XCELIS® Ethanol Solutions

FERMENTATION TROUBLESHOOTING

Understanding and maintaining a healthy fermentation

Introduction

Alcohol fermentation is the process by which yeast (commonly *Saccharomyces cerevisiae*) convert glucose to ethanol and CO_2. This fermentation has been around for thousands of years to produce a variety of alcoholic beverages. More recently, alcohol fermentation has been used for the industrial production of ethanol as a fuel.

The success of a fuel alcohol fermentation is dependent on the dynamic and complex interactions of a variety of fermentation conditions that must be well understood and monitored closely. This guide functions as a tool to help maintain a healthy fermentation.

Goals of Fermentation

In the dry grind industry, saccharification and fermentation primarily occur simultaneously to improve starch hydrolysis rates and ethanol yield as compared to separate systems. The saccharification step uses a gluco-amylase enzyme to hydrolyze, or breakdown, dextrins (shorter starch chains) to glucose. Combining these two steps together is referred to as simultaneous saccharification and fermentation (SSF).

There are four main goals of SSF:

- Dextrin conversion to glucose
- Glucose consumption
- Minimization of byproduct production
- High ethanol production

Factors Affecting Fermentation Performance

Numerous factors contribute to the efficiency of converting starch to ethanol. The majority of these factors cause stress to the yeast or are a result of stress on the yeast. Improper yeast nutrition and/or the presence of environmental stressors, such as osmotic stress (e.g. glucose stress) and heat stress, can potentially lead to poor performance during SSF.

Fermentations that stop early before the available sugar is converted to ethanol are known as stuck ferms, and slow ethanol rates are known as sluggish ferms. Stuck or sluggish fermentations leading to lower ethanol yields and high residual sugars are common indicators of yeast stress. Rarely is only one source of stress present during fermentation, and conditions that are normally below inhibitory levels can induce stress in the presence of other factors/inhibitors.

Detecting Issues in SSF

With so many potential factors that can negatively impact SSF performance, it is inevitable that issues will eventually occur. Being able to quickly detect and resolve issues can save a substantial amount of money and time. Here are two quick guidelines to help detect problems during the process:

1. Gather data under standard/“normal” operations to determine a baseline. Baselines should be updated after any significant process change. It is generally recommended that 1 month of data be used to establish a baseline.
2. Continuously monitor SSF conditions and performance for deviations from the established baseline. Try to identify the cause of variation and make the proper correction(s) to return to standard operations.

The following is a more detailed discussion on issues commonly found during fermentation.

Nutrient Deficiency

Yeast need nitrogen, minerals, and vitamins to convert glucose into ethanol. These nutrients are almost entirely provided by the corn, but certain ones are generally added to the fermenter to improve yield and shorten fermentation times. The most common nutrient added is nitrogen, which can be provided as FAN (free amino nitrogen), urea, or ammonia. Protease enzymes break down proteins in the corn to provide FAN. The resulting FAN can be easily taken up and used by the yeast.

Inadequate nitrogen can severely slow fermentation or result in a stuck fermentation. If this occurs, it is recommended to verify nitrogen addition has not changed and to increase the amount of nitrogen into the process if needed.

Insufficient Enzyme Dosing

Gluco-amylases and accessory enzymes are used during the SSF process to release as much glucose as possible, which can then be converted to ethanol by the yeast. Improper enzyme dosing can lead to a variety of fermentation problems. If too low of a gluco-amylase dose is used, the yeast will have an inadequate supply of glucose and this can result in a sluggish fermentation. Alternatively, if too high of a gluco-amylase dose is used, the yeast may suffer from osmotic stress due to the overabundance of glucose early on. This can result in reduced ethanol production.

It is important to follow the enzyme supplier's recommended dosing strategy. Additionally, ensure that the accurate amount of the enzyme is being added to the fermenter by checking that the target DCS flow rate is correct for the current grind rate. The enzyme dose can also be checked by monitoring if the enzyme tank level is descending at a proper rate.

Microbial Contamination Present

Occasionally growth of unwanted organisms can occur that can result in decreased ethanol titers as the contaminating organism(s) consumes sugar. Bacterial infections can generate lactic and acetic acid, which act as stressors to the yeast. These acids can become even more toxic if the pH of the fermentation decreases as these acids more easily cross the yeast membrane to disrupt cellular function. Infections can be reduced by following the proper plant hygiene protocols, minimizing process upsets, and treating with antibiotics.

Fermentation Byproducts as Inhibitors

Inhibitors can come from a variety of sources in a fuel alcohol plant. Yeast themselves make inhibitors, producing small amounts of acetic acid and fusel alcohols as byproducts during the fermentation process. Fusel alcohols are known to have a distinct unique odor when present. The byproducts may cross the yeast membrane to disrupt cellular function. If inhibitors are not dealt with appropriately they may not only affect the current fermentation, but could also be recycled in water streams to negatively impact the yeast at the beginning of the next ferm. Thus, removal of inhibitors is necessary to produce maximum yields.

Sub-Optimal pH and Temperature Conditions

Conditions should be selected to maintain enzyme and yeast fermentation performance. Two of the most important conditions are temperature and pH. Generally, the typical enzymes and yeast used in the process are robust enough to tolerate slight variations in temperature and pH. However, process excursions may lead to a decreased performance, especially towards the end of fermentation when other stressors are present (e.g. high alcohol concentration). Temperature staging with lower temperature toward the end of ferm can offset the impact of other stressors to result in improved yeast performance. It is important to follow the recommended pH and temperature ranges given by the enzyme and yeast supplier(s).

Quick Tips

As discussed above, elevated organic acids, high residual sugars, and atypical odors are some of the main metrics that can indicate a potential issue in SSF. Here are some quick tips to help troubleshoot when these metrics indicate a possible problem:

- *High levels of organic acids throughout fermentation (acetic and/or lactic acid present)?* Check for an infection
- *High residual glucose present at the end of fermentation?* Evaluate if there are factors that are negatively affecting yeast health (such as environmental stressors or nutrient deficiencies)
- *High residual sugars, but not high glucose, remaining at the end of fermentation?* Evaluate if the gluco-amylase enzyme dosing strategy is adequate, and check that the pH and temperature have not shifted from their target value (which should be set at a value that is appropriate for sufficient enzyme performance)
- *Unusual odor noticeable in fermentation samples?* Check to see if fusels are not being removed effectively downstream

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