Respiration and Fermentation

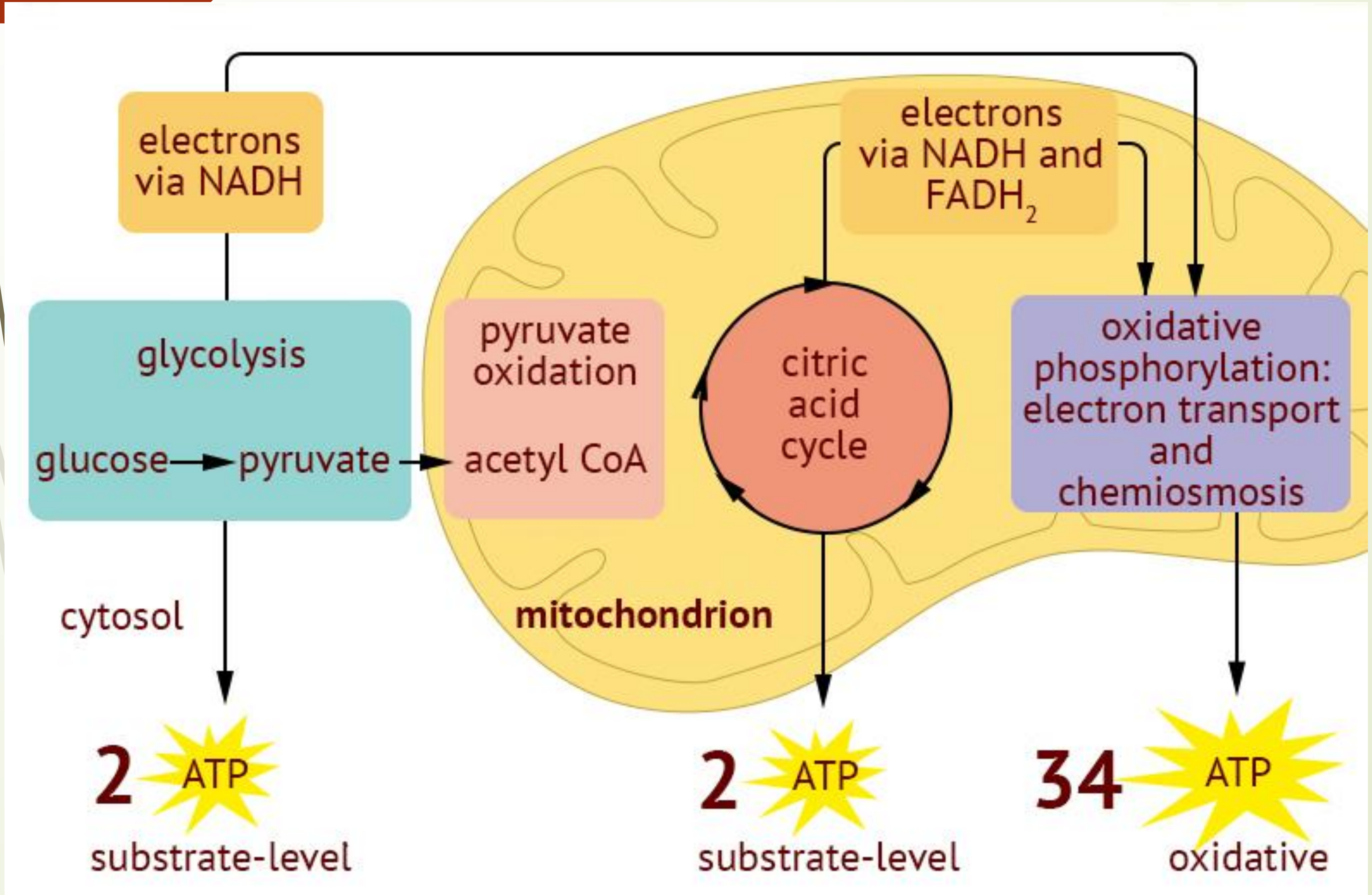
Objectives

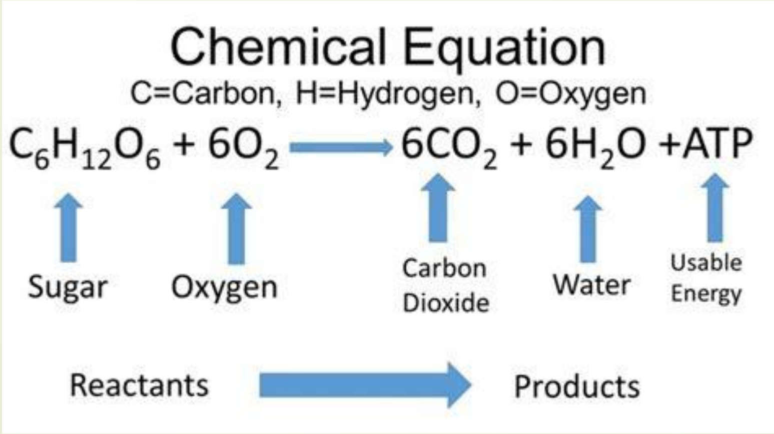
- Determine the rate of cellular respiration by measuring oxygen consumed
- Determine how temperature affects respiration rate
- Describe how yeast metabolizes sugar under differing environmental parameters

Safety

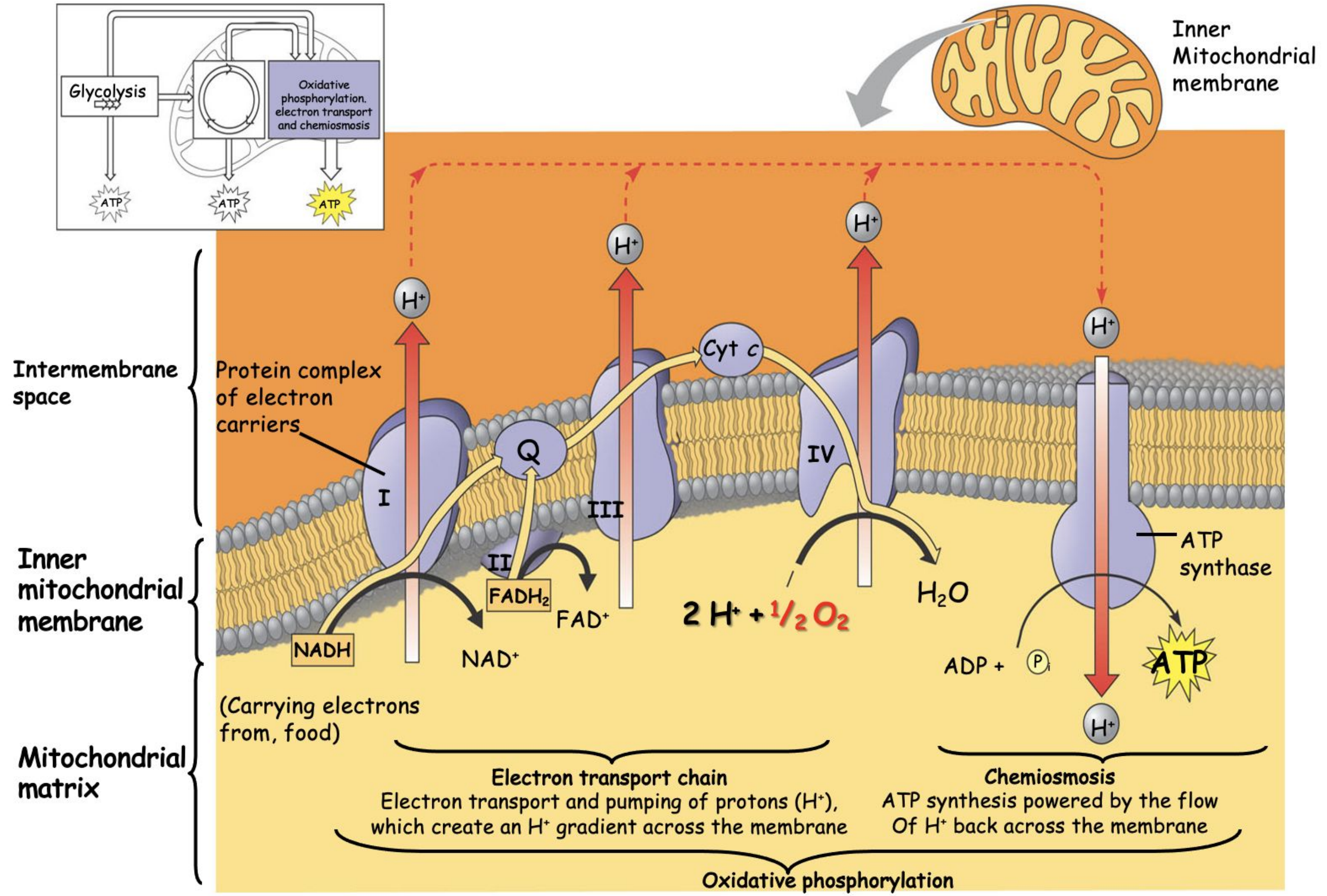
- **Working in groups of 4**
- **Gloves, Goggles, Closed-toe shoes REQUIRED**
- Tip discard containers are for TIPS ONLY!
- Potassium hydroxide soaked cotton balls are disposed of in the **base waste container**
- Yeast and sodium azide must be disposed of in the **toxic waste container** – rinse water, as well!
- Wash hands with soap and water when finished!

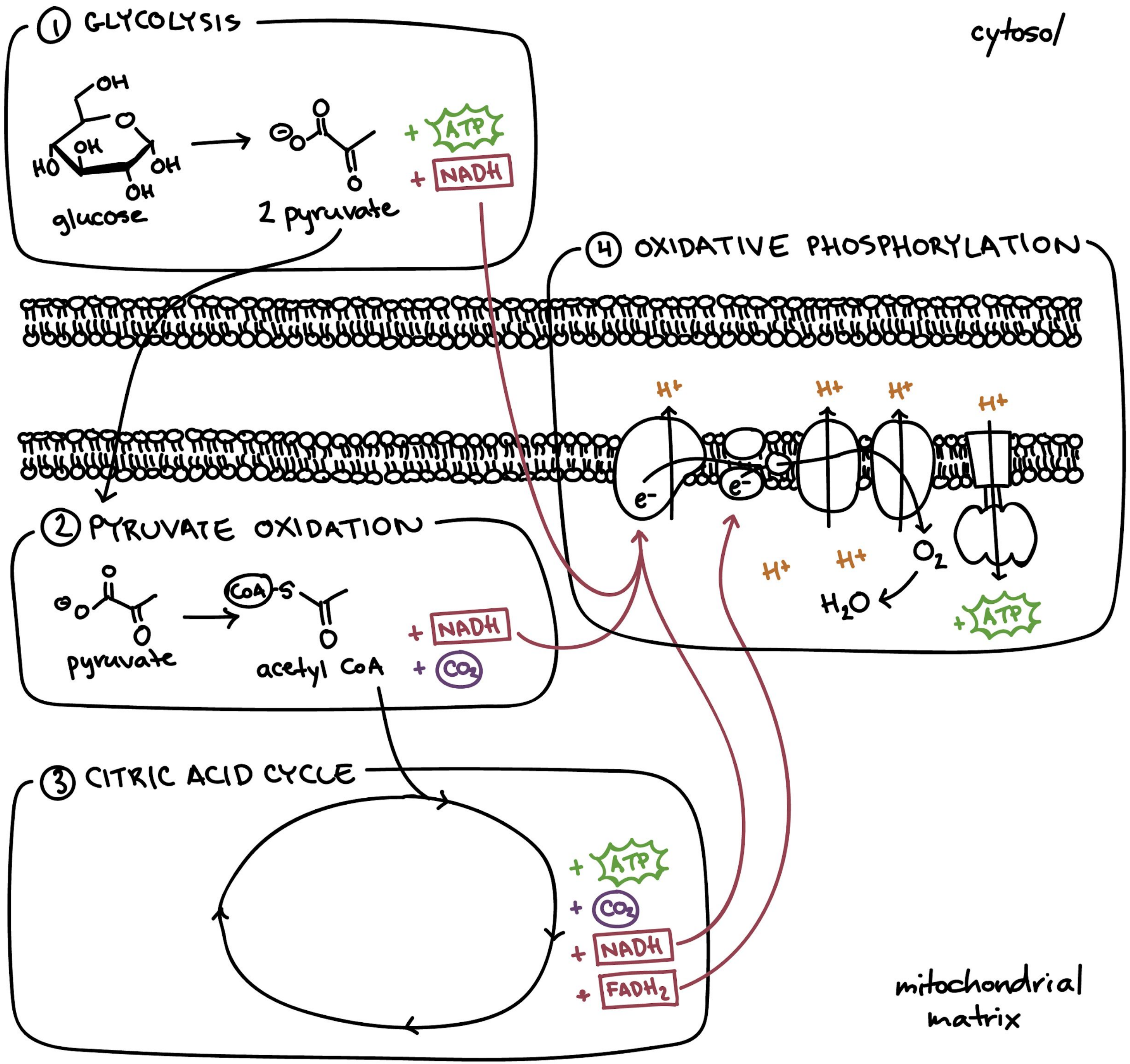
[Cellular Respiration - YouTube]





the electron transport chain




① GLYCOLYSIS
cytosol
OH
O
OH
HO
OH
OH
glucose
2 pyruvate
+ ATP
+ NADH
④ OXIDATIVE PHOSPHORYLATION
H+
H+
H+
H+
e-
e-
H+
H+
O2
H2O
+ ATP
② PYRUVATE OXIDATION
CoA
S
pyruvate
acetyl CoA
+ NADH
+ CO2
③ CITRIC ACID CYCLE
+ ATP
+ CO2
+ NADH
+ FADH2
mitochondrial
matrix

Lab Activity 1: Determine the effect of temperature on respiration rate.

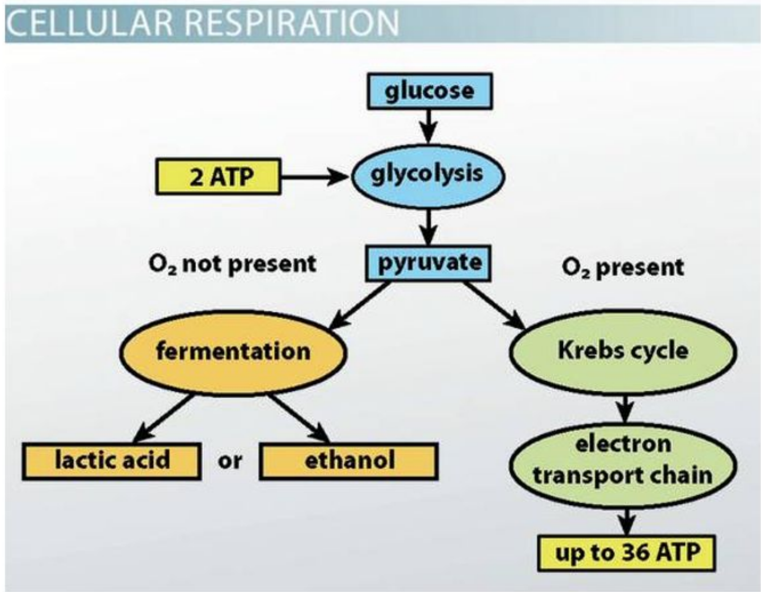
In this experiment you will use a volumetric respirometer to measure the O_2 consumed by the garbanzo beans under different temperatures.

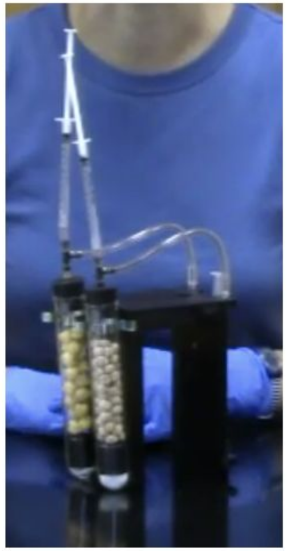
$2KOH$ (liquid) + CO_2 (gas) → K_2CO_3 (solid) + H_2O (liquid)

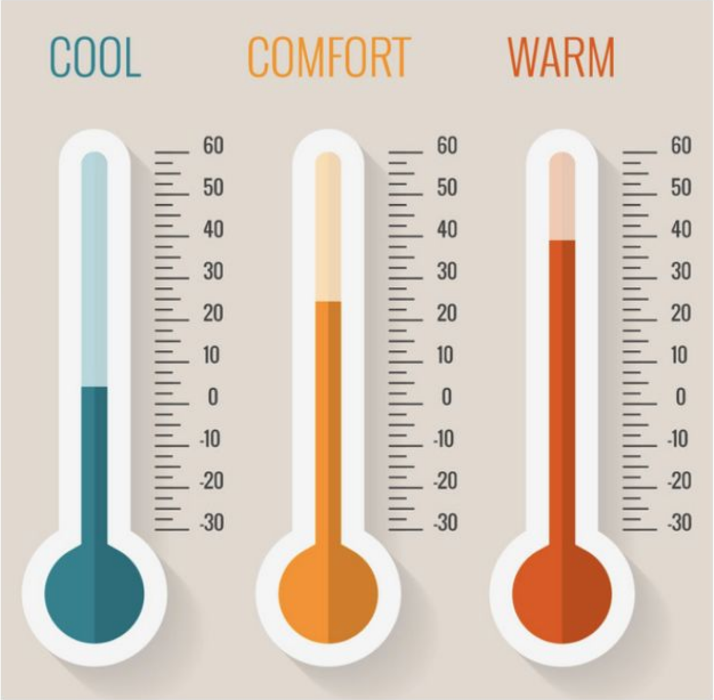
4˚C= 39.2 ˚F

21˚C= 69.8 ˚F

37˚C= 98.6 ˚F







Activity 1 – each group uses only 1 temp.

- To each plastic tube: add soaked beans + KOH cotton ball to one, collect weight. Add dry beans to other + KOH cotton ball
- Equilibrate in assigned temp for 10 minutes (caps loose)
  - Remove from temp bath, tighten caps, equilibrate colored water levels, return to temp
    - **START TIMER & record starting liquid height**
- Record liquid height every 5 minutes (until 30 min)
- Where do you dispose of the KOH cotton balls?
  - Base waste container

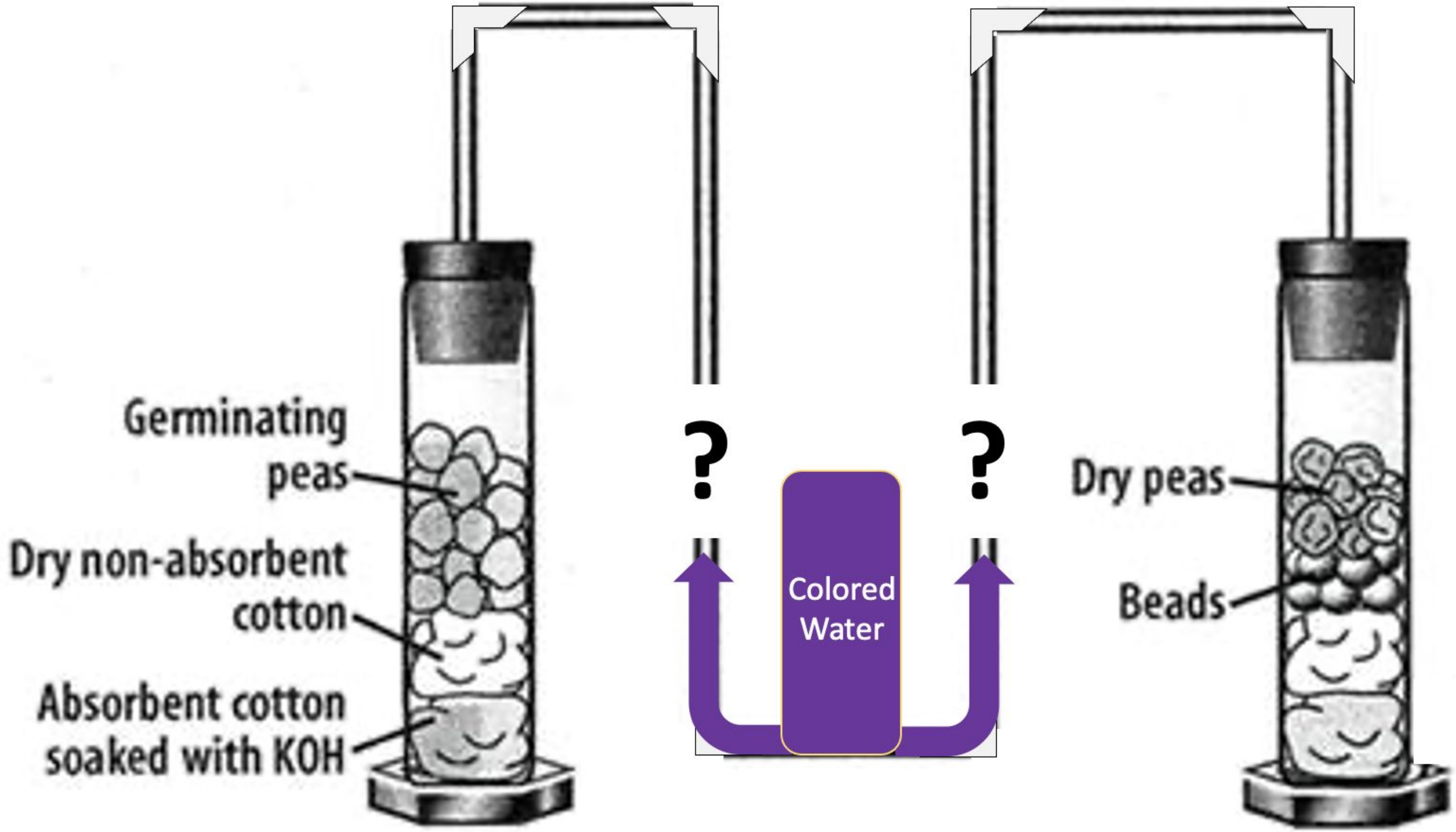
Each group will fill in for 1 temp, then share data.

Lab Activity 1: Table 1

Temperature Conditions	Bean Mass (g)	0 m (mL)	5 min. (mL)	10 min. (mL)	15 min. (mL)	20 min. (mL)	25 min. (mL)	30 min. (mL)
4°C								
Room Temp ??								
37°C								

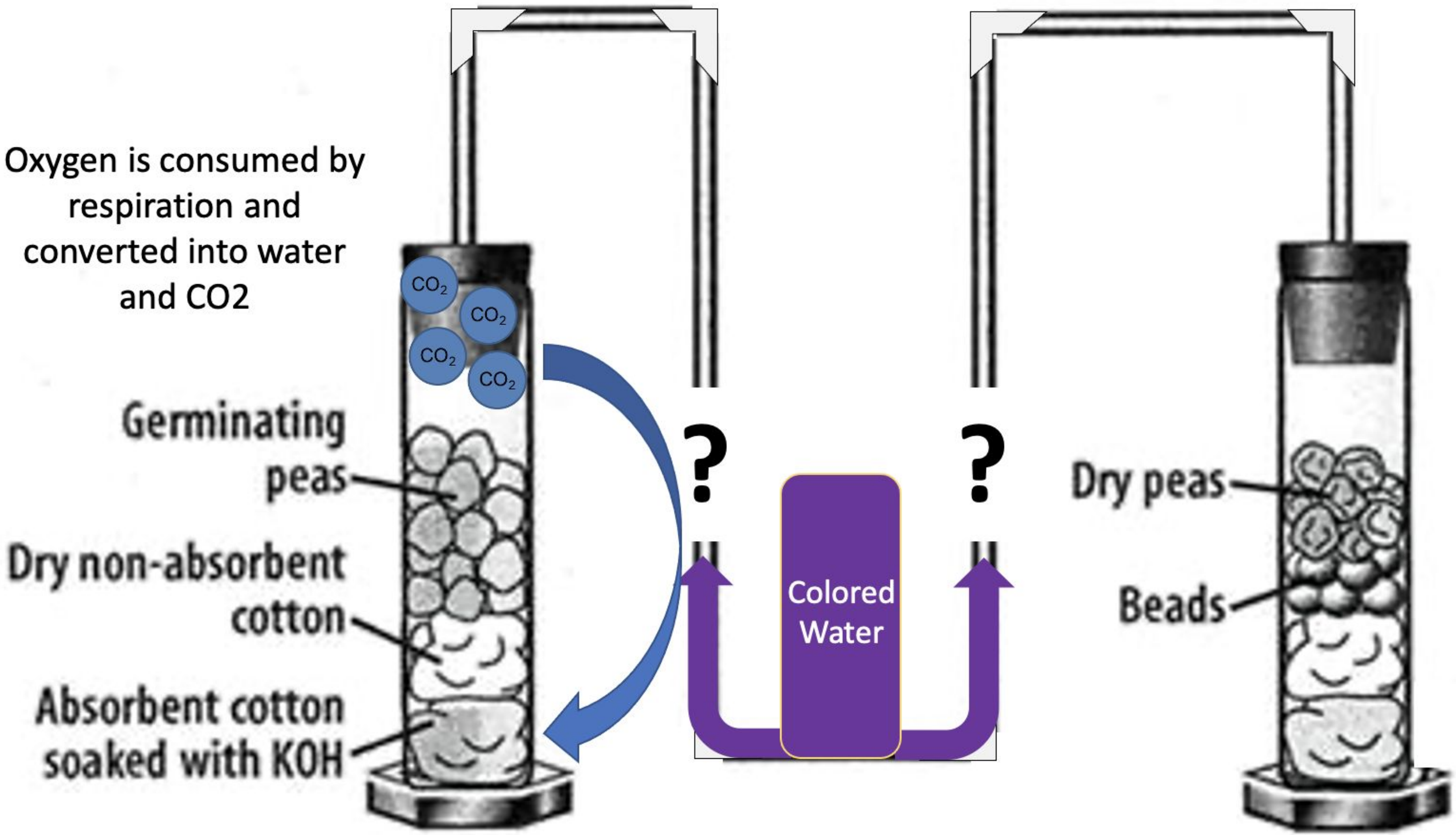
Visual Protocol

$$2KOH\ (liquid) + CO_2\ (gas) \rightarrow K_2CO_3\ (solid) + H_2O\ (liquid)$$



CO_2 Gas is produced from the TCA Cycle

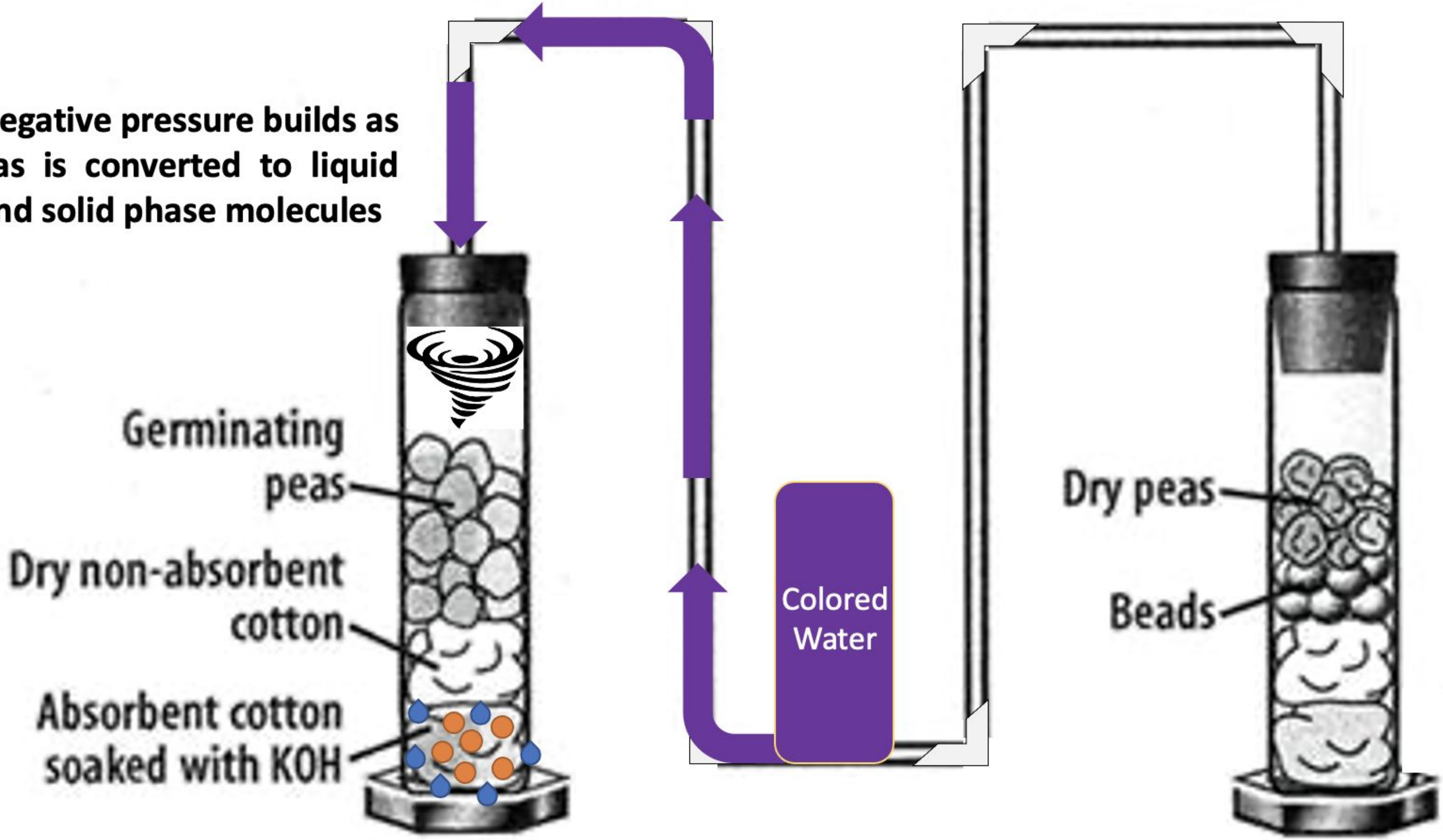
$$2KOH\ (liquid) + CO_2\ (gas) \rightarrow K_2CO_3\ (solid) + H_2O\ (liquid)$$

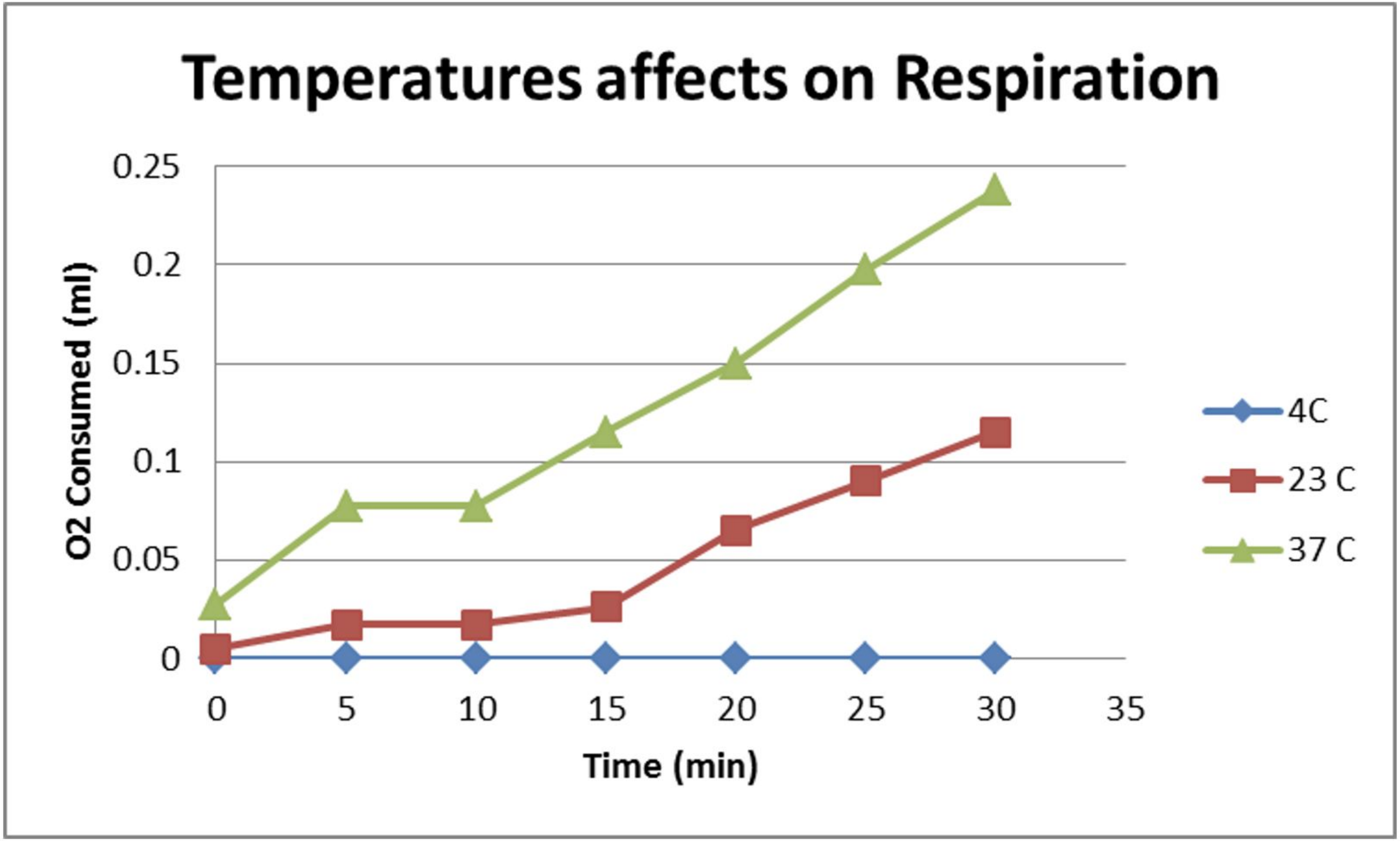


CO_2 is Converted to a Solid Resulting in a Vacuum

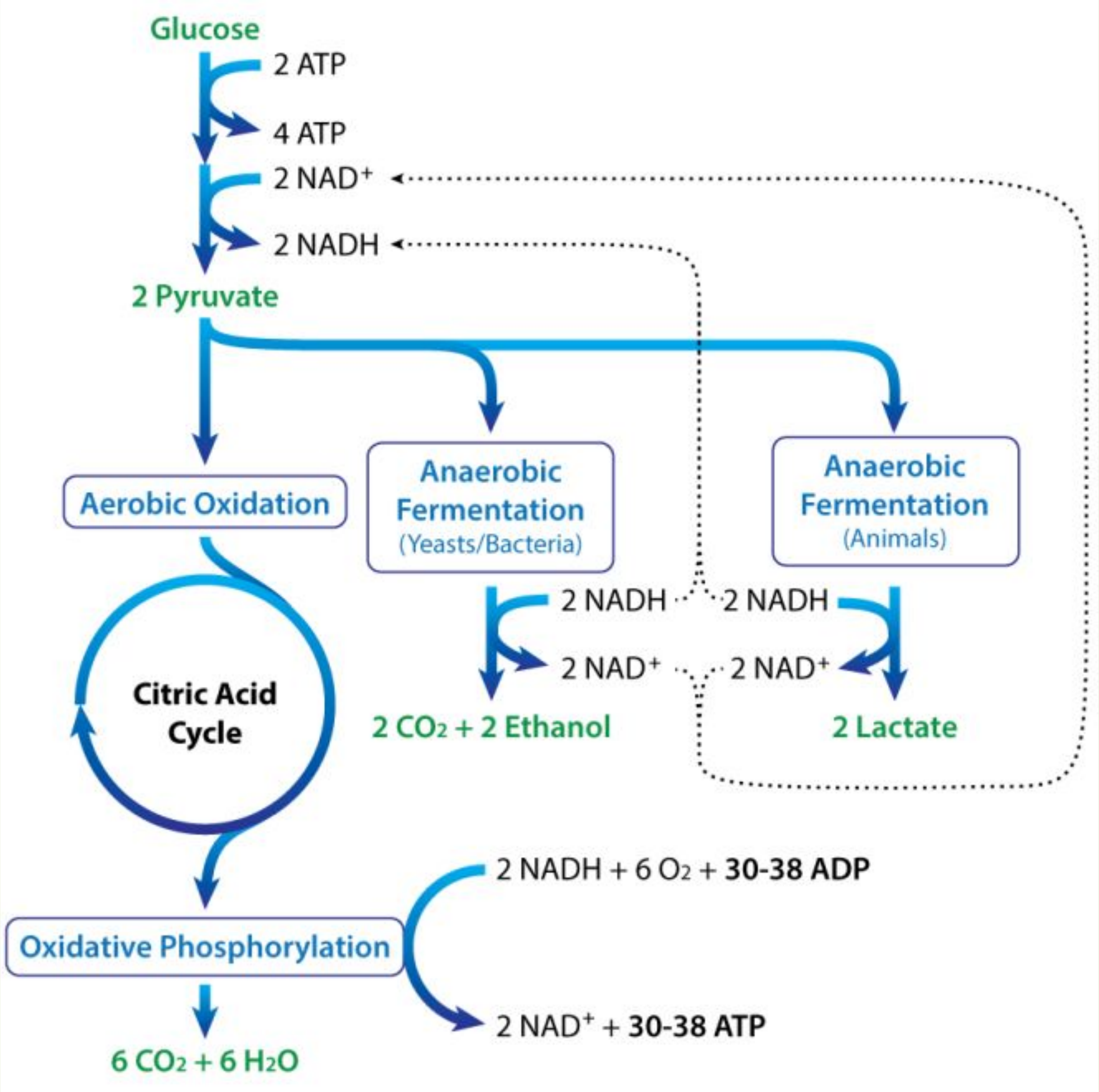
$$2KOH\ (liquid) + CO_2\ (gas) \rightarrow K_2CO_3\ (solid) + H_2O\ (liquid)$$

Negative pressure builds as gas is converted to liquid and solid phase molecules

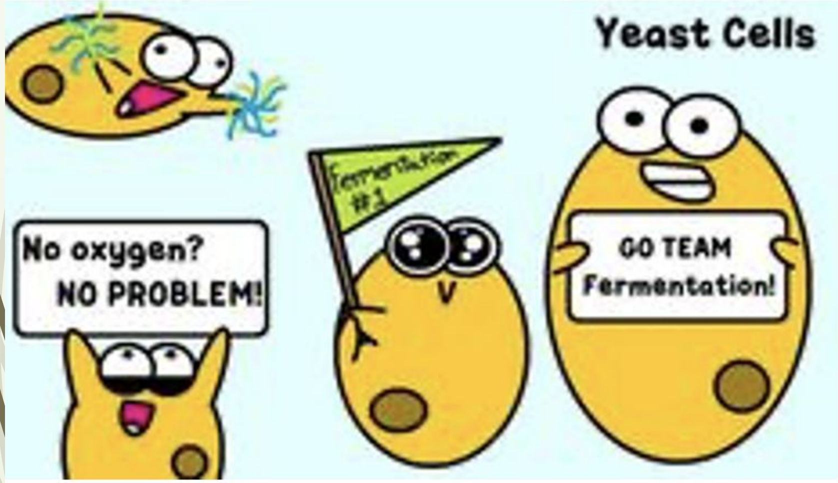


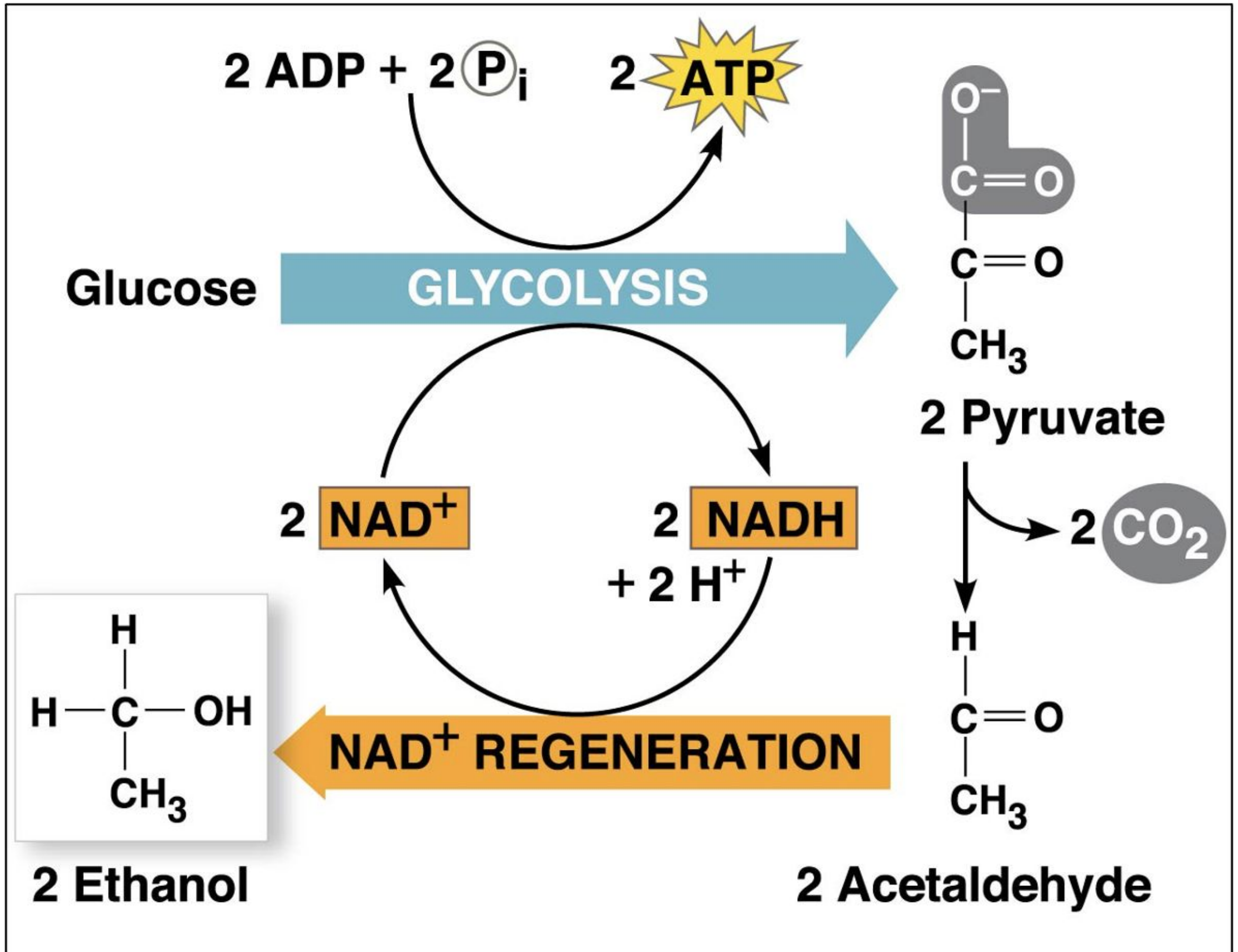


Temperatures affects on Respiration
O2 Consumed (ml)
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
35
Time (min)
4C
23 C
37 C


Glucose
2 ATP
4 ATP
2 NAD+
2 NADH
2 Pyruvate
Aerobic Oxidation
Anaerobic Fermentation (Yeasts/Bacteria)
Anaerobic Fermentation (Animals)
Citric Acid Cycle
2 NADH
2 NAD+
2 NADH
2 NAD+
2 CO2 + 2 Ethanol
2 Lactate
2 NADH + 6 O2 + 30-38 ADP
Oxidative Phosphorylation
2 NAD+ + 30-38 ATP
6 CO2 + 6 H2O

Alcoholic Fermentation

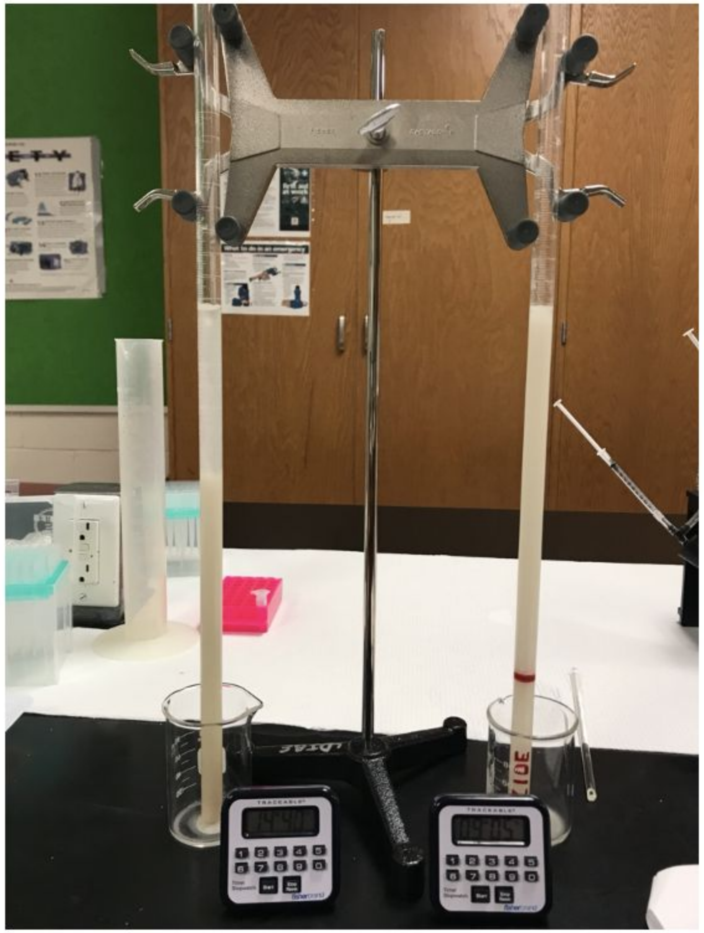




(a) Alcohol fermentation

Lab Activity 2: Determine the optimal sugar for providing energy for starting packaged yeast (*S. cerevisiae*) by measuring CO2 production. (Activities 2 & 3 should be run at the same time!)

You will be using a Geissler burette to measure the volume of CO_2 gas produced by the yeast *Saccharomyces* grown with different sugars and in the presence of the metabolic poison, sodium azide.



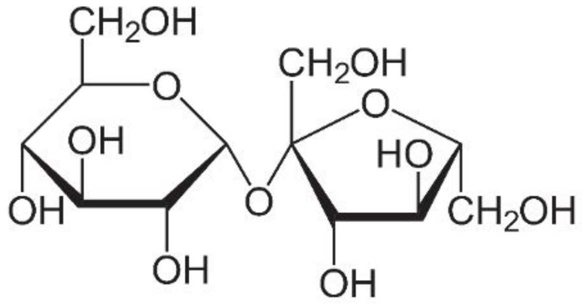
Lab Activity 3: What is the metabolic source of the CO_2; fermentation or respiration?

Think about what the implications are if the ETC is not functioning and compare that to the data you collect.

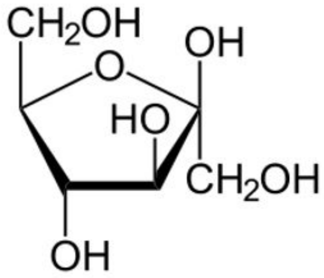
Lab Activity 2 : Determine the optimal sugar for providing energy for starting packaged yeast (S. cerevisiae)

- Yeast (and other organisms) can utilize a vast array of biomolecules as a food source. Here are three sugars that yeast can use as an energy source. Think about what you now know about cellular respiration and fermentation. Do all of these molecules enter those pathways in the same manner? Do they contain the same amount of energy?

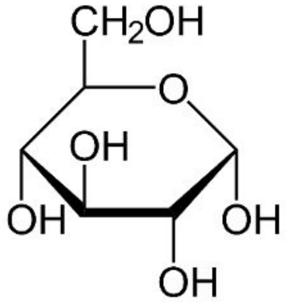
200 mM Sucrose (342 g/mol)



200 mM Fructose (180 g/mol)



200 mM Glucose (180 g/mol)

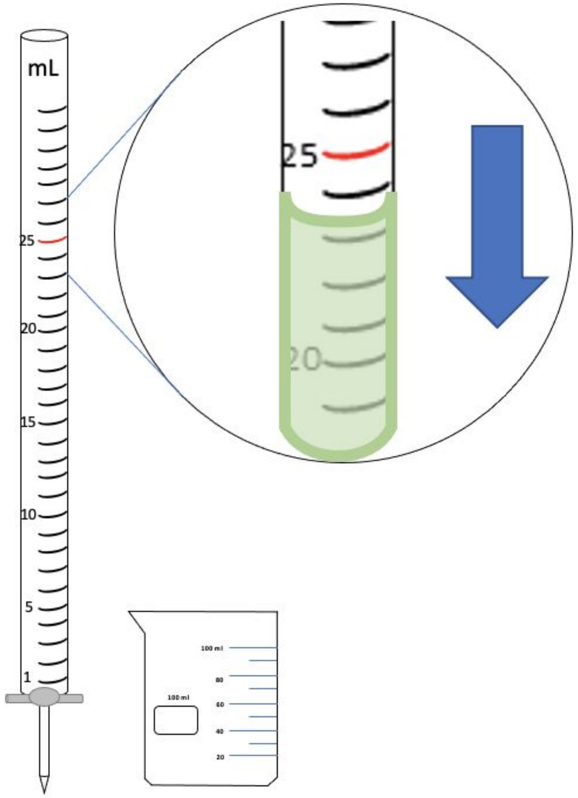


Activity 2 – each group uses only 1 sugar

- Weigh out sugar, dissolve in 75 mL of 37C water
  - Use stir rod to dissolve. Transfer to graduated cylinder, bring to 100mL with 37C water, transfer back to beaker.
- Weigh out 2.5 g yeast, add to sugar solution in beaker, stir 30 sec
- Transfer 25 mL to a CLOSED buret. Flip buret (with beaker as seal). Secure in clamp. Start timer counting up. Measure liquid level every 5 min (until 30 min)
  - Volume displaced is equal to the volume of CO_2

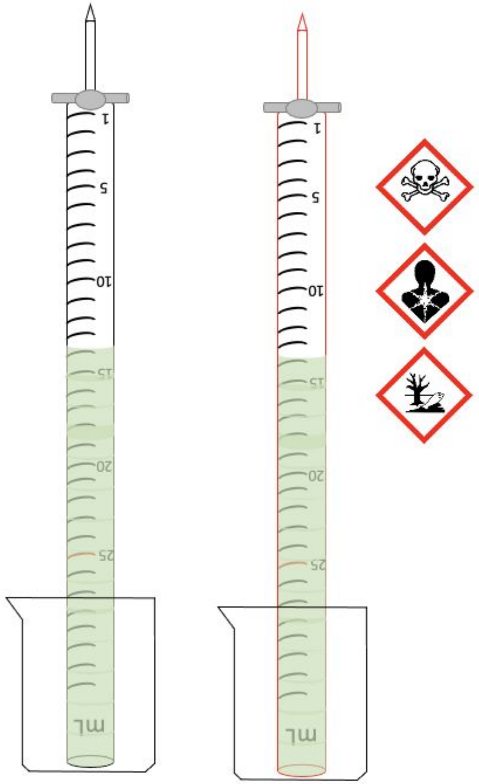
Visual Protocol

The curved surface at the top of the liquid level is called a meniscus. You read the liquid level using the bottom of the meniscus.



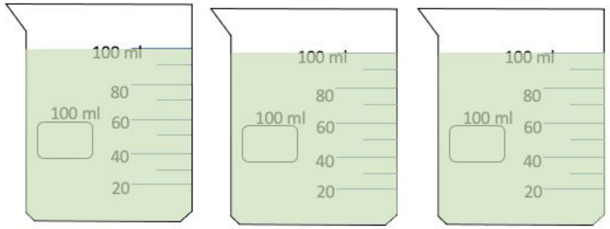
Measure the level of the liquid. The volume of culture displaced is equal to the volume of CO_2.

5.5 min



250 µL 1M Sodium azide

Sugar Yeast solution



200 mM Sucrose

200 mM Fructose

200 mM Glucose

Amount Of Sucrose Sugar Needed

- Volume: 100mL or 0.1L
- Desired Molarity:200mM or 0.2M
- Molecular Weight:342g/mol

- 0.1L*0.2mol/L*342g/mol=6.84grams

Amount Of glucose Sugar Needed

- Volume: 100mL or 0.1L
- Desired Molarity:200mM or 0.2M
- Molecular Weight:180g/mol

- 0.1L*0.2mol/L*180g/mol=3.6grams

Amount of fructose Sugar Needed

- Volume: 100mL or 0.1L
- Desired Molarity:200mM or 0.2M
- Molecular Weight:180g/mol

- 0.1L*0.2mol/L*180g/mol = 3.6grams

Use sucrose, glucose, OR fructose, then share da

Lab Activity 2: TABLE 2 (without azide)

Sucrose	Time (min)	Buret reading	mL CO_2
	0		0
	5		
	10		
	15		
	20		
	25		
	30		

Activity 3 – each group uses only 1 sugar

- Add 25 mL of sugar/yeast solution to red-tape buret
- **CARELFULLY** add 250uL sodium azide to red-tape buret
- Start timer counting up. Measure liquid level every 5 min (until 30 min)
  - Volume displaced is equal to the volume of CO_2

- Where do you dispose of yeast/azide mix in buret?
  - Toxic waste container

Lab Activity 3: TABLE 3 (with azide)

	Time (min)	Buret reading	mL CO_2
Sucrose	0		0
	5		
	10		
	15		
	20		
	25		
	30		