ENZYMES:

ESSENTIAL PROCESS AIDS

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Introduction

For craft distillers working with starch feedstock, enzymes are essential processing aids. Enzymes are required to achieve fermentation rates, yield and completion, in addition to indirectly gaining favorable and desired sensory precursors when moving to distillation. Understanding the roles of enzymes is an important step towards making your desired vodka or whisky.

Various substrates can be used in the production of spirits and different processing methods are often involved. Some raw materials are easier to work than others, as they do not require a lot of processing steps. This is due to them already containing sugars (glucose, fructose, sucrose and/or some others) in forms that can be utilized by yeast. This type of feedstock is called sugar-based and it is represented mainly by various fruits and molasses.

Another basic feedstock type is **starch based**, which is widely used in the production of GNS, vodka, gin and whisky, among other spirits.

It is represented by various cereals (corn, rye, barley, rice, etc.) and some roots, e.g. potatoes. Fermentable sugars in this case are stored in the form of a glucose polymer (chains) – starch. Starch cannot be fermented by yeast directly and must be broken down to fermentable sugars: glucose, maltose. **To break starch down to fermentable sugars, enzymes are required.**

Traditionally, before commercial enzymes appeared on the market, malt-sourced enzymes were used. During the natural malting process in cereals, various enzymes are synthesized, which luckily for us can be used to break down the starch into sugars. This approach is still widely used in the brewing and malt whisky industries today.
Efficient malting is not only costly, and time consuming but malt-sourced enzymes are also very sensitive to pH and temperature, meaning they are complicated to use. As a result, when the enzymes, as we know them today, first appeared on the market, it was able to make distillers' lives easier in addition to allowing the use of a wider range of feedstocks.

Commercial enzymes allow more efficient conversion of starch into fermentable sugars compared to malt sourced enzymes. Some malt whisky producers (outside Scotland) often add commercial enzymes to their process to increase efficiency.

As a yeast producer, here at *Lallemand Craft Distilling* we understand the importance of the correct choice and application of enzymes. Only a balanced application of enzymes and yeast will allow for good efficiency: high ethanol yield, fast kinetics and the desired aromatic profile.

What are Enzymes?

Enzymes, as opposed to yeast, are not living organisms. Enzymes are specific protein molecules and can be sourced from malt or, when produced commercially, from some bacteria and fungi.

The role of enzymes, is as a biochemical catalyst, meaning they can increase the rate of chemical reactions. For example, starch breaking down to dextrins or, peptides breaking down to amino acids. It is also very important to understand that each enzyme can only work on a specific substrate and can only catalyze one type of reaction. In other words, it works as a lock-and-key system, one enzyme for one substrate, one key for one lock (Fig.1).
This means that for any specific need, you will need to use one specific enzyme.

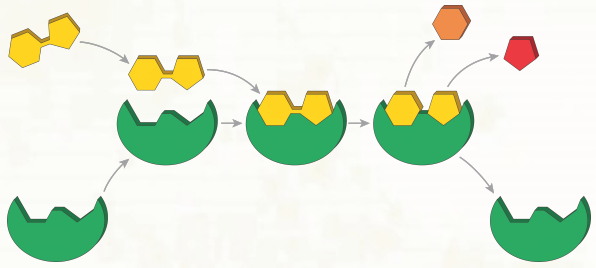

Figure 1: One enzyme- one substrate.

When producing spirits from starch based feedstocks (grains, potatoes, etc.) at least 2 enzymes will be needed for the starch to breakdown to glucose which can then be fermented by yeast: **alpha-amylase** (AA) and **glucoamylase** (GA) (Fig.2).

When using grains, such as rye, barley and oats among others, you may experience problems with non-starch viscosity, therefore it is beneficial to use enzymes for viscosity reduction– **β-glucanase**. The final enzyme is **protease**, which (if proteins are present in the feedstock) can generate nutrition for yeast.

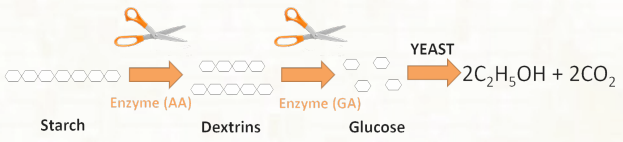

Figure 2: Starch conversion to alcohol.

Alpha-Amylase (AA)

In the presence of water and heat, starch granules swell, a process known as gelatinization. During gelatinization, the viscosity of the mash increases and becomes very difficult to pump. When alpha-amylase is added, the process known as dextrinization (liquefaction) occurs. As a result of this enzymatic hydrolysis, shorter glucose chains (known as dextrins) are obtained and viscosity decreases.

In order to prepare a starch based feedstock for fermentation, it first has to be milled. Milling significantly increases the surface which water and enzymes can reach. Good quality milling and following mashing temperature recommendations (usually in the range 75-90°C) are extremely important to ensure the enzymes work efficiently.

When optimizing your process parameters, (temperature, pH, residence time, and dosage) methods to evaluate enzymes efficiency are needed.

Despite there not being many readily available tools for craft distilleries**, here are some common methods on how to control AA efficiency:**

- *Iodine test:* add a drop of iodine solution to a mash sample and then assess its color (Fig.3). If the starch was not hydrolyzed, the result will be a blue-purple color. By the end of an efficient enzymatic hydrolysis, the result should be a brown to yellow-brown color, meaning that the starch was converted to dextrins and it is now ready for GA.
  Although this test doesn't provide a specific value, it can still be used as a good indicator of AA efficiency.

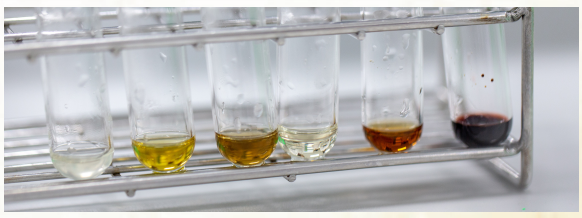

Figure 3: Example of what the results of an Iodine test could look like.

- *Have a look*: the less viscous your mash is and the easier it is to mix or pump, the better your AA is working. The rule of thumb is that if you can pump your mash – AA is working correctly.

Glucoamylase (GA)

As we already know, after alpha-amylase has finished its job, (although it will continue to work slowly during fermentation) the mash contains dextrins, which also cannot be fermented by yeast. These glucose chains are still too big to get inside of the yeast cell for fermentation.

To finally break dextrins down to fermentable sugars a 2nd enzyme, glucoamylase, is added. This process is called saccharification. Glucoamylase works by cutting glucose molecules from the dextrin chain (Fig.4). GA can be added after liquefaction when the mash is cooled down to approximately 58-60°C. After this step, the mash is further cooled down to fermentation temperature and yeast is added. However, these days this method is not recommended as it can result in contamination and osmotic stress to the yeast due to high glucose concentration in the mash.

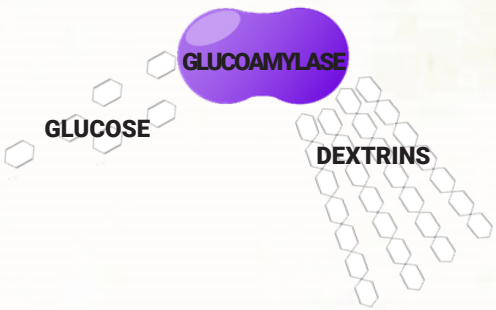

Figure 4: GA removes glucose units from the end of the dextrin molecule, providing fermentable sugars for the yeast.

Today, the most common way to work with GA is **Simultaneous Saccharification and Fermentation (SSF):** in this case, following liquefaction, the mash is cooled down straight to fermentation temperature and GA is added together with yeast directly into the fermenter. When this method is used, GA's activity is reduced compared to when added at 58-60 °C and provides a slower release of glucose. This technique avoids osmotic stress to the yeast and therefore results in better yield. Gradual release of sugar also helps to reduce the contamination risk.

It is very important to have the right dosage of GA in order to achieve good efficiency.

To control GA efficiency in a craft distillery you can use these basic methods below:

- *Evaluate rate of fermentation*: this can be done by a Brix refractometer (Fig. 5) or densimeter (hydrometer). If during fermentation, Brix or gravity is going down too slowly, the reason for this will need to be identified (there are numerous reasons for this occurring).

    * *Too low a GA dosage*: GA is releasing glucose very slowly, meaning the yeast is starving and fermentation will not be complete.

    * *Too high a GA dosage*: even though it seems that this would speed up your fermentation, too much glucose from the start can cause high osmotic stress, meaning the yeast will become suppressed, and therefore in such conditions, the yeast will not be able to ferment efficiently.

- A basic tool that can be used is a glucometer (often used to measure blood sugar levels), to measure glucose concentration in your fermentation (however, you will need a dedicated formula to convert your glucometer readings). We recommend having 2-3% of glucose within first 24-36 hours.

Viscosity Reducing Enzymes

Grains like wheat, barley, rye and oats among others, contain higher amounts of non-starch polysaccharides compared to corn. These components, mainly β-glucans and xylanes, cause significant increases of viscosity during mashing. This increase

makes it difficult to pump the mash, therefore reducing the efficiency of alpha-amylase, and limits your mash solids, often resulting in lowered final alcohol concentration at the end of fermentation. Failure to reduce this viscosity can result in problems in mashing, fermentation and distillation.

To prevent issues with high viscosity when working on such a "difficult" substrate as rye (and others) it is recommended to use a dedicated viscosity reducing enzyme. Commercial enzyme products are usually not pure enzymes. However, products with mixed activities of β-glucanase and xylanase among others, will break down gummy compounds, decrease viscosity and these make your process more efficient.

Evaluation of viscosity reducing enzymes efficiency can be made by looking at viscosity: as its goal is to allow good mixing and pumping.

Protease

Most grains also have proteins which are a good source of nutrition for yeast. But as with starch, the yeast cell cannot utilize proteins directly. In order to make proteins available for yeast, it needs to be broken down into small "bricks" – amino acids. Amino acids are used by yeast as a nitrogen source to build new healthy cells.

If using inorganic nitrogen (for example DAP), an addition of protease is still useful. DAP is consumed by the yeast in the first hours of fermentation, allowing a good start, but protease releases amino acids throughout fermentation. This provides valuable nutrition for the yeast even at the end of fermentation, when the yeast is under many factors of stress (alcohol, organic acids, lack of nutrition), therefore allowing complete fermentation.

There are no specific tools you can use in a craft distillery to assess efficiency of protease, but you can always have a look at fermentation kinetics (shorten fermentation time) and assess how efficient your fermentation finish is: increase of ethanol content and decrease of residual sugars.

Enzymes Operating Ranges

All enzymes have specific temperature and pH ranges within which they can operate. Enzymes suppliers should provide information on best operation practices together with recommended dosing rates. It is very important to follow these values in your process, as when you go outside of these ranges, efficiency might decrease or at some point enzymes could stop working all together.

Conclusion

Enzymes are an important part of the craft spirit making process. Tailoring yeast needs for fermentation with the addition of enzymes to starch-based feedstock is a key aspect for efficient fermentation.

This article presents a general overview of enzymes in relation to how to use, manage and optimize these essential processing aids. The team at LBDS is more than happy to provide further information, advise in greater detail and offer solutions according to your process.