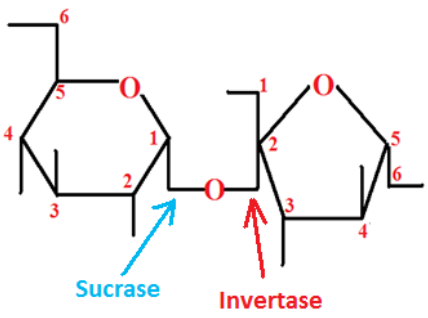
Metabolism: Manometric Measurement of the Fermentation of Sucrose by *Saccharomyces cerevisiae*

## Introduction

Man has been brewing alcoholic beverages for thousands of years (longer than man has been making soap, believe it or not!). Well known liquors, e.g., wine from grapes, vodka from potatoes, whiskey from corn, come from foods with a source of carbohydrate which, while enjoying anaerobic conditions (what are these conditions?), will be metabolized to ethyl alcohol and carbon dioxide.

The source of carbohydrate from plants is, as you recall, starch. As grains begin to sprout (potatoes, as well), enzymes are activated which catabolize (what does this mean?) the starch to glucose. If the process is stopped, e.g., by heating until the "sprouts" are dried, the glucose will not be further catabolized. If the dried residue is rehydrated, has its pH and temperature adjusted to optimal conditions, has yeast added and then the resulting mix isolated from an aerobic atmosphere, then anaerobic fermentation will proceed.

So, what yeast is used for fermenting carbohydrates and what actually happens? The yeast used in this experiment is *Saccharomyces cerevisiae*: common bread-making yeast. The carbohydrate source for this experiment is not glucose, rather it is sucrose: common table sugar, i.e., α-D-glucopyranosyl-α-1→2-α-D-fructofuranoside, and requires hydrolysis for fermentation to proceed. Yeast is the source of invertase. Commercially, invertase is used in candies that have soft centers: the sucrose center is mixed with invertase and then the outer shell is placed around the center. It takes, on average, 2-14 days of storage for the invertase to hydrolyze the sucrose to make the liquid center in the candy.



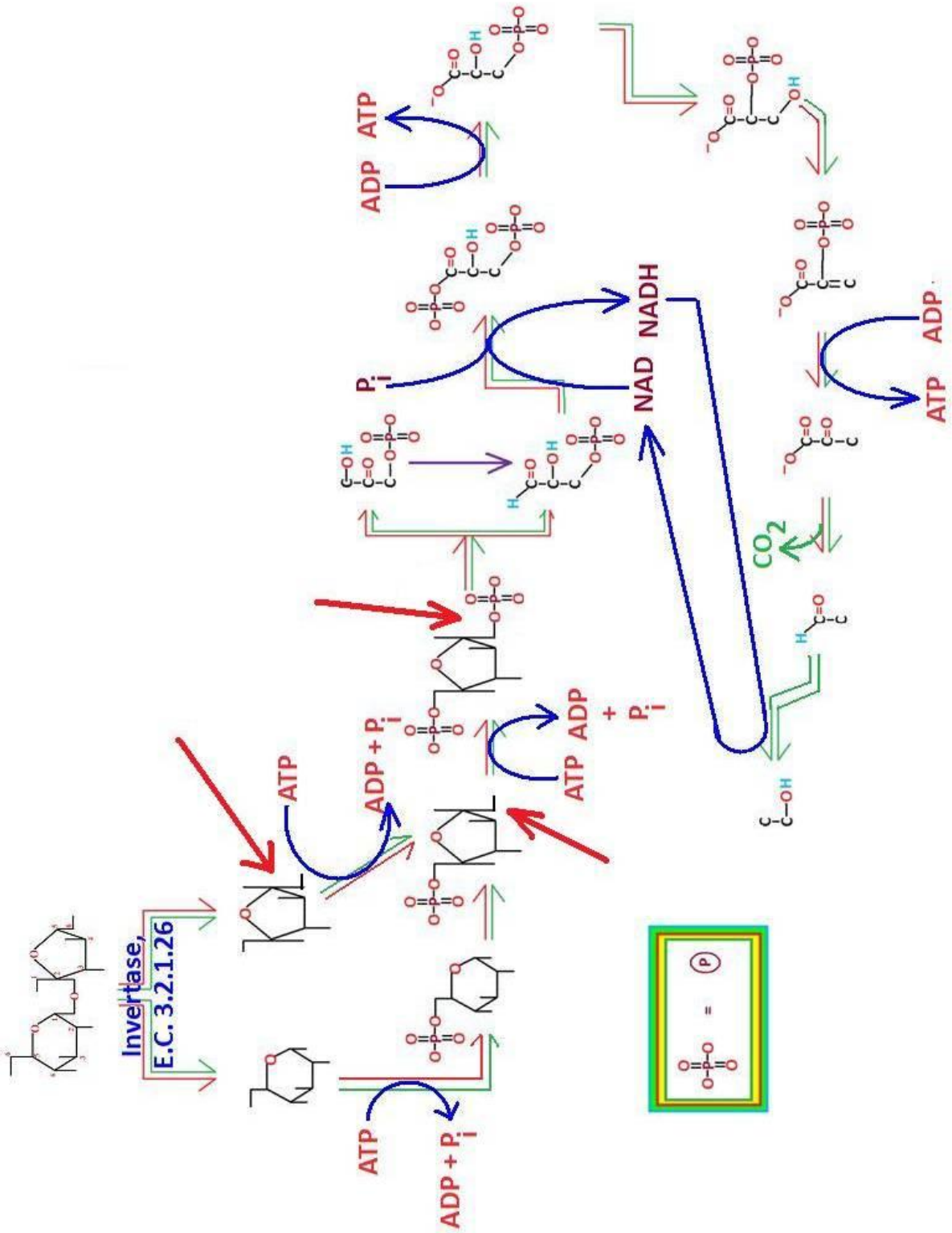

Since a disaccharide is the carbohydrate source for fermentation in this experiment, one would suspect that a disaccharidase would be necessary to initiate the process: and, one would be right: invertase. Invertase (EC 3.2.1.26) is an inter-changeable name for, essentially, sucrase (E.C. 3.2.1.48). Do keep in mind that, while both enzymes yield the both glucose and fructose, their cleavage properties are actually a bit different (figure at right) and, as a result, have different IUB/EC classifications as indicated above.

In addition,

> *Invertase belongs to a class of enzymes known as glycosidases. … these enzymes work by … splitting the bond while other [… enzymes] twist the bond at the same time. These enzymes that twist the bond at the same time as splitting [the molecule] result [in fructose … taking on] a different configuration than when it started so that it is inverted from alpha to beta. … invertase was the first enzyme where this was studied and … is why it was called invertase.* [1]

Furthermore, recent studies [2] have indicated that the role of phosphoglucoisomerase (PGI) in glycolysis (EMP as discussed in lecture) isomerizes α-D-glucose-6-phosphate to β-D-fructose-6-

phosphate, which ties in perfectly with the action of invertase: β-D-fructose is the hydrolytic product (along with α-D-glucose, of course) of invertase action on sucrose. The catabolism of glucose is enzymatically alike between yeast and animals down to pyruvate formation. Once at pyruvate, however, the metabolic similarities under anaerobic conditions cease. In animals, pyruvate is further catabolized to lactic acid (accumulates in muscles and is a cause for cramping). In yeast, the pyruvate is metabolized to ethanol (EtOH) via acetaldehyde and $CO_2$ synthesis and fermentation proceeds anaerobically because the enzymes required to catabolize the glucose are in the yeast. Figure, following page, is the catabolic pathway of EtOH biosynthesis.


Invertase,
E.C. 3.2.1.26
ATP
ADP + P i
ATP
ADP + P i
ATP
ADP + P i
ATP
ADP
P i
NAD
NADH
CO2
ATP
ADP
= P

Note that in the pathway, there are no enzyme names, excepting that of invertase, and there are no co-factors, excepting that of ATP, ADP and NAD/NADH and that there are no names of intermediates. **As part of the assignment for this experiment, label this diagram using information you've learned from lecture for turn in. Don't forget to keep your stoichiometry straight! This will pencil out differently than EMP in lecture, BTW (HINT!).**

The purpose of this experiment is several-fold:

1) To further familiarize the student with carbohydrate metabolism;

2) To reinforce and expand on lecture material for the student's edification;

3) To give the student an opportunity to observe metabolism in action visually (via 'frothing") and instrumentally (using a simple manometer to measure $CO_2$ production);

4) To give the student the opportunity to observe enzyme inhibition under exp[erimental conditions.

## Materials and Methods

### Materials

Students will need to work in groups for this experiment. Each group will need the following supplies:

| | | | | | |
|---|---|---|---|---|---|
| Test tube rack (the larger of the two sizes) | 4-8 disposable Falcon round bottomed tubes | 2 disposable 5 mL pipets | 1 disposable pipet of the bulb-type | 2 auto-pipetters | 1 ring stand (larger of the many sizes) |
| 1 manometer | Bread yeast | Sugar cubes | Timer of some sort | Data sheet (below) | 1-50 mL beaker |
| 1-150 mL beaker | 2 stirring rods | Reagent alcohol | 1-holed rubber stopper with glass tubing | 1-pestle and mortar | |

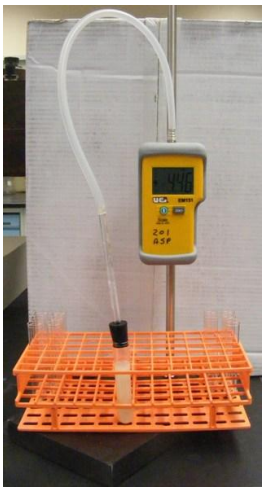
There will be 5 groups as there are only 5 manometers. Each group will need one whole lab bench to spread out on to perform this experiment.

### Methods

Yeast suspension: measure out 3.5 g yeast into a 50 mL beaker and add 50 mL distilled water. Stir **carefully** to make a mixture/slurry. **Stirring with "froth" will denature the enzymes in the yeast**. Set aside.

Sucrose solution: place 8 sugar cubes in a 150 mL beaker and add 80 mL of distilled water. Stir to completely dissolve. Set aside.

Experimental apparatus: assemble the ring stand and obtain the test tube rack and the manometer and 1-holed stopper with glass tubing (this will already be assembled for you). Stick the manometer onto the shaft of the ring-stand (powerful magnet in its back and is why you're using the largest ring stand set-up) and carefully insert the glass tubing into the silicone tubing. It's not necessary to jam it on – insert it ca 3 mm and it will work fine. While holding the stoppered end of the glass tubing in one hand, place the test tube rack on the base of the ring stand with the other and place the stopper in one of the tube positions for support. Set your round bottomed tubes in the test tube rack out of the way of the experiment, but convenient for easy access.



**Read this section first, before you do anything with it.**

Trial 1 -- Control

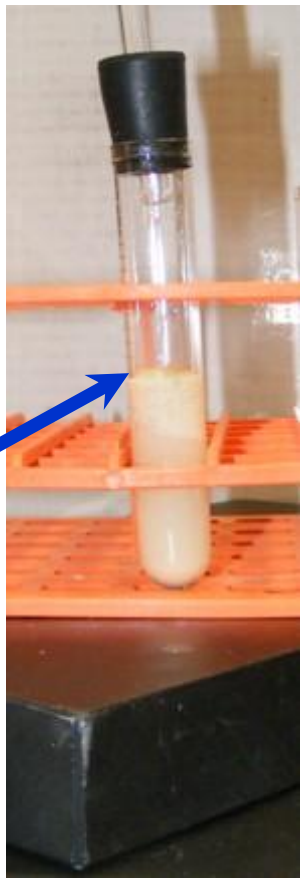
Place one of your round bottomed tubes in the front of the test tube rack closest to you. Assemble one auto-pipetter and pipet 0.5 mL sucrose into it. Expectorate the sucrose into your round bottomed tube. Set the auto-pipetter aside.

Assemble the other auto-pipetter; stir the yeast slurry for re-suspension; auto-pipet 4.5 mL yeast into the pipet. Expectorate the yeast suspension into the round bottomed tube that you placed the sucrose in with a little force – not too much, just enough to mix the two fluids.

Set the second auto-pipetter aside, and, fairly quickly, insert with a twist and some pressure (not a lot!) the stopper from the manometer tubing into the tube (make sure the neck of the tube is NOT cracked or split – this will ruin the experiment). Press the green button on the manometer to turn it on (it should read "0" values and have "mBar" as the units in the upper portion of the screen), start your stop-watch and record the first reading at time zero. Continue recording the pressure readings every 30 seconds for 30 minutes OR until the readout says "OL". "OL" means Over Limit. When "OL" is in the screen, simply take the stopper out of the tube and turn the instrument off.

If the manometer does not read "mBar", press the green button until it does. Your apparatus will look something like the figure at right.

When the trial is completed, remove the stopper and hold the green button down until the screen reads "OFF". Also note that in this trial, you'll be able to observe the effect of $CO_2$ generation, figure at lower right.



Trial 2 – Effect of Solid Sucrose on Fermentation

Place one of your round bottomed tubes in the front of the test tube rack closest to you. Take one (1) sugar cube and place it in the mortar. Pulverize it with the pestle and place the "powdered sugar" into the bottom of a second round bottomed tube.

Use your previously assembled auto-pipetter, pipet 0.5 mL sucrose into the tube (on top of the solid sucrose). Expectorate the sucrose into your round bottomed tube. Set the auto-pipetter aside.

Stir the yeast slurry for re-suspension; use your previously assembled auto-pipetter to auto-pipet 4.5 mL yeast into the pipet. Expectorate the yeast suspension into the round bottomed tube that you placed the sucrose in with a little force – not too much, just enough to mix the two fluids.

Do NOT mix this tube after you've added any liquids to it!

Set the second auto-pipetter aside, and, fairly quickly, insert with a twist and some pressure (not a lot!) the stopper from the manometer tubing into the tube (make sure the neck of the tube is NOT cracked or split – this will ruin the experiment). Press the green button on the manometer to turn it on (it should read "0" values and have "mBar" as the units in the upper portion of the screen), start your stop-watch and record the first reading at time zero. Continue recording the pressure readings every 30 seconds for 30 minutes OR until the readout says "OL". "OL" means Over Limit. When "OL" is in the screen, simply take the stopper out of the tube and turn the instrument off.

If the manometer does not read "mBar", press the green button until it does.

When the trial is completed, remove the stopper and hold the green button down until the screen reads "OFF".

Trial 3 – Effect of EtOH on Fermentation

Place one of your round bottomed tubes in the front of the test tube rack closest to you. Using the one disposable pipet (bulb-type), obtain 1 mL of reagent alcohol (EtOH) and pipet it into the bottom of a second round bottomed tube.

Use your previously assembled auto-pipetter, pipet 0.5 mL sucrose into the tube (on top of the solid sucrose). Expectorate the sucrose into your round bottomed tube. Set the auto-pipetter aside and give the tube a quick couple of flicks to mix the sucrose with the EtOH.

Stir the yeast slurry for re-suspension; use your previously assembled auto-pipetter to auto-pipet 4.5 mL yeast into the pipet. Expectorate the yeast suspension into the round bottomed tube that you placed the sucrose in with a little force – not too much, just enough to mix the two fluids.

Do NOT mix this tube after you've added the yeast suspension to it!

Set the second auto-pipetter aside, and, fairly quickly, insert with a twist and some pressure (not a lot!) the stopper from the manometer tubing into the tube (make sure the neck of the tube is NOT cracked or split – this will ruin the experiment). Press the green button on the manometer to turn it on (it should read "0" values and have "mBar" as the units in the upper portion of the screen), start your stop-watch and record the first reading at time zero. Continue recording the pressure readings every 30 seconds for 15 minutes. After the 15 minute period simply take the stopper out of the tube and turn the instrument off.

If the manometer does not read "mBar", press the green button until it does.

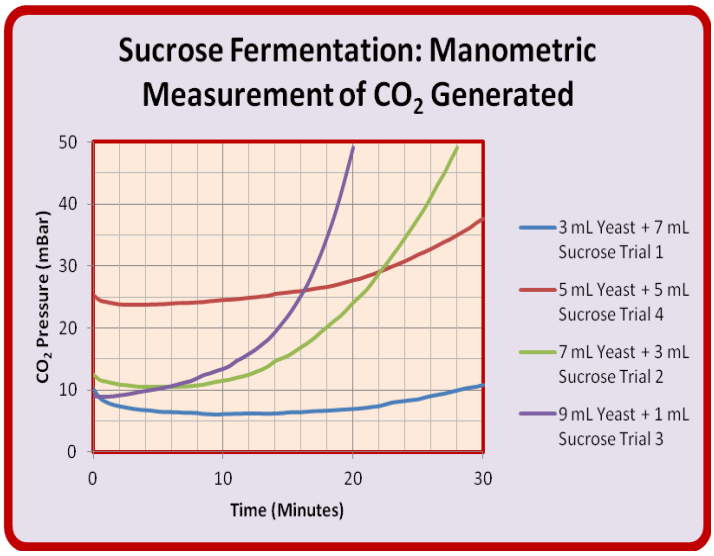
When the trial is completed, remove the stopper and hold the green button down until the screen reads “OFF”.

In all instances, each tube from each trial may be dumped and rinsed down the sink. NOW start your experiment.

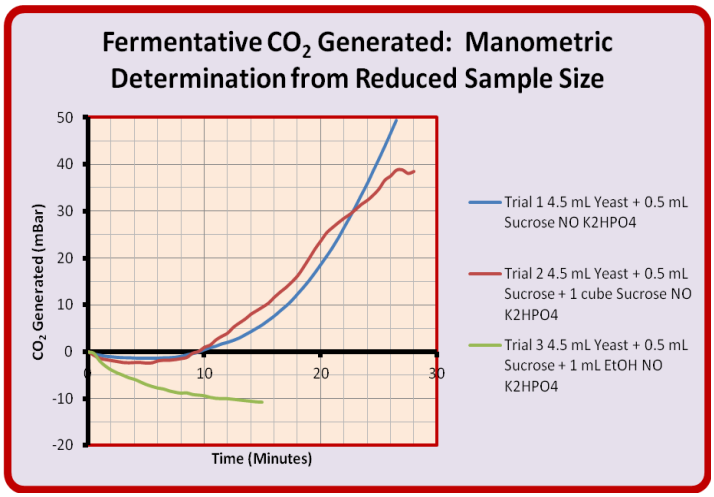
Results

Your results need to be graphed using some spreadsheet program (Excel with smooth scatter plots work quite nicely).

In developing this experiment, several approaches were taken and graphed, image at upper right.




In addition, experimental results for this exact experiment were graphed, image at right.




Your data, when graphed, will resemble this last graphic, i.e., the graphic at immediate right. Attach your graph to your experiment for turn-in. This experiment is another exception to the “turn-it-in-before-you-leave” rule. It is due **at the beginning** of your next lab period and is worth 25 points.

Discussion and Questions

When developing this experiment from scratch, it was necessary to determine what the ideal reaction conditions would be. One usually begins “somewhere” and observes and records data to get a “feel” for which direction to go (Scientific Method: Observe, Test, Hypothesize, Test, Theorize). The approaches indicated in the top right graphic are to simply find under what conditions the invertase will operate optimally with a minimum of reagents. This approach used only yeast, sucrose, water and dipotassium hydrogen phosphate. It was clear, once plotted and analyzed, that the best conditions were to run the experiment with a maximum amount of yeast suspension and a minimum amount of the sucrose solution. Following are student questions to be completed for turn-in.

Student Question: How were these conditions determined?

| Time (Min) | Trial 1 Control | Trial 2 Solid Sucrose | Trial 3 EtOH Addition | Time (Min) | Trial 1 Control | Trial 2 Solid Sucrose | Trial 3 EtOH Addition |
|---|---|---|---|---|---|---|---|
| 0 | | | | 15.5 | | | |
| 0.5 | | | | 16 | | | |
| 1 | | | | 16.5 | | | |
| 1.5 | | | | 17 | | | |
| 2 | | | | 17.5 | | | |
| 2.5 | | | | 18 | | | |
| 3 | | | | 18.5 | | | |
| 3.5 | | | | 19 | | | |
| 4 | | | | 19.5 | | | |
| 4.5 | | | | 20 | | | |
| 5 | | | | 20.5 | | | |
| 5.5 | | | | 21 | | | |
| 6 | | | | 21.5 | | | |
| 6.5 | | | | 22 | | | |
| 7 | | | | 22.5 | | | |
| 7.5 | | | | 23 | | | |
| 8 | | | | 23.5 | | | |
| 8.5 | | | | 24 | | | |
| 9 | | | | 24.5 | | | |
| 9.5 | | | | 25 | | | |
| 10 | | | | 25.5 | | | |
| 10.5 | | | | 26 | | | |
| 11 | | | | 26.5 | | | |
| 11.5 | | | | 27 | | | |
| 12 | | | | 27.5 | | | |
| 12.5 | | | | 28 | | | |
| 13 | | | | 28.5 | | | |
| 13.5 | | | | 29 | | | |
| 14 | | | | 29.5 | | | |
| 14.5 | | | | 30 | | | |
| 15 | | | | | | | |

Student Question: Offer an explanation for the different appearances of the lines in the top right graphic, previous page, for the 3 mL Yeast vs the 9 mL Yeast suspension $CO_2$ generation.

The second graphic on the previous page shows the application of the data from the first graphic, top right, previous page. In these instances, though, only 4.5 mL Yeast suspension were used, as well as 0.5 mL Sucrose.

Student Question: Suggest a reason for why the volumes were altered for this experiment (HINT: wait until after you've completed your experiment to answer this).

When comparing the Control curve in the second graphic with the curve for the solid sucrose, the lines do have a different appearance.

Student Question: Suggest reasons that might explain this phenomenon using what you know about proteins, enzymes and metabolic pathways.

Furthermore, when comparing the EtOH added sample curve to the Control curve, they are remarkably different.

Student Question: Explain the discrepancies between these two lines using what you know about proteins, enzymes and metabolic pathways.

Student Question: In the second graphic did you notice the notation that there was no $K_2HPO_4$ used? Provide two (2) explanations why someone would want to use this compound for a fermentation experiment.

References

Linked in experimental write-up

This page built 16 August 2012.