CHEMISTRY OF FOOD FERMENTATION



Fermentation has been used by humans for the production of food and beverages since the Neolithic age. For example, fermentation is employed for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt (see fermentation in food processing), as well as for producing alcoholic beverages such as wine (see fermentation in winemaking) and beer. Fermentation can even occur within the stomachs of animals, such as humans. Auto-brewery syndrome is a rare medical condition where the stomach produce brewer's yeast that break down starches into ethanol; which enters the blood stream.

Fermentation is a form of anaerobic digestion that generates adenosine triphosphate (ATP) by the process of substrate-level phosphorylation. The energy for generating ATP comes from the oxidation of organic compounds, such as carbohydrates. In contrast, during respiration the energy for ATP formation is derived from hydrogen protons gradient that is generated across the inner mitochondrial membrane via electron transport chain, in a process called oxidative phosphorylation.

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to aerobic respiration, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect). The antibiotic activity of hops also inhibits aerobic metabolism in yeast. Fermentation uses an endogenous, organic electron acceptor. A widely used endogenous electron acceptor is pyruvate. During fermentation, pyruvate is metabolized to various compounds through several processes:

- Ethanol Fermentation also referred to as alcoholic fermentation, is the conversion of pyruvate into ethanol and carbon dioxide
- Lactic Fermentation (short for lactic acid fermentation) refers to two means of producing lactic acid:
 - 1. Heterolactic Fermentation is the production of lactic acid as well as other acids and alcohols.
 - 2. Homolactic Fermentation is the production of lactic acid from pyruvate

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, lactose, and hydrogen gas (H₂). However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

Comparison of <u>aerobic respiration</u> and most known fermentation types in <u>eucaryotic</u> cell. Numbers in circles indicate counts of carbon atoms in molecules, C6 is glucose $C_6H_{12}O_6$, C1 carbon dioxide CO₂. <u>mitochondrial</u> outer membrane is omitted.

Fermentation products contain chemical energy (they are not fully oxidized), but are considered waste products, since they cannot be metabolized further without the use of oxygen.



The chemical equation below shows the alcoholic fermentation of glucose, whose chemical formula is $C_6H_{12}O_6$. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:

 $\textbf{C}_6\textbf{H}_{12}\textbf{O}_6 \rightarrow \textbf{2} \ \textbf{C}_2\textbf{H}_5\textbf{OH} \textbf{+} \textbf{2} \ \textbf{CO}_2$

C₂H₅OH is the chemical formula for ethanol.

Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules. This is known as glycolysis.

Lactic Acid Fermentation

Lactic acid fermentation is the simplest type of fermentation. In essence, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces (i.e. transfers electrons to) nicotinamide adenine dinucleotide (NAD⁺), forming NADH. However there is a limited supply of NAD⁺ available in any given cell. For glycolysis to continue, NADH must be oxidized (i.e. have electrons taken away) to regenerate the NAD⁺ that is used in glycolysis. In an aerobic environment, where oxygen is available, oxidation of NADH is usually done through an electron transport chain in a process called oxidative phosphorylation, but oxidative phosphorylation cannot occur in anaerobic environments (i.e. where oxygen is absent) due to the pathway's dependence on oxygen as the terminal electron acceptor. Instead, the NADH donates its extra electrons to the pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD⁺ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate.

In heterolactic acid fermentation, one molecule of pyruvate is converted to lactate; the other is converted to ethanol and carbon dioxide. In homolactic acid fermentation, both molecules of pyruvate are converted to lactate. Homolactic acid fermentation is unique because it is one of the only respiration processes to not produce a gas as a byproduct.

Homolactic fermentation breaks down the pyruvate into lactate. It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some kinds of bacteria (such as lactobacilli) and some fungi. It is this type of bacteria that converts lactose into lactic acid in yogurt, giving it its sour taste. These lactic acid bacteria can be classed as homofermentative, where the end-product is mostly lactate, or heterofermentative, where some lactate is further metabolized and results in carbon dioxide, acetate, or other metabolic products.

The process of lactic acid fermentation using glucose is summarized below. In homolactic fermentation, one molecule of glucose is converted to two molecules of lactic acid:

$C_6H_{12}O_6 \rightarrow 2 CH_3CHOHCOOH.$

or one molecule of lactose and one molecule of water make four molecules of lactate (as in some yogurts and cheeses):

$\label{eq:c12} C_{12}H_{22}O_{11} + H_2O \rightarrow 4 \ CH_3CHOHCOOH.$

In heterolactic fermentation, the reaction proceeds as follows, with one molecule of glucose being converted to one molecule of lactic acid, one molecule of ethanol, and one molecule of carbon dioxide:

$\textbf{C}_6\textbf{H}_{12}\textbf{O}_6 \rightarrow \textbf{CH}_3\textbf{CHOHCOOH} + \textbf{C}_2\textbf{H}_5\textbf{OH} + \textbf{CO}_2$

Before lactic acid fermentation can occur, the molecule of glucose must be split into two molecules of pyruvate. This process is called glycolysis.

To extract chemical energy from glucose, the glucose molecule must be split into two molecules of pyruvate. This process generates two molecules of NADH and also four molecules of adenosine triphosphate (ATP), yet there is only net gain of two ATP molecules considering the two initially consumed.

$C_6H_{12}O_6$ + 2 ADP + 2 P_i + 2 NAD⁺ \rightarrow 2 CH_3COCOO^- + 2 ATP + 2 NADH + 2 H_2O + 2H⁺

The chemical formula of pyruvate is CH_3COCOO^- . P_i stands for the inorganic phosphate. As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD⁺ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.

Aerobic Respiration

In aerobic respiration, the pyruvate produced by glycolysis is oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and is not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD⁺; lactic acid fermentation is one of these pathways.

Hydrogen Gas Production in Fermentation

Hydrogen gas is produced in many types of fermentation (mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, glyoxylate fermentation), as a way to regenerate NAD⁺ from NADH. Electrons are transferred to ferredoxin, which in turn is oxidized by hydrogenase, producing H₂. Hydrogen gas is a substrate for methanogens and sulfate reducers, which keep the concentration of hydrogen low and favor the production of such an energy-rich compound, but hydrogen gas at a fairly high concentration can nevertheless be formed, as in flatus.

As an example of mixed acid fermentation, bacteria such as *Clostridium pasteurianum* ferment glucose producing butyrate, acetate, carbon dioxide and hydrogen gas:^[13] The reaction leading to acetate is:

$\textbf{C}_6\textbf{H}_{12}\textbf{O}_6\textbf{+4}\textbf{H}_2\textbf{O}\rightarrow\textbf{2}\textbf{C}\textbf{H}_3\textbf{C}\textbf{O}\textbf{O}^{-}\textbf{+2}\textbf{H}\textbf{C}\textbf{O}_3^{-}\textbf{+4}\textbf{H}^{+}\textbf{+4}\textbf{H}_2$

Glucose could theoretically be converted into just CO_2 and H_2 , but the global reaction releases little energy.

Methane Gas Production in Fermentation

Acetic acid can also undergo a dismutation reaction to produce methane and carbon dioxide:

$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$ $\Delta G^\circ = -36$ kJ/reaction

This disproportionate reaction is catalyzed by methanogen archaea in their fermentative metabolism. One electron is transferred from the carbonyl function (e^- donor) of the carboxylic group to the methyl group (e^- acceptor) of acetic acid to respectively produce CO₂ and methane gas.

