Pre-lab: Energy Metabolism in Yeast

After reading over the lab, complete the questions below before coming to lab!

1. This week's lab covers glycolysis and cellular respiration. This process is a complex chain of chemical reactions that can be simplified into an overall reaction summary equation. Read the lab, find the summary equation for cellular respiration.

   Cellular Respiration summary equation:

2. State a hypothesis regarding the effects of temperature, substrate concentration, or enzyme inhibitors on the rate of glycolysis in yeast?

3. Explain the difference between fermentation and aerobic respiration

4. Why are we using methylene blue in part II?

Energy Metabolism in Yeast

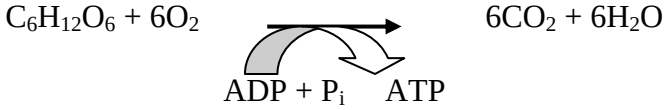
GOALS: After completing this lab, a student should be able to:

- Understand HOW and WHY temperature, substrate concentrations, and inhibitor molecules affect glycolysis and the citric acid cycle in yeast.
- Use the scientific method to investigate biological processes.
- Calculate a rate constant from experimental data

OVERVIEW:

Cellular respiration converts the stored energy from sugar molecules into a more useful form, **ATP**, which is used to fuel chemical reactions throughout the cell. **ATP**, or adenosine triphosphate, is the energy "currency" of the cell, because the cell can't "spend" sugar molecules directly to run its chemical reactions, but instead must convert sugar to a usable form of energy that the cell can use to run its chemical reactions.

Cellular respiration is a complicated process that in a simplified form looks like this:



Most organisms (plants, animals, most protists, and many fungi **including the yeast we will be using today**) use this process of cellular respiration. If oxygen is not available, most organisms can temporarily use anaerobic respiration to gain some ATP, but the efficiency drops dramatically. **Anaerobic respiration** yields only 2 ATP per glucose molecule, whereas **aerobic respiration** (with oxygen) can yield as much as approximately 36 ATP per glucose molecule! **Remember that rapidly growing yeast will deplete oxygen and switch from aerobic respiration to anaerobic respiration (fermentation) over time.** This is because the cells can't get rid of the surplus electrons through the electron transport chain (and thus can't recycle NADH and $FADH_2$ to NAD^+ and FAD^+). In our experiment today we will use an artificial electron acceptor, methylene blue, to help us assess the influence of small organic acids on the rate of respiration

Part I: Glycolysis

In the first part of today's lab we will examine one of the energy yielding reactions of the normal brewing and bakers yeast *Saccharomyces cerevisiae.* **Work in groups of four** to set up an experiment to test your hypotheses regarding the effects of substrate concentrations, temperature, or enzyme inhibitors on glycolysis. **After deciding upon the experimental set up, present your proposal to Ben or Rebecca before you begin your experiment.**

You will have the following solutions and equipment available for your experiment:

- 0.5 M glucose (a normal substrate for glycolysis)
- 0.5 M fructose (another substrate for glycolysis)

- 0.1 M sodium fluoride (the fluoride ion binds Mg++ which is an important cofactor for enzymes used in phosphate transfer reactions during glycolysis)
- 0.5 M sodium citrate (sodium citrate is an inhibitor of the enzyme phosphofructokinase).
- 0.1 M xylose (xylose is a 5-carbon sugar that inhibits glycolysis in the yeast *S. cerevisiae*)

- 10 ml glass pipets (tips sealed), approximately 12 per group
- Clean test tubes
- Test tube racks
- An incubator at 37 degrees
- Ice buckets
- Gauze and rubber bands

General directions for glycolysis reaction setup:

1. Use small lumps of floral clay to seal the tips of the 10 mL pipettes.

2. Add approximately 6 mL of yeast solution to the open end of each of the 10 mL pipettes (up to the number 4). Be sure to insert the tip of the Pasteur pipette *below* the constriction.

3. Add some volume of sugar solution (0 to 5 mLs)

4. If using an inhibitor, add up to 1 mL of inhibitor solution

5. Add water to bring the volume in each pipet to the same level.

6. Cover the open end of each pipette securely with Parafilm and invert several times to mix the yeast and test solutions.

7. Remove the Parafilm. Invert one of the test tubes over the open end of the pipette and quickly invert both. Your instructor will demonstrate this step. A bubble of air will move into the pipette when you do this.

8. Place your "respirometer" (this is what you just made) in the test tube rack. Repeat for the other test solutions.

9. Allow 10 minutes for the respirometers to equilibrate. Take a reading from each pipette at this time

(Time 0) and about every 5 minutes for 35 to 45 minutes. Remember that each space on the pipette equals 0.1 mL. You want to take the DIFFERENCE between the initial reading (Time 0) and each subsequent reading (mL at Time0 - mL at Time1; mL at Time0 - mL at Time2, etc.). Record this data in a table.

Experimental Setup: (fill in the table with your proposed test conditions) **Checked_____**

Hypothesis:

Prediction:

Respirometer #	Yeast suspension	Sugar solution	Inhibitor	Water	Incubation Temp.
1	6 mL	5 mL Glucose	none	1 mL	
2	6 mL	None	none	6 mL	
3	6 mL				
4	6 mL				
5	6 mL				
6	6 mL				
7	6 mL				
8	6 mL				
9	6 mL				
10	6 mL				
11	6 mL				
12	6 mL				

Volume of CO_2 produced (NOTE: This table is not complete)

Respirometer #	T_0	T_1	T_2	T_3	...
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

Part II: Citric Acid Cycle

Examination of succinic acid dehydrogenation.

In the second part of today's lab we will examine another energy yielding reaction of the normal brewing and bakers yeast *Saccharomyces cerevisiae.* One of the reactions in the citric acid cycle is the dehydrogenation of succinic acid to produce fumaric acid. One of the first discoveries about this cycle was that certain small organic acids increased the rate of aerobic respiration. Other small acids did not speed the reaction and instead acted as inhibitors. We will check this with our yeast suspensions today. The added methylene blue will act as an artificial indicator and electron acceptor. Oxidized methylene blue is blue, but when reduced it becomes colorless. Here we will use it in place of the normal electron acceptor FAD. So, the speed at which the blue color disappears is an indication of the metabolic rate of the yeast (or at least the activity of the citric acid cycle).

Work in groups of four to set up an experiment to test your hypotheses regarding the effects of temperature, succinic acid, and/or malonic acid on cellular respiration. **After deciding upon the experimental set up present your proposal to Ben or Rebecca before you begin your experiment.**

You will have the following solutions and equipment available for your experiment:

- 0.5 M glucose
- 0.5 M fructose
- 0.5 M succinic acid
- 0.5 M malonic acid
- Methylene Blue
- Mineral oil
- Clean test tubes
- Test tube racks
- An incubator at 37 degrees
- Ice buckets

General Experimental Setup

1. Obtain and label 8 small test tubes.

2. Prepare tubes as outlined in the next table. (you will decide with your team what to add to tubes E through H)
3. Add 100 μl of 0.1% methylene blue to each tube and mix well. The solution should be a light blue in color.
4. Hold each tube at a slight angle and add 500 μl mineral oil to form a thin film over the top of the solution. This will slow the diffusion of oxygen into the system.

5. Place tubes in 37° C incubator and observe at 5 minute intervals. Note any color changes.

Experimental Setup for citric acid cycle **Checked _____**

Hypothesis:

Prediction:

Tube #	Yeast suspension	glucose	fructose	succinic acid	malonic acid	water	Incubation Temp.
A	-	-	-	-	-	3 mL	37° C
B	1 mL	1 mL	-	1 mL	-	-	37° C
C	1 mL	1 mL	-	1 mL	-	-	37° C
D	1 mL	1 mL	-	0.5 mL	0.5 mL	-	37° C
E	1 mL						
F	1 mL						
G	1 mL						
H	1 mL						

Record the length of time it took each tube to turn from blue to colorless:

Tube #	Time to colorless (minutes)
A	
B	
C	
D	
E	
F	
G	
H	

LAB WRITEUP

After completing your experiment on **yeast glycolysis**, answer the following questions:

1. For each trial you completed, create a spreadsheet with columns of concentration, natural log of concentration, one-divided by concentration, and time. Attach the spreadsheets to this packet.

2. Create a graph of gas concentration verses time for one of your trials. Describe the linearity of the curve. When was the instantaneous rate fastest? Slowest? Attach your graph to this packet.

3. For each trial you completed, determine the rate constant using graphical methods similar to the kinetics CAL workshop. (In the workshop you examined linearity for the two simplest relationship cases. In this lab write-up you will look at the three simplest relationship cases.) Create three graphs for each trial: one of concentration verses time, one with natural log of concentration verses time, and one with one-divided-by-concentration verses time. Be sure to include the R^2 value and the equation of the linear trend line on your graphs. Briefly explain how you identified the graph to use for rate constant determination. Attach all of your graphs to this packet.

4. How was the rate constant affected by your changing variable? (This answer could be different based on what your experiment was designed to test: temperature? Inhibitors? Etc.)

5. What were the conditions in your experiment that allowed the maximum and minimum amount of glycolysis to occur?

6. Were your hypotheses supported or rejected?

After completing your experiment on the **citric acid cycle**, answer the following questions:

7. Which conditions led to the fastest conversion? Which conditions were the slowest?

8. Look up the structures of succinic and malonic acids. Are there any similarities?

9. Give the equation for the reaction of the citric acid cycle that is examined here. What is the product?

10. Why was it important to block oxygen diffusion for this experiment?