# Cellular Respiration and Fermentation

## Learning Goals:

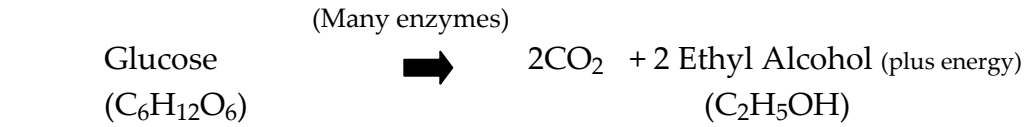
After completing these laboratory experiments you will be able to:

1. Describe in detail the process of glycolysis.
2. Compare and contrast anaerobic and aerobic metabolism of glucose.
3. Design experiments to determine effective substrates for metabolic processes.

## Introduction

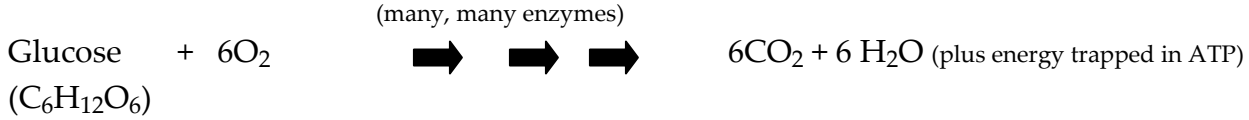
All living organisms require an input of energy for their basic needs. Energy is used for the synthesis of biomolecules, maintaining osmotic gradients, and movement. The chemical changes and processes that occur in living cells are called metabolism. Metabolic activity usually results in the production of and/or uptake of a gas; for example, carbon dioxide evolution in fermenting organisms and oxygen uptake in respiring organisms.

It is very likely that early living cells evolved under anaerobic conditions -- that is, with no molecular oxygen ($O_2$) in the atmosphere. All living cells today have mechanisms for obtaining energy from organic molecules <u>without</u> $O_2$ (anaerobic metabolism). In some organisms the process results in the production of ethyl alcohol (used in wine & beer manufacture). This process is known as alcoholic, or ethanolic fermentation.




The organism traps some of the released energy in ATP molecules which then supply that energy to various energy-consuming activities necessary for life.

Later on oxygen began to accumulate in the atmosphere, as a result of photosynthesis, from the newly evolved photosynthetic organisms, and many new organisms developed metabolic pathways which degraded the molecules produced by anaerobic metabolism, utilizing $O_2$ as a "dump" for hydrogen atoms. The overall process (aerobic respiration), which utilizes oxygen, extracts much more energy from the original food molecules than does fermentation, which is anaerobic. When glucose is the starting molecule:



## Procedure: Effect of pH on the Rate of Fermentation in Yeast.

In this lab exercise, you will study the effect of pH on the rate of the overall process of anaerobic fermentation, using a solution of glucose and yeast organisms in various buffers each with a different pH. As a measure of the rate of the reaction, you will be measuring the amount of $CO_2$ produced.

l. Examine the Smith fermentation tubes and figure out how you will measure the $CO_2$ produced by the yeast. Label 7 smith tubes as listed below with numbers (l to 7 and correlating pH), and calibrate the closed portions of each tube by taping a ruler to it so you can measure in l mm intervals or 5 mm, whatever seems feasible.

Tube 1 pH 3
Tube 2 pH 4
Tube 3 pH 5 Fill the tubes after you mix up each treatment in a beaker.
Tube 4 pH 6
Tube 5 pH 7
Tube 6 pH 8
Tube 7 pH 9

2. Your Lab Instructor will prepare the yeast solution (7% warm glucose + 30g yeast/1000ml) and mix it before the students pour off 50ml or more of active yeast solution for their experiment. The solution must be swirled to mix it up, prior to pouring off or groups may get varying amounts of yeast, too much or not enough. (This is true for your individual experimental set ups too.) Set up 7 beakers, one labeled for each buffer you have. You will add 5 ml of your yeast solution to each beaker, **but be sure to swirl and mix up the yeast solution before pipetting** it into each beaker. Wear gloves and add l5 ml of the appropriate buffer solution to each labeled beaker and mix the yeast and buffer by swirling the beaker, be careful with the more acidic (lower pH) solutions since they may damage clothes and irritate skin , wear gloves (Be sure to wipe up spills and use care handling).

Beaker 1 5ml yeast solution and l5 ml pH 3 buffer
Beaker 2 5ml yeast solution and l5 ml pH 4 buffer
Beaker 3 5ml yeast solution and l5 ml pH 5 buffer
Beaker 4 5ml yeast solution and l5 ml pH 6 buffer
Beaker 5 5ml yeast solution and l5 ml pH 7 buffer
Beaker 6 5ml yeast solution and l5 ml pH 8 buffer
Beaker 7 5ml yeast solution and l5 ml pH 9 buffer

3. Mix the solutions in each beaker very well. Then test the pH with pH paper, record results in table I below. Two partners should each independently test each pH, and if results do not agree with each other repeat pH determinations.

4. Now transfer the 20ml of solution in each beaker to the appropriately labeled smith tube.

5. Let the entire set of Smith tubes equilibrate for about l0 minutes (If you place the tubes in a 37°C water it will speed up the reaction). As you prepare to start the experiment invert the tubes simultaneously as possible, so that only the future $CO_2$ gas generated is trapped in the smith tube. Record the time (this is the beginning of the experiment).

6. Measure the length of the tube occupied by gas at l0 minute intervals for 40 minutes (or 60 minutes, if time permits). Record measurements in table II below.

7. At the end of the experiment the pH of each solution should be measured again (record in table I). Yeast cells produce acids as well as alcohol, and the buffering agent used may not be able to keep the pH constant. If the pH of any solution has changed during the experiment, use the average pH of that solution when you plot your results (Graph 2, below).

8. All glassware must be thoroughly washed, ideally in hot soapy solution and rinsed well. Replace tubes and beakers where you found them on your bench at the start of the lab. Pipets can go in the dirty pipet bin on the instructor's table.

Table I -- pH Measurements

| Tube # | stated pH of buffer | Starting Measure of pH | End Measure of pH | Average pH (beginning+end/2) |
|---|---|---|---|---|
| 1 | 3 | | | |
| 2 | 4 | | | |
| 3 | 5 | | | |
| 4 | 6 | | | |
| 5 | 7 | | | |
| 6 | 8 | | | |
| 7 | 9 | | | |

Table II – Length of Tube occupied by gas. (mm)

| Tube # | 0 min. | l0 min. | 20 min. | 30 min. | 40 min. | 50 min. | 60 min. |
|---|---|---|---|---|---|---|---|
| 1 | 0mm | | | | | | |
| 2 | 0mm | | | | | | |
| 3 | 0mm | | | | | | |
| 4 | 0mm | | | | | | |
| 5 | 0mm | | | | | | |
| 6 | 0mm | | | | | | |
| 7 | 0mm | | | | | | |

# Follow-Up Assignment Guidelines.

Prepare two graphs of your results. Be sure that they are correctly formatted, and accurately display the data you collected.

- Plot on one graph the amount of gas produced in each tube (dependent variable) vs. time of incubation (independent variable). The slope of the curve indicates the rate of the reaction -- the steeper the curve, the faster the reaction. Which tube had the fastest rate of reaction? Which had the slowest?

- Plot on a second graph the amount of gas produced after 40 minutes incubation (or 60 minutes - whatever the maximum time of your experiment was) versus the average pH of the solution. In this graph the independent variable is pH, so it is to be plotted along the x axis.

Then provide concise answers to the following. If there is time, these questions can be discussed in lab.

1. Based on your graphed data, what is the effect of pH on fermentation in yeast?

2. Based on what you know about cell biology and metabolism, what do you think could be a possible mechanism for any effect seen? Speculation is OK here, as long as you can back up your speculation with some plausible biology from the textbook or lecture.