Dealing with Elevated Fermentation Temperatures and Heat Stress

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As the temperature and humidity levels rise in the spring and summer months, ethanol plants must utilize their cooling towers to effectively deal with negative temperature effects on fermenta-tion and ethanol yields. Temperature control during fermentation is critical for preventing yeast stress and impacting ethanol yield. To operate smoothly in higher temperatures, planning and pro-cess adjustments are required.

How does heat stress impact yeast health and performance?

Any environmental condition deviating from normal conditions can be considered a stressor. The inability to precisely control fermentation temperature is the most common factor impacting etha-nol yield. Elevated fermentation temperatures are often a result of higher solids content, additional nitrogen, and more extensive ethanol production. Most of the heat generated during fermentation takes place between 10 and 30 hours into fermentation when the yeast activity is highest. Within the first 30 hours of fermentation the heat released can be up to 44,000 BTU (46,500 kJ) per 100 lbs of ethanol or 7450 BTU (7857 kJ) per 56-lb bushel (25.4 kg) of fermented corn. Heat removal from fermentation is often a bottleneck for most plants. It is imperative that plant incorporate enough cooling capacity to account for peak summer rates of production at the highest sugar levels possible.

The optimum fermentation temperature for yeast growth and activity is 32°C to 35°C (90°F to 95°F). *Saccharomyces cerevisiae* is tolerant of higher temperatures in the early stages of growth, but as ethanol levels rise and other conditions of stress occur the yeasts become even more stressed and many of the cells begin to die. The maximum temperature is not as critical on yeast health and activity as is the length of time spent at the higher temperature. For cells exposed to elevated growth temperatures there are a variety of possible target sites for heat-induced injury including proteins which can aggregate or denature, cell membrane damage, leading to permeability changes and ion leakage, ribosome breakdown, and DNA strand breakage. It has been speculated that the cell membrane is the target site for thermal damage and can ultimately lead to cell death. More recently it has been proposed that an important component of heat injury is the effect on cell membranes leading to increased fluidity and the permeability of the membrane to protons and other ions. Increased levels of ions can lead to delayed effects resulting in alteration of the composition of mem-brane proteins as well as lipid saturation.

1. Heat Shock Proteins (Hsp)

The heat shock response in yeast has been extensively studied. Yeast cells exhibit a rapid molecular response when they are exposed to elevated temperatures. Sub-lethal heat shock of yeast cells lead to the induction of synthesis for a specific set of proteins, the highly conserved group of heat shock proteins (Hsp). Chaperoning Hsp proteins prevent protein aggregation, ensure proper folding or re-folding of denatured proteins, and assist in the degradation of stress-damaged proteins. At normal cell growth conditions, the Hsp enzymes are expressed at low levels, but they are strongly induced when temperatures are elevated. Yeast cells respond by accumulating putative protecting compounds such as trehalose, enzymes such as catalase, and mitochondrial superoxide dismutase, which permits trapping of superoxide radicals that increase under heat shock conditions.

The response of yeasts cells to elevated temperature that is not lethal leads to the rapid induction of substantially increased thermotolerance up to 45°C (113°F). This initial heat stress is accompanied by an accumulation of trehalose which, together with a specific Hsp, acts synergistically to confer thermos-protection by inducing heat shock proteins. These proteins are produced at high rates for about 30 min, then the rates decline to steady-state levels. Subsequently the cells will recover, resume growth at the elevated temperature and maintain thermotolerance. Since this process involves the shift of carbon metabolism away from ethanol fermentation towards increased glycolysis and accumulation of trehalose, ethanol yield will be decreased.

2. Trehalose

Trehalose is a non-reducing disaccharide that accumulates in yeast cells under conditions that reduce their growth rate. Trehalose is mostly produced and accumulates late in fermentation when stressors are high. This can often be seen in an elevated DP2 peak late in fermentation. Under stressful conditions yeast can accumulate trehalose up to 15% of the cell dry mass. While trehalose plays an important role in thermotolerance, it cannot assist in refolding damaged proteins. Trehalose is more effective in protecting proteins against denaturation and aggregation because of its unusual ability to alter the water environment surrounding proteins. Ethanol can substitute for water and alter the positioning of molecules on the cell membrane, influencing the interactions between lipids and proteins, and ultimately damage the structure and function of the membrane. During ethanol stress, trehalose functions as a chemical co-chaperone, which means that the increased trehalose prevents protein denaturation and the aggregation of misfolded proteins in the cell membrane. At high concentrations of ethanol, trehalose will displace the ethanol on the yeast membrane, and the subsequent formation of hydrogen bonds between the hydroxyl groups of trehalose and the polar groups of lipids stabilize the cell membrane. Therefore, the accumulation of trehalose may create an optimal intracellular environment under ethanol stress conditions.

Trehalose also acts in vitro to protect enzymes from heat, and heat shock causes a very rapid accumulation of the disaccharide in the cytoplasm. Trehalose will accumulate transiently following heat shifts, and at temperatures above 40°C (104°F) it can accumulate to very high levels. It has been suggested that under conditions of heat stress there may be a recycling of trehalose since both systems for synthesis and degradation of trehalose are activated by mild heat stress and salt shock.

Under conditions of starvation, neutral trehalase is the main enzyme produced by the yeast to degrade accumulated trehalose to help restore nutrients such as metabolizable nitrogen compounds, phosphate and sulfate to cells starved for nutrients in the presence of glucose, or by adding fermentable sugars to cells in stationary phase.

What are some recommendations for dealing with temperature stress?

The easiest way to reduce fermentation temperature is to reduce the sugar level going into fermentation, thereby reducing yeast growth and activity. Another way to deal with higher temperatures is to incorporate temperature staging. Temperature staging is where the temperature is gradually reduced to lower levels than typical later in fermentation to remove the temperature stress and avoid premature yeast death. Here are some more recommendations that should be considered and implemented.

1. Chiller inspection

The cooling towers often do not provide sufficient cooling to run fermentation and downstream processes at the same capacity as during cooler months. To supplement the cooling requirements, many plants have chillers, which are refrigeration systems that focus cooling within the process. These chillers require a large amount of electricity to run, which adds to operational costs. However, the cost of operating a chiller is minimal when compared to lost production caused by fermenting at too high a temperature. It is recommended to have chillers inspected and in good working order prior to the hotter months.

2. Prepare for increased copper levels

Chillers can increase the copper content of cooling water blow-down, the water drained from cooling towers to remove mineral build-up. It is important to know your permitted copper levels prior to starting chillers. It is also recommended to discuss your permitted copper levels with water treatment vendors to avoid potential mishaps that could arise from the elevated copper levels.

3. Allocate chilled water resources

Since fermentation will demand much of the available chilled water, it is important to reserve some for distillation exchangers. It is important to confirm that the plant is effectively balancing cooling water between fermentation and downstream processes. We recommend plants develop a heat exchange strategy by mapping out cooling tower control valves and open percentages. The plan should include prioritizing chilled water to fermentations during the highest metabolic state, which typically occurs 12 to 24 hours into fermentation.

4. Standardize chiller water allocation procedure

Standard procedures for all parts of the plant are important for reducing process variability. Minimizing the chance of excessively hot fermentations, temperatures ≥96°F (35.5°C), is critical. Hot fermentations will impact yeast growth and cause conditions that are more favorable to bacterial infection. Hot fermentations can lead to lower ethanol yield, increased organic acid concentrations, and higher remaining sugars.

5. Avoid repeatedly turning chiller on and off

Once the chiller is turned on, it is important to minimize the number of times the chiller is turned off and on. The greatest cost associated with running a chiller is the high peak electrical demand needed to turn it on. Once the chiller is on, it is recommended to leave it on.

6. Decrease corn solids loading

To help control yeast metabolism and fermentation temperatures, it is recommended to reduce corn solids. Checking the temperature forecast every 24 hours, along with planning solids loading accordingly, can help avoid yeast temperature stress. The higher the temperature, the lower the solids loading should be. It is also recommended to maintain the temperature of the mash entering the fermenter at 88°F (31°C). Starting at a lower temperature will help prevent the fermentation from getting to 96°F (35.5°C), thereby reducing the potential stress on the yeast.

7. Monitor supplemental nitrogen

The best way to ensure appropriate fermentation conditions is to give the yeast what they need at the time it is needed. During warmer months, plants must pay closer attention to how nitrogen is being dosed. Nitrogen will accelerate yeast metabolism and is critical in mitigating yeast stress. However, if too much nitrogen is dosed early on in fermentation, more heat will be produced. Regular monitoring and adaptation can help reduce temperature stress by slowing the fermentation down. It is recommended to consider using a protease to supply amino nitrogen that the yeast can utilize. This can also help combat heat stress, while reducing the need for supplemental nitrogen.

References

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