XCELIS® Ethanol Solutions

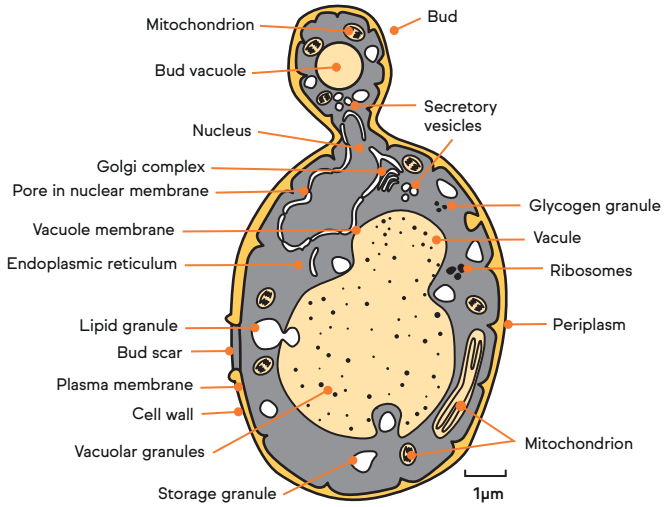
YEAST NUTRITION AND HEALTH

The requirements for yeast growth in the dry grind process

Introduction to yeast

Yeast are single cell (unicellular) organisms that carry out the process of converting glucose to ethanol and carbon dioxide. Yeast reproduce by budding and are eukaryotic members (contain membrane bound nucleus) of the fungus kingdom.

The yeast *Saccharomyces cerevisiae*, also known as brewer's yeast or baker's yeast, is commonly used in the dry grind ethanol process.



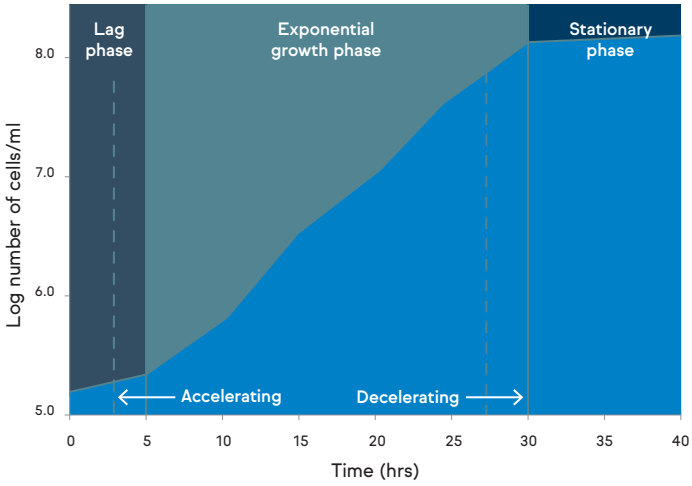
Lifespan and aging cycle

Yeast cells have a finite replicative lifespan that is affected by genetics and the environment. It is measured by the number of times a cell divides. During replication, a new bud/daughter cell is formed. The new cell divides when it reaches a critical size and the cycle repeats until the division limit of the cell is reached.

Factors that impact growth

During conditions suitable for growth, the following yeast growth phases are observed during fermentation:

- Lag phase: yeast cells mature and acclimate to the environment
- Exponential growth phase: yeast counts rapidly increase as cells grow and divide (i.e. where ethanol production increases)
- Stationary phase: growth slows down and yeast cells enter into a nondividing state



The primary factors that affect the growth of yeast include:

- Carbohydrates (e.g. glucose)
- Nitrogen
- Vitamins and minerals
- Water and oxygen/sterols and unsaturated fatty acids
- Suitable environment

If yeast nutrition is not maintained then the fermentation will suffer and result in lower ethanol rates and lower ethanol yield. The following is a more detailed discussion on the nutrients and environmental factors that impact growth.

Nutrient: carbohydrates

Glucose is the main carbohydrate source for yeast in the dry grind ethanol process. The long starch chains must be hydrolyzed (i.e. broken apart using water molecules) to make glucose readily available to the yeast. This is typically done by using an alpha-amylase in liquefaction to make shorter starch chains/dextrins followed by using a glucoamylase in the saccharification and fermentation (SSF) step to release glucose units.

Nutrient: nitrogen

The useable forms of nitrogen for yeast include ammonium ions, urea, amino acids, and small peptides (short chains of amino acids). Amino acids are taken up by the yeast in a specific order. For instance, the amino acid lysine is preferentially utilized/consumed by yeast compared to the amino acid proline. Proteases are enzymes that can help convert unavailable nitrogen in the form of protein to the more available nitrogen sources for yeast (smaller peptides and/or amino acids). The relative availability of different amino acids and other nitrogen sources may dictate the progress of a fermentation.

Nutrients: vitamins and minerals

Vitamins and minerals are crucial for optimal growth. Examples of vitamins include biotin and pantothenic acid. Examples of minerals include potassium, phosphorous, sulfur, and zinc. Providing adequate nutrition can alleviate some of the negative stress effects such as high alcohol and temperature. Yeast food with a combination of vitamins and minerals is commonly used in fermentation instead of adding each nutrient in separately. This can be beneficial as it is often difficult to track down if there is a specific nutrient deficiency.

Nutrients: water and oxygen/sterols and unsaturated fatty acids

Water and oxygen are both factors that influence cell growth. It is important to consider what is in the plant's water source (especially recycled water) that may be beneficial or harmful to yeast viability, such as ions. Oxygen is needed during the cell growth for synthesis of the cell membrane (sterols and unsaturated fatty acids (UFAs)); however, these sterols and UFAs may already be present, in which case oxygen isn't as critical. With oxygen present, yeast convert glucose and oxygen to carbon dioxide, water, and energy in a process known as aerobic respiration. This is essential in the propagation step of a dry grind plant to increase yeast biomass. When oxygen is removed, yeast convert glucose to ethanol, carbon dioxide, and some energy in a process known as anaerobic fermentation.

Aerobic respiration:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{Energy}$$

glucose oxygen carbon dioxide water

Anaerobic fermentation:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{Energy}$$

glucose ethanol carbon dioxide

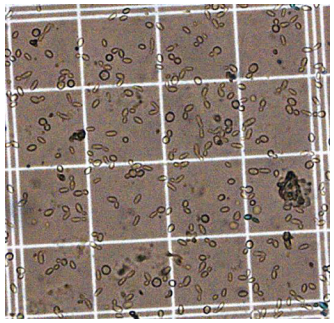
Environmental impacts on growth

Temperature, pH, microbial contamination, osmotic stress (e.g. high ethanol or salt concentration), and nutrient stresses (e.g. low nitrogen resulting in nutrient deficiency, osmotic stress from high sugar concentration) are all examples of potential environmental factors that can affect yeast health. For instance, typically a pH range of 3.5–5.0 and temperature range of 28–34°C is acceptable, although all conditions must be considered for optimal growth. These environmental factors can impact both yeast viability (number of cells alive vs. dead) and vitality (state of activity level/health of the yeast).

Healthy yeast = successful fermentations

Understanding the nutrients and environmental requirements is important for achieving the healthiest growing yeast and the most successful fermentations. Tools are available to help accomplish this, such as high performance liquid chromatography (HPLC) to monitor glucose and the use of a microscope to monitor yeast cell counts, budding, and viability.

Overall, the best results will come from growing and producing as many healthy cells for as long as possible.



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