

# Biol 105 Biofuel Lab Web Resource Page

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## Who is interested in yeast?

Lots of people, actually. Scientists performing **basic research** and **applied research** study yeast cells for many reasons. Yeast have come to serve as a '**model**' **organism** – an organism about which we can learn fundamental biological principles that also apply to other organisms – for the study of basic eukaryotic cell biology. On one hand, as a single-celled organism their biological functions are much easier to unravel than those of more complex multicellular life forms. They are also easy to grow and can be stored for long periods of time in a dormant state (such as the activated dry yeast sold in grocery stores for baking). Furthermore, because yeasts have a relatively small **genome**, they have been widely used to decipher the genetic basis of cellular processes.

Obviously, biologists working in biofuel, distillery, brewery and baking industries also have good reasons to study the characteristics of yeast. The activities of yeast cells form the very foundation of these industries, and improving ethanol production and the fermentation process in general helps these industries to be more cost efficient and commercially competitive. And ethanol is not the only marketable product derived from industrial fermentation. Many large industries also collect and compress the CO<sub>2</sub> released during fermentation and sell it for a variety of uses, such as for carbonating beverages. Another byproduct of industrial fermentation is the yeast cells themselves, which typically triple in biomass during the fermentation process. Rather than being discarded, the 'waste' yeast are sold as nutritional supplements for animal food stock and as flavoring agents in soups, sauces and snacks such as potato chips.

## More information about fermentation

In principle, biofuels can be produced from a variety of raw materials, and ideally we would use raw materials (such as plant biomass) that do not compete with food sources. Unfortunately, current technology relies mainly upon grains such as corn as the raw material. Corn contains lots of starch that can be readily broken down into glucose and converted into ethanol by yeast cells.

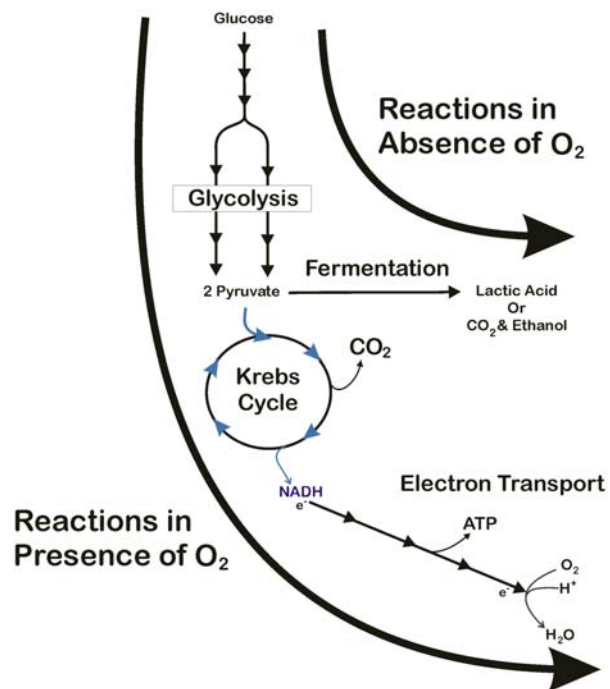
Biofuel industries continuously seek to optimize yeast fermentation to maximize the yield of ethanol. The scientists and engineers responsible for improving industrial processes also must have a detailed knowledge of the cellular process that is responsible for the conversion of sugar into ethanol, and you also should have some knowledge of this process to understand the results of this exercise.

Fermentation is a cellular process related to cellular respiration, the enzymatic reactions that extract energy from molecules such as carbohydrates, and make that energy available in the form of ATP (Figure 1). The three principal stages of cellular respiration are called **glycolysis**, **Krebs cycle**, and the **electron transport chain**. In most higher organisms, cellular respiration is an aerobic (oxygen requiring) process.

If oxygen is not available, the electron transport pathway and the Krebs cycle will shut down, just as an electric power plant that could not dispose of its waste products would eventually cease operations. (Each day a coal-burning electric power plant produces tons of fly ash - the powdery ash left after the coal burns - which must be hauled away continuously for disposal in landfills. If the dump trucks that haul away this waste were to stop running, the power plant would soon become so inundated with fly ash that it would have to shut down.) In a similar manner, if oxygen is not available, the whole process of cellular respiration will grind to a halt.

In the absence of oxygen most organisms can survive for short periods of time by activating a process called fermentation, which allows glycolysis to continue and produce small amounts of ATP. A byproduct of this pathway is lactic acid in animals, and ethanol and CO<sub>2</sub> in yeasts. Some cells can function for awhile on the energy provided by fermentation; for example, during strenuous activity, muscle cells continue to function for a short period of time in this manner. However, the amount of ATP produced anaerobically is too low to support the tremendous energy demands of our bodies, and this is why higher organisms suffocate in the absence of oxygen.

**Figure 1.** Overview of cellular respiration and fermentation pathways.



## How do yeast cells survive via fermentation?

Single celled organisms can survive on fermentation alone for several reasons. On one hand, the energy needs of a single cell are much lower than those of a complex, multicellular organism. Also, yeast cells can import and metabolize carbohydrates very rapidly – in effect, compensating for the inefficiency of fermentation by processing greater quantities of carbohydrate. As a result, yeasts release great quantities of CO<sub>2</sub> and ethanol during fermentation, which makes these organisms particularly useful to the biofuel industry.

## More information about factors that affect fermentation

### Temperature and Biological Stress

Temperature is carefully regulated during industrial fermentation processes since it can dramatically affect efficiency of ethanol production. High temperature is an example of a biological 'stress' factor – a physical condition outside the normal for an organism that threatens its health. It is now well recognized that cells of essentially all organisms respond to stress factors by producing protective proteins called 'stress proteins', also widely known as 'heat-shock proteins' since they were first discovered as a response to high temperature. It is now known that stress proteins are produced in response to wide variety of stress conditions, and yeast has served as an important model organism for studying their function.

Temperature effects can also be considered from a biochemical perspective. Essentially all chemical interconversions that occur in cells are catalyzed by **enzymes**. Enzymes are proteins – long chains of amino acids folded into a complex three-dimensional shape. They are called **catalysts** because they increase the rate at which chemical reactions occur. The rate of an enzymatic reaction is also temperature dependent. Within a certain range, higher temperatures increase enzyme activity. However, proteins (including enzymes) are structurally damaged (**denatured**) by higher temperatures (this is the type of damage that stress proteins serve to mitigate).

### Type of carbohydrate

Depending upon the initial raw material that is used, many different types of carbohydrates might be fermented in biofuel production. Glucose, obtained from the starch, is the most abundant sugar when grains are the raw material; however, a wide variety of other types of carbohydrates occur in grains. There is much interest in using plant **biomass** (such as corn stalks and other bulk plant materials) as the raw material to reduce competition between biofuel and food production. However, along with glucose, plant biomass contains many other types of sugars (e.g., xylose, galactose and rhamnose) that are not as readily fermented by yeasts.

Many carbohydrates are not used as an energy source because yeast cells lack the enzymes necessary to convert them into a form that can be used by the enzymes of glycolysis. For example, few organisms (including yeasts) can use cellulose as a nutritional source because their cells do not produce the enzymes that can release the glucose subunits. Another important factor that determines whether a carbohydrate can serve as an energy source is a cell's ability to import the carbohydrate through the cell membrane into the cytoplasm. This process requires special carbohydrate-transporting proteins in the cell membrane, and like all enzymes, these transporters are very specific to the type of carbohydrate that they can pass into the cytoplasm. If its cells lack the necessary transport protein, an organism can starve in a sea of food.

## Carbohydrate concentration and osmolarity

Outwardly, one might assume that increasing the amount of the sugar that is available to yeast cells would increase the rate of fermentation; however, the relationship is actually far more complicated. In general, and over a certain range, increasing the concentration of the sugar will increase the rate of fermentation. Low concentrations will 'limit' the rate of fermentation; that is, the cells could carry out fermentation at a higher rate if more carbohydrate were available. As the concentration of carbohydrate is increased, the rate of fermentation will also increase, but at some sufficiently high level, the concentration of sugar will exceed the assimilative capacity of the cells and the rate of fermentation will level off.

The relationship between sugar concentration and fermentation rates is actually somewhat more complicated than that described above for a variety of reasons. The concentration of carbohydrate in the solution also affects the 'osmolarity' of the solution (the concentration of all dissolved substances), which has effects independent of the particular type of sugar involved.

## pH

All organisms and cellular processes are affected by **pH** (the concentration of  $H^+$  ions in the liquid environment). For this reason, the pH of the cell's internal cytoplasm is closely regulated. The pH of the external environment also affects cell growth and metabolism, and cells can grow and carry on fermentation best within a certain range of values. The external pH affects the rate at which molecules can be imported into the cell, and extreme pH values will cause damage to the cell membrane and cell death. Yeast cells have been used as a model by which we can better understand how other organisms respond to changes in the pH of their habitat, such as changes to water and soil pH resulting from acid precipitation.

## Salt concentration

Many organisms require Na in their diet; however, there can be too much of a good thing, and too much salt can inhibit essential enzymes and damage the structure of the cell membrane. How do yeast cells respond to different concentrations of salt (NaCl)? At high concentrations salt causes 'stress' reactions within yeast cells, and yeast cell has served as a "model organism" for studying cellular responses to stress. Salt stress is of particular concern for agriculture in arid regions, where extensive irrigation has led to **salinization** of the farm lands. Salinization of farm land impairs the productivity of many crops, leading to economic hardship and famine.

## Ethanol concentration

Understanding the effect of ethanol on yeast cells is very important to distillery and ethanol production industries. Ethanol, a mere waste product to yeast cells, merits higher esteem from manufacturers and consumers of alcoholic beverages, and is the essence of biofuel. During biofuel fermentation, the rising concentration of ethanol tends to have an inhibitory effect on the fermentation process. This effect is partially due to **feedback inhibition**, whereby accumulation of the end products of a process tends to slow the process itself. However, ethanol also has other more complex effects on the cells that are not fully understood.

## Materials on reserve in the library

### I. General characteristics of yeasts

**Carlile MJ, Watkinson SC. 1994. The Fungi. New York (NY): Academic Press; 482 p.**

A good source for general information about yeasts in general; however, of greatest pertinence is the section describing characteristics of *Saccharomyces*, the genus of yeast we use in this lab.

**Davenport RR. 1980. An introduction to yeasts and yeast-like organisms. In: Skinner FA, Passmore SM, Davenport RR, editors, Biology and Activities of Yeasts. New York: Academic Press; p 1-27.**

A general overview of yeasts, from which you can cite information about the general properties of yeasts and about *Saccharomyces*, the genus of yeast we use in this lab, such as its occurrence in the environment. There is a nice overview of factors that affect yeast growth on page 6.

**Hagler AN, Adhearn DG. 1987. Chapter 6. Ecology of aquatic yeasts. In: Rose AH, Harrison JS, editors. The Yeasts. Vol. I. The Biology of Yeasts. New York(NY); Academic Press. 285 p.**

Provided here are several pages from this chapter that include brief descriptions of some factors (NaCl, temperature, and pH) that affect yeasts.

**Spencer JFT, Spencer DM, de Figueroa LIC. 1997. Chapter 5. Yeasts as living objects: Yeast nutrition. In: Spencer JFT, Spencer DM, editors. Yeasts in Natural and Artificial Habitats. Berlin (Germany); Springer-Verlag. 381 p.**

A description of organic and inorganic nutritional requirements of yeasts.

### II. Effects of physical parameters on yeast growth and fermentation

**Note:** Do not get ‘lost in detail’ when examining the articles. Often what you are looking for will be in the introduction, figures and their descriptions, and/or in the discussion -- learn to peruse an article, take notes, and record the source and page number for the information. Also, speak with your instructor for help when reviewing the articles.

#### Carbohydrate type

**D'Amore T, Russell I, Stewart GG. 1989. Sugar utilization by yeast during fermentation. Journal of Industrial Microbiology 4: 315 – 323.**

Discussion of glucose, fructose and sucrose fermentation separately and combined.

**Spencer JFT, Spencer DM, de Figueroa LIC. 1997. Chapter 5. Yeasts as living objects: Yeast nutrition. In: Spencer JFT, Spencer DM, editors. Yeasts in Natural and Artificial Habitats. Berlin, Germany: Springer-Verlag. 381 p.**

A description of organic and inorganic nutritional requirements.

**van Maris AJA, Abbott DA, Bellissimi E, van den Brink J, Kuyper M, Luttik MAH, Wisselink HW, Scheffers WA, van Dijken JP, Pronk JT. 2006. Alcoholic fermentation of carbon sources in biomass hydrolysates by *Saccharomyces cerevisiae*: current status. Antonie van Leeuwenhoek 90: 391–418.**

Although this article is somewhat lengthy, and includes much detail beyond what you need to be concerned about, you will find summaries of how well yeasts can ferment different types of carbohydrates.

## Glucose concentration

**D'Amore, T. 1992. Improving Yeast Fermentation Performance. Journal of the Institute of Brewing 98: 375-382.**

Describes the effects of different variables on yeast fermentation. Figure 2A nicely shows the effect of increasing concentration of glucose. As described above, carbohydrate concentration also affects osmolarity of the solution, which also affects fermentation. Figure 2B looks at the effect of osmolarity by increasing the concentration of sorbitol (a sugar that is not fermented) while keeping the concentration of glucose constant.

**D'Amore T, Panchal CJ, Russeil I, Stewart GG. 1988. Osmotic pressure effects and intracellular accumulation of ethanol in yeast during fermentation. Journal of Industrial Microbiology 2: 365-372.**

This article focuses on the effects of osmolarity, which is closely tied to the concentration of sugar. Table 1 shows a nice summary of the effect of different concentration of glucose on fermentation and ethanol production. The authors give concentrations of glucose as “grams/Liter”, which can be converted to percent by dividing by 10; thus,  $100 \text{ g/l} \div 10 = 10\%$ .

**Zhao Y, Lin Y-H. 2003. Growth of *Saccharomyces cerevisiae* in a chemostat under high glucose conditions. Biotechnology letters 25:1151-1154.**

A study of fermentation rates, measured as ethanol production, at different glucose concentrations. Note: a chemostat is a large fermentation chamber that maintains stable conditions. The authors give concentrations of glucose as “ $x \text{ g/l}^{-1}$ ” which means “ $x$  grams per liter”; thus,  $100 \text{ g/l}^{-1} = 100 \text{ g/L} = 2\text{g}/20 \text{ ml} = 10\%$ . Yeast ‘biomass’ refers to the weight of the cells produced, which is a measure of their growth.

## Ethanol concentration

**D'Amore, T. 1992. Improving yeast fermentation performance. Journal of the Institute of Brewing 98: 375-382.**

This article describes the effects of several variables on yeast fermentation. Figure 1 and the accompanying text presents the effect of ethanol on two different *Saccharomyces* species. The units of ethanol concentration are given as g/L (the weight of ethanol / liter of solution) and can be converted to percent v/v units by dividing by 7.8 (e.g.,  $100 \text{ g/l} \div 7.9 = 12.6\%$ ).

**Kalmokoff ML, Ingledew WM. 1985. Evaluation of ethanol tolerance in selected *Saccharomyces* strains. ASBC Journal 43:189-196.**

Presents effects of ethanol on a variety of growth parameters.

**Ough CS. 1968. Fermentation rates of grape juice. III. Effects of initial ethyl alcohol, pH and fermentation temperature. Am J of Enology and Viticulture 17:74-81.**

This study examines the effect of pH and temperature in the presence of different initial concentrations of ethanol. Tables 3 and 4 show the effects of initial ethanol concentration on the rate of fermentation at three different temperatures and 3 different pHs.

**Wasungu KM, Simard RE. 1982. Growth characteristics of baker's yeast in ethanol. Biotechnology and Bioengineering 24:1125-1134.**

The beginning of the section on ‘Influence of Ethanol Concentration’ is very informative.

## Osmolarity

**D'Amore, T. 1992. Improving Yeast Fermentation Performance. Journal of the Institute of Brewing 98: 375-382.**

Describes the effects of different variables on yeast fermentation. Figure 2A nicely shows the effect of increasing concentration of glucose. As described above, carbohydrate concentration also affects osmolarity of the solution, which also affects fermentation. Figure 2B looks at the effect of osmolarity by increasing the concentration of sorbitol (a sugar that is not fermented while keeping the concentration of glucose constant).

**D'Amore T, Panchal CJ, Russeil I, Stewart GG. 1988. Osmotic pressure effects and intracellular accumulation of ethanol in yeast during fermentation. Journal of Industrial Microbiology 2: 365-372.**

Authors use sorbitol to alter osmolarity of solutions containing glucose, and Figure 1 shows a nice summary of the effect on fermentation.

**Meikle AJ, Reed RH, Gadd GM. 1988. Osmotic adjustment and accumulation of organic solutes in whole cells and protoplasts of *Saccharomyces cerevisiae*. J of General Microbiology 134:3049-3060.**

(Selected pages) Introduction describes many issues relating to the effect of tonicity (osmotic pressure) on cells. References also very useful.

**Morris GJ, Winters L, Coulson GE, Clarke KJ. 1986. Effect of osmotic stress on the ultrastructure and viability of the yeast *Saccharomyces cerevisiae*. Journal of General Microbiology 129: 2023-2034.**

While the procedures and results of this article are rather advanced, the discussion presents a readable explanation of why high osmolarity has deleterious effects on the cells.

## pH

**Betts GD, Linton P, Betteridge RJ. 1999 Food spoilage yeasts: effects of pH, NaCl and temperature on growth. Food Control 10: 27-33.**

Note that this article deals with the effects of these parameters on growth (not fermentation specifically) of different types of yeast, including *Saccharomyces cerevisiae*; however, you can discuss how these effects likely also apply to fermentation.

**Ough CS. 1968. Fermentation rates of grape juice. III. Effects of initial ethyl alcohol, pH and fermentation temperature. Am J of Enology and Viticulture 17:74-81.**

This study examines the effect of pH and temperature in the presence of different initial concentrations of ethanol. Tables 3 and 4 show the effects of initial ethanol concentration on the rate of fermentation at three different temperatures and 3 different pHs.

**Parsons RV, McDuffie NG, Din GA. 1984. pH inhibition of yeast ethanol fermentation in continuous culture. Biotechnology Letters 10: 677 – 680.**

An interesting study of the effect of pH on yeast fermentation.

**Wasungu KM, Simard RE. 1982. Growth characteristics of baker's yeast in ethanol. Biotechnology and Bioengineering 24:1125-1134.**

Describes effects of pH, temperature and ethanol on yeast growth. Figure 2 shows the effects of pH on growth at 30°C.

## Salt concentration

**Betts GD, Linton P, Betteridge RJ. 1999 Food spoilage yeasts: effects of pH, NaCl and temperature on growth. Food Control 10: 27-33.**

Note that this article deals with the effects of these parameters on growth (not fermentation specifically) of different types of yeast, including *Saccharomyces cerevisiae*; however, you can discuss how these effects likely also apply to fermentation.

**Gaxiola R, de Larrinoa IF, Villalba JM, Serrano R. 1992. A novel and conserved salt-induced protein is an important determinant of salt tolerance in yeast. EMBO Journal 11: 2157-3164.**

Introduction offers a nice overview of topics and references pertaining to salt-stress. Actual research may be too technical for use in this course.

**Morris GJ, Winters L, Coulson GE, Clarke KJ. 1986. Effect of osmotic stress on the ultrastructure and viability of the yeast *Saccharomyces cerevisiae*. Journal of General Microbiology 129: 2023-2034.**

This article studies the affect of osmolarity created by different concentrations of salt. While the procedures and results of this article are rather advanced, the discussion presents a readable explanation of the effects of high salt concentrations on the cells.

**Wei C-J, Tanner RD, Malaney JW. 1982. Effect of sodium chloride on bakers' yeast growing in gelatin. Appl Environ Microbiol 43: 757-763.**

This article finds is that the effects of salt on yeast grown in gelatin is very similar to that on yeast grown in a normal broth culture, so their results are pertinent to our biofuel lab exercise.

## Temperature

**Betts GD, Linton P, Betteridge RJ. 1999 Food spoilage yeasts: effects of pH, NaCl and temperature on growth. Food Control 10: 27-33.**

Note that this article deals with the effects of these parameters on growth of different types of yeast, including *Saccharomyces cerevisiae*.

**Ough CS. 1968. Fermentation rates of grape juice. III. Effects of initial ethyl alcohol, pH and fermentation temperature. Am J of Enology and Viticulture 17:74-81.**

This study examines the effect of pH and temperature in the presence of different initial concentrations of ethanol. Tables 3 and 4 show the effects of initial ethanol concentration on the rate of fermentation at three different temperatures and 3 different pHs.

**Wasungu KM, Simard RE. 1982. Growth characteristics of baker's yeast in ethanol. Biotechnology and Bioengineering 24:1125-1134.**

Describes effects of pH, temperature and ethanol on yeast growth. Figure 1 shows the effects of temperature on growth.

**Watson, K. 1987. Chapter 3. Temperature relations. In: Rose AH, Harrison JS, editors. The Yeasts. Vol. II. Yeasts and the environment. New York (NY): Academic Press; 285 p.**

This chapter has a nice overview of the influence of temperature on yeast growth. You may consider including information about the classification of yeast as psychrophilic, mesophilic or thermophilic, and how results of your experiments relate to these classifications. You should also consider including in your discussion section information about the effects of low and high temperatures on cells.

### III. Information about biofuel production

**Berry DR. 1982. The biology of yeasts. London (England): Edward Arnold (Publishers) Ltd.; 57 p.**

[Chapters 8 - Yeasts and Industry] This chapter discusses the uses of yeasts in various types of industries. Very interesting especially if you are unaware of the many uses of yeasts.

**Demirbas A. 2009. Biofuels. In: Demirbas A, editor. Biofuels: Securing the planet's future energy needs. London (England): Springer; 336 p.**

[Chapter 3 – Introduction to Biofuels] This chapter has a nice introduction to various topics concerning biofuels.

**Groom MJ, Gray EM, Townsend PA. 2008. Biofuels and biodiversity: Principles for creating better policies for biofuel production. Conservation Biology 22: 602-609.**

Much of this article discusses the potential impacts of expanded biofuel production on natural ecosystems and biodiversity.

**Pimentel D. 2003. Ethanol Fuels: Energy balance, economics, and environmental impacts are negative. Natural Resources Research 12: 127-133.**

This article compares the relative cost and energy input during ethanol production and the energy content and cost of the final product. Interesting article.

**Reijnders L, Huibregts, MAJ. 2009 Biofuels for road transport: A seed to wheel perspective. London (England); Springer. 170 p.**

Chapter 6 – Frequently Asked Questions in the Biofuel debate .

**Wang M, Saricks C, Wu M. 1999. Fuel ethanol produced from midwest U.S. corn: Help or hindrance to the vision of Kyoto? J. Air & Waste Management Association 49:756-772.**

A study of the air pollutants emitted during the production and use of fuel ethanol.

### Seeking other information resources

You are encouraged to seek other articles for use with your lab report; however, you should confirm with your instructor the suitability of any additional information sources. Remember that web sites are not suitable sources.

Suggested literature databases: Electronic Journal Center (for online journals), Electronic Book Center (for online books), Biosis and BioAbstracts (database of scientific literature), Ohiolink, McCat (Dawes Library; for on-campus holdings).

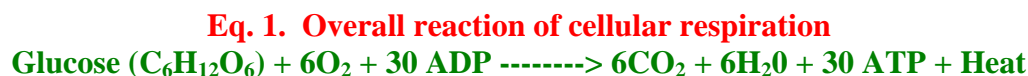
key words: try different combinations of: cell physiology, yeast, *Saccharomyces*, biofuels, fermentation, alcohol or ethanol production/manufacture, stress, and other words pertaining to your group's experiment.

## More information about cellular respiration

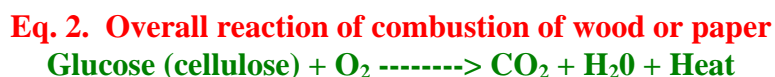
### What are the overall reactions and functions of cellular respiration?

For the Biofuel lab exercise you are not responsible for the details of the three stages of cellular respiration (glycolysis, Krebs cycle, and electron transport), but further information about these processes is provided below as a reference and for students wishing a deeper understanding.

The overall reaction of cellular respiration can be simplified as follows:



Outwardly, this reaction appears very similar to the combustion of wood or paper – indeed there are some intriguing similarities. Wood and paper are also made of glucose – in the form of cellulose – and when burnt, O<sub>2</sub> is used and the end products are CO<sub>2</sub> and H<sub>2</sub>O.

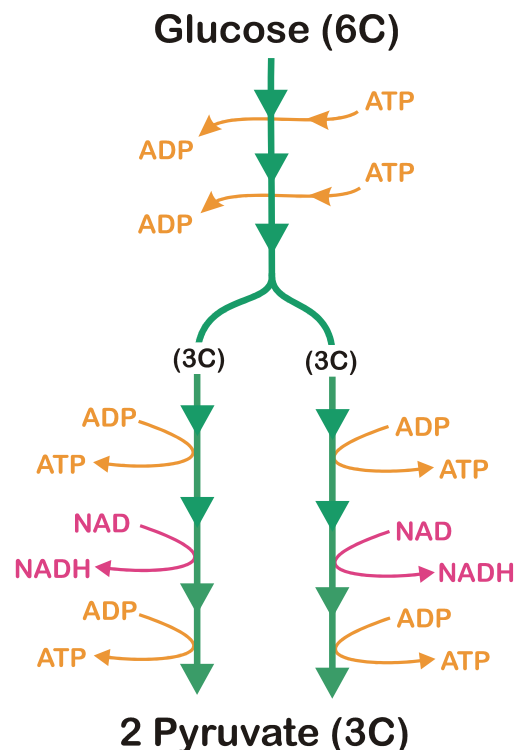


Obviously, there are a number of significant differences between these two processes. Combustion begins when the temperature is raised sufficiently high to cause O<sub>2</sub> to react directly with the atoms of cellulose. During the reaction much heat is released, and this causes more of the carbohydrate to burn, triggering an uncontrolled chain-reaction. Eventually, the majority of the energy stored in the carbohydrate is given off as heat. This is very advantageous when the purpose is to use the heat for warming ourselves or cooking food, but not very useful if we desire to store the energy for future use.

Thus, combustion is not very useful inside of cells, and despite alleged reports, ‘spontaneous combustion’ of people does not occur. As shown in Eq. 1, some heat is given off during cellular respiration – approximately 50% of the energy in glucose is released as heat. This heat is not merely lost, but rather is used to warm the cells and bodies of organisms. In cold weather, we maintain a body temperature of 98.6<sup>o</sup>F with the heat given off during cellular respiration (and maybe an occasional cup of hot chocolate). Some species can actually increase the amount of heat that is released during cellular respiration; skunk cabbage is one of the first species to emerge early each spring, often melting overlying snow with heat generated through cellular respiration.

But allowing all of the energy produced during cellular respiration to radiate away as heat would not meet the other energy needs. Cells retain energy for other

**Figure 2.** Overview of glycolysis. Energy from 2 ATP molecules is first added to the glucose to allow subsequent energy release. Two ATP can be produced per NADH through the action of the electron transport pathway.



uses and do so by storing energy temporarily in ATP. If we consider fats and polysaccharides as a long-term 'bank' of energy, then ATP is the 'pocket change' of energy currency. Organisms can withdraw fats and polysaccharides from their reserves when necessary, although such withdrawals do take time. Just as we find it convenient to quickly reach into a pocket and slip a few quarters into a vending machine, ATP is readily available to cells for rapid, immediate energy needs. (Imagine how inconvenient it would be if you had to go to the bank every time you wanted a candy bar.) We can infer from the above information that approximately 50% of the energy of the carbohydrates oxidized during cellular respiration is retained in ATP. This is the energy used by cells for all of their activities, from the synthesis of DNA and protein, to beating of flagella, muscle contraction and generation of nerve impulses.

### What are the functions of the glycolysis, Krebs cycle and electron transport pathways?

Each of the steps of cellular respiration serve specific functions. It is believed that glycolysis was the first ATP generating pathway to evolve. It exists in almost the exact form in all organisms, from primitive bacteria to higher animals and plants. In one manner of thinking, glycolysis initiates cellular oxidation of carbohydrates like a match triggers combustive oxidation of paper. When applied to a sheet of paper, a flame raises the temperature of the paper high enough to trigger combustion, which then spreads to envelop the rest of the sheet, releasing a much greater amount of heat. Likewise, glycolysis begins with a transfer of energy (from two ATP molecules) to the glucose molecule (Figure 2). This causes the glucose to become structurally unstable and allows it to be enzymatically split into two 3-carbon products. Through subsequent enzymatic reactions, energy is then extracted from these 3-carbon molecules. During glycolysis, enough energy is extracted to yield 4 ATP molecules (but with only a net yield of 2 ATP). Energy in the form of electrons is also held in a molecule called **NADH**, and these electrons later can be used to produce ATP in the mitochondria during electron transport. When glycolysis is completed, the carbohydrate will have been oxidized to two 3-carbon molecules called **pyruvate**.

By itself, glycolysis is not a very efficient process – approximately 95% of the energy of the original glucose molecule remains in the two pyruvates. The Krebs cycle and electron transport pathways (Figure 3) provide a means by which this energy can be further extracted. When pyruvate moves into the mitochondria, it is oxidized and enzymatically reduced by one carbon atom, which is released as CO<sub>2</sub>. The remaining 2-carbon molecule (acetyl-CoA) then enters the Krebs cycle, during which two more CO<sub>2</sub> molecules are released. During these oxidation steps in mitochondria, a total of 8 NADH molecules, 2 FADH molecules (which is functionally similar to NADH) and 2 ATP are produced per pyruvate.

During the process of 'electron transport' energy from electrons held in NADH and FADH are used to produce approximately 23 additional ATP. Overall, per glucose molecule, 30 ATP can be produced: 2 directly from the Krebs cycle, 23 during electron transport, and 5 through the combined actions of glycolysis and electron transport (3 from the NADH and 2 ATP produced directly during glycolysis).

The CO<sub>2</sub> that we exhale in our breath is produced during the breakdown of pyruvate in mitochondria; it is generated from carbon and oxygen atoms that already existed in the glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) molecule. The oxygen required during cellular respiration is only used in the final step of electron transport where it serves as the **terminal electron acceptor**. After yielding all of the energy that can be used for ATP production, the electrons are transferred to oxygen atoms, which then combine with H<sup>+</sup> atoms to yield H<sub>2</sub>O.

**Figure 3.** Overview of Krebs cycle and electron transport pathways of cellular respiration. Note that pyruvates are oxidized to  $\text{CO}_2$  just prior to and during the Krebs cycle. Although a couple of ATP arise during the Krebs cycle, the majority of ATP are produced from the NADH and FADH during electron transport.

