#  Factors Affecting Enzymatic Activity in Fermentation

**Objectives:**

1. To understand the factors that increase or decrease enzymatic activity.

2. To understand how and why fermentation occurs.

3. To understand how cellular respiration and photosynthesis require and produce different materials.

Cellular respiration is that portion of metabolism used to extract the potential energy stored in the chemical bonds of glucose. **Glycolysis** is the first metabolic pathway of cellular respiration. It consists of a series of enzymatic reactions designed to break the carbon backbone of glucose in half. The net reaction for glycolysis is shown below.

Glucose

(*a six-carbon molecule*)

2 molecules of pyruvic acid

(*two 3-carbon molecules*)

2 ADP

2 ATP

2 NAD+

2 NADH

As shown above, a portion of the chemical energy extracted in this process is used immediately to convert ADP to ATP. The remainder of the energy, in the form of “high energy electrons”, is captured in the formation of NADH. NADH is an **electron carrier**. It will deliver the electrons to the final pathway of cellular respiration, the **electron transport chain**. The energy extracted from those electrons will eventually be used to form more ATP.

Once NADH has delivered its electrons to the electron transport system, it is converted back to NAD+. The NAD+ is returned to the glycolysis pathway to be converted into NADH once more. This is important because the cell does not have an unlimited supply of NAD+. If the supply of NAD+ were to be used up, glycolysis would stop, preventing further steps in respiration from occurring, and preventing further production of ATP.

If NADH is to be able to deliver high-energy electrons to the electron transport chain, there must be some molecule available to receive the electrons once they exit that system – a **final electron acceptor**. In eukaryotic cellular respiration, the final electron acceptor is oxygen. This is the reason **aerobic** organisms, like humans, require oxygen. When oxygen is scarce, the electron transport chain can’t accept more electrons. Under those conditions, NADH will not be converted back to NAD+ and the supply of NAD+ will be used up, and respiration will stop.

Many organisms have developed a way to deal with this situation. When oxygen is not available, they use an additional reaction to regenerate enough NAD+ to keep glycolysis in operation. This allows a small amount of ATP to be made even if the electron transport chain is not operating. This process is called **fermentation**. This takes place in an **anaerobic** environment (does not require oxygen).

Yeasts (single-celled fungi) are examples of organisms that carry out a fermentation pathway which results in the production of alcohol. Fermentation as carried out by yeast is of great economic importance. This reaction produces the alcohol in wine and other alcoholic beverages and the CO2 that causes bread to rise.

When yeast cells are given glucose but no oxygen, they carry out glycolysis as normal. After that, instead of sending pyruvic acid to the next steps in the respiration pathway, they use NADH to convert pyruvic acid to ethyl alcohol and carbon dioxide. This process is valuable to the yeast cell because it ensures that there is enough NAD+ to keep glycolysis going and that the cell will make at least some ATP. The reaction below shows the glycolysis and fermentation pathways that yeast perform.

Glucose

(*a six-carbon molecule*)

2 molecules of pyruvic acid

(*two 3-carbon molecules*)

2 ADP

2 ATP

2 NAD+

2 NADH

2 molecules of ethyl alcohol

(*two 2-carbon molecules*)

PLUS 2 molecules of CO2 *(two 1-carbon molecules)*

2 NADH

2 NAD+

**Glycolysis**

**Fermentation**

Fermentation replenishes the NAD+ needed for glycolysis to occur

**Enzyme activity in glycolysis and fermentation pathways**

As for all metabolic pathways, specific enzymes are required for each step in the glycolysis and fermentation pathways. Glycolysis requires ten distinctive types of enzymes. An eleventh type of enzyme is required to convert pyruvate, the product of glycolysis, to CO2 and ethyl alcohol during the fermentation pathway.

How efficiently an enzyme works to catalyze a reaction depends upon the concentration of the reactants (substrates) and the temperature. When substrate concentration is low, low quantities of the product will be made. At high concentration, higher quantities of the product can be made. Similarly, each enzyme has a specific optimal temperature range. At temperatures that are too low, the enzyme will not function properly and little to no product will be made. As the temperature increases, the enzyme’s activity will increase and more of the product will be made. However, at temperatures that are too high, the enzyme may become denatured, causing its activity, and the production of the product, to halt.

In this experiment we will use baker’s yeast cells as a model system, and investigate the influence of temperature, substrate concentration, and inhibitors on the activity of the yeasts’ glycolysis and fermentation enzymes. One of the products of alcohol fermentation is CO2. During this exercise, you will ***measure the production of CO2 to assess the activity of the enzymes*** involved in the glycolysis and fermentation pathways.

# Exercise 1: Effect of *substrate concentration* on enzyme activity

All enzyme reactions rely on physical contact between the substrate and the enzyme. For this reason, increasing the number of substrate molecules (glucose in this case) will have a direct effect on the rate of appearance of product (CO2).

**Obtain the following materials from the supply table:**

5 syringe/centrifuge tube set-ups (labeled 1-5)

1 large test tube rack

5 small collection cups to mix liquids before putting into syringe (labeled 1-5)

**Procedure:**

1. Measure the following volumes of water and glucose into the empty collection cups per the table below. Add yeast to all of the cups. Draw liquid up into the appropriate syringe. Expel air from the syringe. ***(Note: Each syringe should contain 20mL of liquid.)***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube # | Deionized H2O (mL) | 5% Glucose (mL) | Yeast Suspension (mL) | CO2 produced (cm3) |
| 1 | 10 | 0 | 10 |  |
| 2 | 8 | 2 | 10 |  |
| 3 | 5 | 5 | 10 |  |
| 4 | 2 | 8 | 10 |  |
| 5 | 0 | 10 | 10 |  |

2. Place each syringe into the correct corresponding centrifuge tube. Place the entire syringe/centrifuge set-up into the large test tube rack.

3. Place the rack of tubes in the 37oC incubator.

4. After one hour, remove tubes from incubator, determine amount of CO2 production, and record in table above.

**Exercise 2: Effect of *enzyme inhibitor* on enzyme activity**

In the following exercises we will inhibit glycolysis. We will use sodium fluoride (NaF) as an inhibitor of the enzymes in this pathway, preventing the formation of pyruvate*. FLUORIDES ARE POISONOUS TO HUMANS, SO HANDLE THE SOLUTIONS CAREFULLY IN THIS EXERCISE. WASH YOUR HANDS IMMEDIATELY AFTER HANDLING THESE SOLUTIONS.*

**Obtain the following materials from the supply table:**

5 syringe/centrifuge tube set-ups (labeled 1-5)

1 large test tube rack

5 small collection cups to mix liquids before putting into syringe (labeled 1-5)

**Procedure:**

1. Measure the following volumes of water, glucose, and sodium fluoride into the empty collection cups per the table below. Add yeast to all of the cups. Draw liquid up into the appropriate syringe. Expel air from the syringe. ***(Note: Each syringe should contain 20mL of liquid.)***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Tube # | Deionized H2O (mL) | 5% Glucose (mL) | NaF (mL) | Yeast Suspension (mL) | CO2 produced (cm3) |
| 1 | 10 | 0 | 0 | 10 |  |
| 2 | 5 | 5 | 0 | 10 |  |
| 3 | 0 | 5 | 5 of 0.01 M | 10 |  |
| 4 | 0 | 5 | 5 of 0.05 M | 10 |  |
| 5 | 0 | 5 | 5 of 0.10 M | 10 |  |

2. Place each syringe into the correct corresponding centrifuge tube. Place the entire syringe/centrifuge set-up into the large test tube rack.

3. Place the rack of tubes in the 37oC incubator.

4. After one hour, remove tubes from incubator, determine amount of CO2 production, and record in table above.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |

 **Exercise 3: Effects of *temperature* on enzyme activity**

**Obtain the following materials from the supply table:**

5 syringe/centrifuge tube set-ups (labeled 1-5)

1 large test tube rack

5 small collection cups to mix liquids before putting into syringe (labeled 1-5)

**Procedure:**

1. Measure the following volumes of water and glucose into the empty collection cups per the table below. Add yeast (which has been kept at each of the temperatures) to the appropriate cup. Draw liquid up into the appropriate syringe. Expel air from the syringe. ***(Note: Each syringe should contain 20 mL of liquid.)***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube # (temperature) | Deionized H2O (mL) | 5% Glucose (mL) | Yeast Suspension (mL) | CO2 produced (cm3) |
|  1 (5o) | 5 | 5 | 10 |  |
|  2 (20o) | 5 | 5 | 10 |  |
|  3 (37o) | 5 | 5 | 10 |  |
|  4 (45o) | 5 | 5 | 10 |  |
|  5 (60o) | 5 | 5 | 10 |  |

2. Place each syringe into the correct corresponding centrifuge tube. Place the entire syringe/centrifuge set-up into the large test tube rack.

3. Place the tubes in the appropriate temperature locations.

4. After one hour, collect tubes from their temperature locations, determine amount of CO2 production, and record in table above.

 **Questions**

1. Define the following terms: glycolysis, cellular respiration, aerobic, anaerobic, and fermentation.

2. What does a cell gain by doing fermentation?

3. What is a substrate? What is a product? What is an enzyme?

4. What gas was collected in the closed end of the fermentation tube?

5. Which living organism provided the enzyme for the fermentation experiment?

6. What was the function of tube 1 in exercises 1 and 2? Be specific.

7. What was the function of glucose in these experiments?

8. What is the effect of sodium fluoride (NaF) in this experiment?

9. A change in color of the bromothymol blue (from blue to yellow) indicated the presence of what substance?

10. In exercise 4, why were the 2 flasks placed in the dark?