrations by the mutant yeast cells, but the amount of CO_2 formed per cell should be less than the wild type strain (Hohmann and Cederberg 1990).

Methods

The mutant and wild type yeast cells were kept in a flask at room temperature (approximately 25ºC) with aluminum foil covering the opening to prevent contamination. We were provided YPD yeast culture with 0.11 M glucose. We tested three different concentrations of glucose, 0.11, 0.50, and 1.00 M on the wild type and yeast strains. The optimal glucose concentration for production of CO_2 by the wild-type strain is 0.50 M, whereas 1.00 M concentration of glucose is ideal for observing the Crabtree effect (C. Pollock, Lab Instructor, personal communication). We also had negative control treatments (without yeast cells) at 0.11, 0.50, and 1.00 M. There were four replicates for each treatment of both strains.

We used yeast culture with a cell concentration of 10^7 cells/mL, and this concentration was determined by using a haemocytometer. We aimed for a cell count of magnitude 10^8 - 10^9 cells/mL because this is the optimal cell count for observing CO_2 production (C. Pollock, Lab Instructor, personal communication). If the cell count was too low, we concentrated the cells by

performing a centrifugation on both yeast strains at maximum speed for 5 minutes. Approximately 800 mL of wild type yeast culture and 480 mL of mutant yeast culture were centrifuged because the cell count was not close to the optimal magnitude. Both yeast cultures were suspended in 140 mL of YPD medium after centrifugation. The pellet was mixed with the yeast growth medium at speed 6 on a vortex mixer for both yeast cultures.

We weighed 3.50 g of glucose and added it to 50 mL of YPD culture to make the 0.5 M treatment, and added 8.01 g of glucose to 50 mL culture to make the 1.0 M treatment. After adding the glucose we thoroughly vortexed the yeast solution to ensure complete mixing.

Yeast solutions were added into a respirometer to monitor the CO_2 recovery. The small test tubes were marked with 0.5 mL increments using a micropipette as shown in Figure 2. The large 20 mL test tube was inked 2 cm from the bottom to indicate the point to which the yeast solution should be added. The respirometers were placed in a water bath at a temperature of 30ºC as shown in Figure 3. We noted the time each respirometer was placed in the rack and recorded observations for initial bubbling indicating rapid CO_2 production. We observed the production of CO_2 for 40 minutes but recorded the volume every 5 minutes to ensure consistency. After conducting a trial experiment, we deduced that 40 minutes was an ideal length of time for observing CO_2 production. When the murky yeast solution exceeded the 2 cm line on the large test tube, we found it difficult to measure the CO_2 production because it was hard to see the increments on the small test tube. Therefore we removed the excess yeast until it levelled with the 2 cm mark on the larger test tube.

Figure 2. Small test tube labelled with 0.50 mL increments.

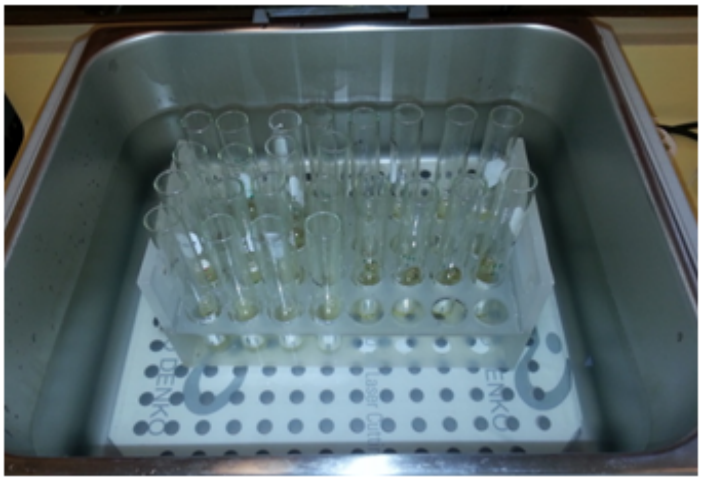

Figure 3. The set-up of the respirometers in the 30ºC hot water bath.

The amount of CO_2 produced after 40 minutes from each set of replicates was averaged for both mutant and wild type, and this average was divided by the total number of cells used in the mutant and wild type yeast cultures, respectively. The 95% confidence intervals were calculated for CO_2 production per cell for both wild type and mutant to see significance between each concentration of glucose. A two-sample t-test was conducted to determine significance between the means of the wild type and mutant, at $\alpha = 0.05$.

Results

There was no significant difference in CO_2 production between the 0.11 M and 0.50 M glucose concentrations for the wild type yeast since the confidence interval of 0.50 M overlaps the mean of the 0.11 M (Figure 4). However, there was a significantly lower amount of CO_2

made at 1.00 M glucose compared to the 0.11 M, in particular 3.4 times less CO_2 was made per cell. The overall trend for the wild type is that as glucose concentrations increase the production of CO_2 per cell decreases.

For the mutant yeast, none of the CO_2 amounts made per cell was significantly different from each other but there was a slight decrease in CO_2 production as glucose concentration was increased, in particular 1.4 times less CO_2 was made per cell at 1.00 M compared to 0.11 M.

There was no CO_2 produced in the procedural controls of each glucose concentration. The values obtained for the treatments were the direct result of yeast cells producing CO_2 and not any external factors (i.e. a possible reaction of glucose with the components of the growth medium).

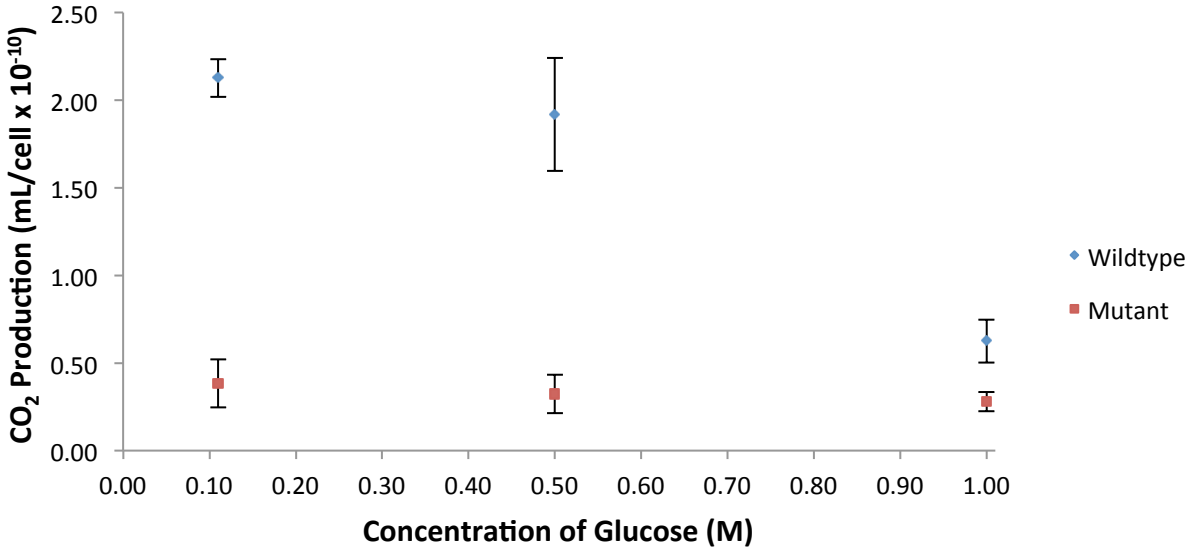

Figure 4. Mean amount of CO_2 produced per cell at 0.11, 0.50, and 1.00 M glucose concentrations for the wild type and mutant yeast, *S. cerevisiae*, after 40 minutes. Error bars represent 95% confidence intervals. The differences in mean CO_2 production between wild type and mutant are significant (P-value < 0.05, n=4).

A greater amount of CO_2 was produced by the wild type yeast cells (Figure 4). A two-sample t-test was done on the means of the mutant and wild type at each concentration, and it

was observed that the t-values were higher than the critical t-value. This indicates that the P-values are less than 0.05; therefore, there is a significant difference between the means of wild type and mutant at each concentration.

Sample calculations

Glucose concentrations

Existing concentration of glucose = $\left(\frac{20\text{ g}}{\text{L}}\right)\left(\frac{1\text{ mol}}{180.16\text{ g}}\right) = 0.11\text{ M}$

To make the glucose concentration of 50 mL of yeast to 1.00 M:

$$1.00\text{ M} = \left(\frac{1\text{ mol}}{\text{L}}\right)\left(\frac{180.16\text{g}}{1\text{ mol}}\right) = 180.16\frac{\text{g}}{\text{L}}$$

Existing concentration + x = Desired concentration, where x is the amount of glucose to add to the yeast.

$$\frac{20\text{ g}}{\text{L}} + \left(\frac{\text{x g}}{0.050\text{ L}}\right) = \frac{180.16\text{ g}}{\text{L}}$$

$$\text{x} = \left(\frac{180.16\text{ g}}{\text{L}} - \frac{20\text{ g}}{\text{L}}\right)(0.050\text{ L}) = 8.01\text{ g}$$

Number of cells producing the CO_2 inside the small test tube of respirometer (wild type yeast cells at 0.11 M glucose)

$$\text{Number of cells} = \left(\frac{2.85\text{ x }10^9\text{cells}}{\text{mL}}\right)(4\text{ mL}) = 1.14\text{ x }10^{10}\text{ cells}$$

Amount of CO_2 per cell (wild type yeast cells at 0.11 M glucose)

Replicate 1 produced 2.6 mL of CO_2,

$$\text{Amount of CO}_2\text{ per cell} = \frac{2.6\text{ mL}}{1.14\text{ x}10^{10}\text{cells}} = 2.281\text{ x }10^{-10}\frac{\text{mL}}{\text{cell}}$$

95% Confidence Intervals for mean CO_2 production per cell (wild type yeast cell at 0.11 M glucose)

Mean CO_2 per cell, $\bar{\mathbf{Y}} = \frac{(2.281 + 2.105 + 2.018 + 2.105)\text{x }10^{-10}}{4} = 2.127\text{ x }10^{-10}\frac{\text{mL}}{\text{cell}}$

Variance, $s^2 = \frac{\Sigma(Y^2) - n\bar{Y}^2}{n-1}$

$= \frac{\left((2.281^2 + 2.105^2 + 2.018^2 + 2.105^2) \times 10^{-20}\right) - (4)(2.127 \times 10^{-10})^2}{4-1}$

$= 1.21832 \times 10^{-22}$

Standard error in $\bar{Y}$, $SE_{\bar{Y}} = \frac{s}{\sqrt{n}}$

$= \frac{\sqrt{1.21832 \times 10^{-22}}}{\sqrt{4}}$

$= 5.5189 \times 10^{-12}$

95% C. I. $= 1.96 \times SE_{\bar{Y}} = (1.96)(5.5189 \times 10^{-12}) = 1.0817 \times 10^{-11} \frac{mL}{cell}$

Upper limit = $\bar{Y}$ + 95% C.I.

$= 2.127 \times 10^{-10} + 1.0817 \times 10^{-11}$

$= 2.24 \times 10^{-10} \frac{mL}{cell}$

Lower limit = $\bar{Y}$ - 95% C.I.

$= 2.127 \times 10^{-10} - 1.0817 \times 10^{-11}$

$= 2.02 \times 10^{-10} \frac{mL}{cell}$

Two-sample t-test to compare means of the wild type and mutant at 0.11 M glucose

$t = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$

Where $\bar{Y}_1$ is the mean of the wild type values and $\bar{Y}_2$ is the mean of the mutant value.

$s^2 = \frac{\Sigma\left(Y - \bar{Y}_1\right)^2 + \Sigma\left(Y - \bar{Y}_2\right)^2}{n_1 + n_2 - 2}$

$\Sigma (Y - \bar{Y}_1)^2 = ((2.281 - 2.127)^2 + (2.105 - 2.127)^2 + (2.018 - 2.127)^2 + (2.105 - 2.127)^2) \times 10^{-20})$

$= 3.65497 \times 10^{-22}$

$\Sigma (Y - \bar{Y}_2)^2 = 5.88235 \times 10^{-22}$

$s^2 = \frac{3.65497 \times 10^{-20} + 5.88235 \times 10^{-22}}{4+4-2} = 1.5895522 \times 10^{-22}$

$$t = \frac{\bar{Y}1 - \bar{Y}2}{\sqrt{s^2\left(\frac{1}{n1}+\frac{1}{n2}\right)}} = \frac{2.127 \times 10^{-10} - 3.824 \times 10^{-11}}{\sqrt{1.5895522 \times 10^{-22}\left(\frac{1}{4}+\frac{1}{4}\right)}} = 19.57$$

Critical t-value for a two-sided test at 95% confidence level with 6 degrees of freedom = 2.447. t > t-critical, therefore P-value < 0.05 and we conclude that the means of the wild type are significantly different from the mutant.

Discussion

Following the analysis of our results, we rejected our first null hypothesis that at high glucose concentrations CO_2 production will remain the same or that there will be an increase in CO_2 production in the wild type yeast, *S. cerevisiae*. Therefore, we provided support for our alternate hypothesis that the wild type yeast will produce less CO_2 at high glucose concentrations (≥ 0.50 M).

We failed to reject our second null hypothesis that at high glucose concentrations CO_2 production will remain the same or that there will be an increase in CO_2 production in the mutant yeast, *S. cerevisiae*. Therefore, we could not provide support for our alternate hypothesis that the mutant yeast will produce less CO_2 at high glucose concentrations (≥ 0.50 M).

The difference between means of the wild type and mutant were significantly different, with the mutant yeast producing less CO_2 than the wild type. This was an expected result, as the *PDC1* mutant has reduced fermentative metabolism and this results in a lower amount of CO_2 being made (Hohmann and Cederberg 1990).

As explained in the introduction, when yeast cells are in a high glucose environment they utilize their fermentative pathway to produce CO_2 and ethanol (Deken 1965). Because the pyruvate obtained from glycolysis is not sent to the tricarboxylic acid cycle in this process, less CO_2 should be made via fermentation than oxidative phosphorylation (Pronk *et al.* 1996). That is the trend seen for the wild type yeast in Figure 4, where at 1.00 M glucose significantly less CO_2

is produced than at 0.50 and 0.11 M. However, the amount of ATP produced from one mole of glucose via fermentation is much less than that produced via oxidative phosphorylation (Nelson and Cox 2008). Thus a question that arises from this switch to fermentation is why would yeast opt for a process that is less efficient than oxidative phosphorylation in producing ATP? This may be a possible evolutionary adaptation in yeast to ward off other microorganisms from using its food source.

A product of fermentation is ethanol, which is toxic to many microorganisms, but relatively nontoxic to yeast (Thomson *et al.* 2005). Under high glucose concentrations the increased use of the fermentation process allows for the accumulation of ethanol, and it has been proposed that this accumulation is employed by yeast to protect its carbon source from ethanol sensitive microorganisms (Thomson *et al.* 2005). This adaptation may have occurred around the Cretaceous period (145-65 million years ago) when fleshy, high sugared fruits first began to grow and yeast and other microorganisms started using these fruits as a carbohydrate source (Thomson *et al.* 2005).

Regarding the mutant yeast, CO_2 production did decrease slightly at higher glucose concentrations, but these decreases were not significant and this led us to not reject our second null hypothesis. Considering that pyruvate decarboxylase is an essential enzyme in fermentation, a non-functioning pyruvate decarboxylase would lead to very small or no CO_2 production—potentially leading to cell death (Pronk *et al.* 1996, Dijken *et al.* 1993). However, CO_2 was produced and the reason for this lies in the discovery of a gene very similar to *PDC1*, known as *PDC5* (Hohmann and Cederberg 1990). This gene codes for the PDC5 protein, an isoenzyme of PDC1, which catalyzes the same reaction as PDC1 and is activated by high glucose concentrations in the same way (Seeboth *et al.* 1990). Hohmann and Cederberg (1990) report

that when *PDC1* is mutated *PDC5* is activated and pyruvate decarboxylase activity is still present. However, 80 percent of normal pyruvate activity is observed when only *PDC5* is activated (Hohmann and Cederberg 1990). Since the PDC5 enzyme can function like PDC1, it would be expected that PDC1 mutant yeast cells would show the same trend as the wild type. Thus, mutant yeast cells would be expected to have decreasing levels of CO_2 at higher glucose concentrations (Seeboth *et al.* 1990, Nelson and Cox 2008).

However this expectation was not observed in this experiment—there was no difference in CO_2 production when glucose concentration was increased to 0.50 M or 1.00 M from 0.11 M. An explanation for this may be found in the regulatory role of ethanol on *PDC5*. What may have happened in these mutant yeast cells is that the ethanol produced decreased the activity of *PDC5* in order to prevent ethanol from reaching toxic levels (Seeboth *et al.* 1990). Although yeast cells are relatively resistant to ethanol, they do have a tolerance limit of 20 g/L (Morais *et al.* 1996). Such high concentrations of ethanol are unlikely to been produced in this experiment and so a more likely reason may be that the equipment used in this experiment was not sensitive enough to detect the changes in CO_2 production of the mutant yeast.

One limitation of this study was the way the CO_2 was measured. Although a micropipette was used to make the marking on the small test tube of the respirometer, only 0.50 mL intervals were made (Figure 2). It would have been too time consuming to measure out smaller intervals (for example 0.10 mL) for 24 test tubes. The consequence of this was that when the CO_2 level was between two intervals, the measurement was slightly subjective; for example, a measurement of 2.2 mL could have been 2.3 mL. A future consideration would be to use a more sensitive instrument, such as filling a syringe halfway, and measuring the amount by which the syringe moves.

An assumption made was that the number of yeast cells stayed the same during the time measurements were taken. This is because it takes approximately 90-140 minutes for yeast cells to grow, however the experiment was conducted in 40 minutes (Sherman 2002). But yeast cells can be at different stages of development and some yeast cells could have been growing and reproducing throughout the experiment. This would have increased the cell number thus leading to slightly more CO_2 production in each treatment. Also, it was assumed that the concentrations of glucose stayed the same throughout the experiment, but yeast metabolized the glucose and therefore its concentration would have decreased over time. Therefore, glucose was being used up, so the actual measurements of CO_2 would have decreased slightly over time.

A possible future experiment could be to measure the ethanol production instead of CO_2 production. CO_2 is produced by both oxidative phosphorylation and fermentation therefore the exact switch to fermentation cannot be discerned from the CO_2 production data. Ethanol is not a product of oxidative phosphorylation, so using ethanol production as the response variable can provide information such as at what concentration of glucose yeast cells begin fermentation.

Conclusion:

When the concentration of glucose increased in wild type *S. cerevisiae,* the production of CO_2 decreased due to a decrease in oxidative phosphorylation and an increase in fermentation. However the same trend seen in the wild type yeast cells was not observed for the mutant as there was no significant difference in CO_2 production between different glucose concentrations. Consequently, we rejected our first null hypothesis, whereas we failed to reject our second null hypothesis.

Acknowledgements

We would to like to thank Dr. Carol Pollock for approving and providing feedback throughout our experiment, Mindy Chow for preparing our yeast cultures as well as setting up

our equipment, and Katelyn Tovey for providing assistance with data analysis. We would also like to thank The University of British Columbia for providing the opportunity to take Biology 342.

Literature Cited

Deken, R. 1965. The Crabtree effect: A regulatory system in yeast. Journal of General Microbiology, **44**: 140-156.

Dijken, J. P. V., Weusthuis, R. A., and Pronk, J. T. 1993. Kinetics of growth and sugar consumption in yeasts. Antoine Van Leeuwenhoek International Journal of General and Molecular Microbiology, **63**: 343-352.

Hohmann, S. and Cederberg, H. 1990. Autoregulation may control the expression of yeast pyruvate decarboxylase structural genes *PDC1* and *PDC5*. Journal of Biochemistry, **188**: 615-621.

Meijer, M. M. C., Boonstra, J., Verkleij, A. J., and Verrips, C. T. 1998. Glucose repression in *Saccharomyces cerevisiae* is related to glucose concentration rather than the glucose flux. The Journal of Biological Chemistry, **273** (37): 24102-24107.

Morais, P. B., Rosa, C. A., Linardi, V. R., Carazza, F., and Nonato, E. A. 1996. Production of fuel alcohol by *Saccharomyces* strains from tropical habitats. Biotechnology Letter, **18** (11): 1351-1356.

Nelson, D.L., and Cox, M.M. 2008. Lehninger. Principles of biochemistry. W. H. Freeman and Company, New York.

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

Seeboth, P. G., Bohnsack, K., and Hollenberg, C. P. 1990. *pdc10* Mutants of *Saccharomyces cerevisiae* give evidence for an additional structural *PDC* gene: Cloning of *PDC5*, a gene homologous to *PDC1*. American Society for Microbiology, **172** (2): 678-685.

Sherman, F. 2002. Getting started with yeast. Methods in Enzymology, **350**: 3-41.

Thomson, J. M., Gaucher, E. A, Burgan, M. F., De Kee, D. W., Li, T. Aris, J. P., and Benner S. A. 2005. Resurrecting ancestral alcohol dehydrogenases from yeast. Nature Genetics, **37** (6): 630-635.