

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 CO₂ 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 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. When **biomass** (bulk plant matter) is the raw material an even wider variety of sugars may be present for yeast to ferment. Can different types of sugars be equally well fermented by yeast cells?

Carbohydrates include such molecules as glucose and fructose (simple carbohydrate subunits called **monosaccharides**) sucrose (common table sugar, which is composed of one glucose subunit and one fructose subunit) and lactose (found in milk), and starch, a large **polysaccharide** – a carbohydrate containing many monosaccharide subunits. Generally, glycolysis is said to begin with glucose, but cells often can use other carbohydrates as energy sources as well. However, 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

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 ‘tonicity’ of the solution (the concentration of all dissolved substances), which has effects independent of the particular type of sugar involved. Furthermore, sugar concentration also affects the tendency of yeast cells to employ aerobic versus anaerobic respiration.

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. NaCl at a concentration of 2% will cause ‘stress’ reactions within yeast cells (see discussion of **biological stress** above). 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. Yeast have served as a model organism for studying cellular responses to salt stress. Scientists hope that knowledge of how organisms respond to salt stress and why some organisms are more tolerant than others will lead to the development of salt-tolerant crop plants.

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

Berry DR. 1982. The Biology of Yeasts. London: Edward Arnold (Publishers) Ltd. 57 p.

[Chapters 3 (Nutrition and Metabolism of yeast)] This chapter briefly describes some yeast nutritional requirements and metabolic processes.

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. 310 p.

A general overview of yeasts, their environmental distribution, means of identification, etc.

II. Effects of physical parameters on yeast

Carlile MJ, Watkinson SC. 1994. The Fungi. New York: Academic Press. 482 p.

(Selected pages) A good source for general information about yeasts.

D'Amore, T. 1992. Improving Yeast Fermentation Performance. Journal of the Institute of Brewing 98: 375-382. Describes the effects of different variables, such as glucose, ethanol and magnesium concentration, on yeast 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.

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.

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.

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.

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: Academic Press. 285 p.

(Selected pages) Some brief descriptions of some factors (NaCl, temperature, pH and phosphate) that affect aquatic yeasts. Although *Saccharomyces* is not an aquatic yeast, responses of aquatic yeast can provide an interesting comparison.

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.

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.

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

(Selected pages from chapter 3) These pages describe some of the effects of temperature on yeast cell structure and function.

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.

III. More information about biofuel production and industrial uses of yeasts

Berry DR. 1982. The Biology of Yeasts. London: Edward Arnold (Publishers) Ltd. 57 p.

[Chapter 8 (Yeasts in Industry)]. This chapter provides a nice overview of industrial applications and processes involving yeasts.

Chang T. 1999. U.S. Consumption of alternative fuels growing. Oil and Gas J. 97:37-39.

(Full text of the article is available through ProQuest.)

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.

Rose AH. 1980. Recent research on Industrially Important Strains of *Saccharomyces cerevisiae*. In: Skinner FA, Passmore SM, Davenport RR, editors. Biology and Activities of Yeasts. New York: Academic Press. p 103-121. 310 p.

A nice discussion of the properties desirable of yeasts used for industrial purposes. Focus tends to be on beverage industries.

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.

Online bibliographic resources

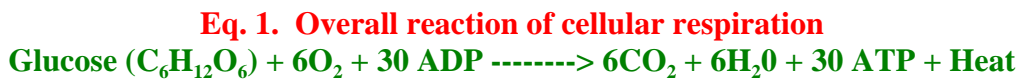
Biosis and BioAbstracts (databases of scientific literature), Electronic Journal Center (for online journals), McCat (Dawes Library; for on-campus holdings).

key words: try different combinations of: cell physiology, yeast, *Saccharomyces*, biofuels, alcohol or ethanol production/manufacture, cell or animal physiology, stress, and other words pertaining to the particular topic that you are studying in your student-designed experiment.

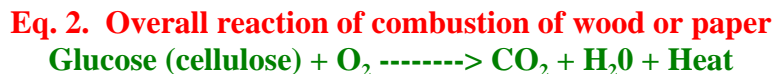
More information about cellular respiration

What are the overall reaction and function of cellular respiration?

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, O₂ is used and the end products are CO₂ and H₂O.



Obviously, there are a number of significant differences between these two processes. Combustion begins when the temperature is raised sufficiently high to cause O₂ 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° 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 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

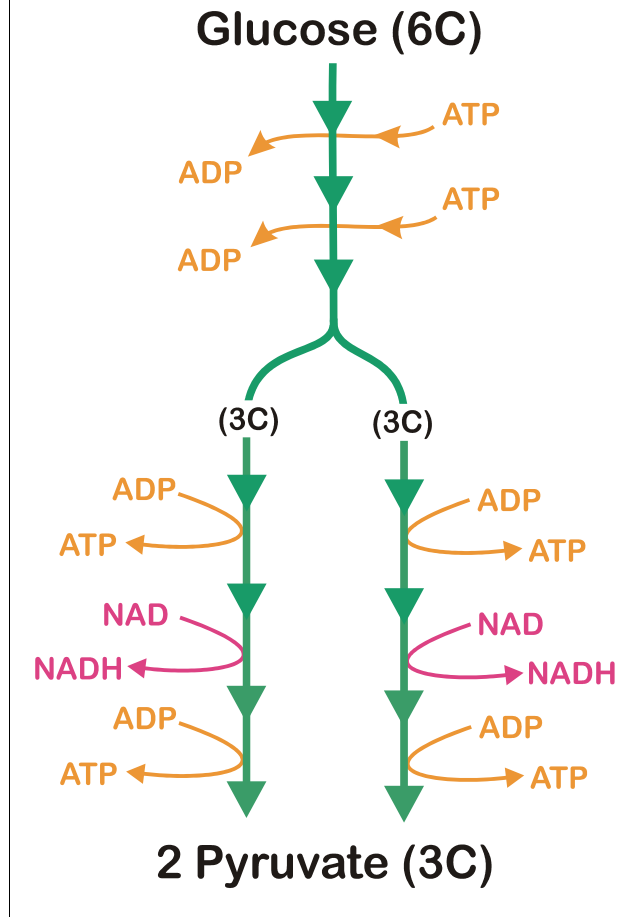
machine, ATP is readily available to cells for rapid, immediate energy needs. (Imagine how inconvenient it would be if you had to go to the bank every time you wanted a candy bar.) We can infer from the above information that approximately 50% of the energy of the carbohydrates oxidized during cellular 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 1). 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 2) 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 CO₂. The remaining 2-carbon molecule (acetyl-CoA) then enters the Krebs cycle, during which two more CO₂ molecules are released. During these oxidation steps in mitochondria, a total of 8 NADH molecules, 2 FADH molecules (which is

Figure 1. 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.



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_2 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 ($\text{C}_6\text{H}_{12}\text{O}_6$) 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^+ atoms to yield H_2O .

Figure 2. Overview of Krebs cycle and electron transport pathways of cellular respiration. Note that pyruvates are oxidized to 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.

