Termamyl® SC

A novel alpha-amylase for liquefaction of whole grain

Presented at the 1999 Starch Convention in Detmold, Germany, by Larry W. Peckous, Novozymes Biochem

Introduction

Novozymes has designed a new alpha-amylase specifically for the liquefaction of whole-grain mashes in the production of potable and fuel ethanol. Fuel ethanol producers no longer have to be satisfied with enzymes designed for starch wet-milling, which often perform poorly in the highly demanding dry-mill environment.

Starch enzymes in the fuel ethanol industry

There are many examples of enzymatic solutions to the challenges found in the starch wet-milling industry. The starch industry has new blends of enzymes to produce exceptionally high levels of dextrose, maltose or cyclodextrins. There are new isomerases, transferases and debranching enzymes. However, the industry that processes whole-grain mashes into whisky, vodka, neutral spirits and fuel ethanol has received far less attention. Despite the fact that the fuel ethanol industry processes approximately 100,000 metric tons of grain per day worldwide, the whole-grain (or dry-milling) industry has had to make do with enzymes designed for the starch industry. Although enzymes designed for the starch wet-milling industry can be made to work in whole-grain applications, they are inefficient.

In recent years, the dry-milling process has become automated and highly controlled. This improvement in process consistency has highlighted the importance of thorough liquefaction to the efficiency of the whole-grain fermentation process. Unfortunately, liquefaction enzymes designed for the starch wet-milling industry often have a hard time standing up to the stressful conditions found in whole-ground grain processing and inconsistent starch conversion results.

Some of the concerns of the dry-milling industry for a liquefaction amylase include consistent conversion at decreased calcium ion levels and at lower pH values. Further, the industry wants to see rapid viscosity reduction in the mash, energy cost reductions and efficient utilization of recycle streams. Table 1 contrasts the needs and concerns of these two starch-processing industries.

Need or concern	Wet-miller	Dry-miller
Low calcium requirement	Lowers ion exchange cost	Decreases Ca-oxalate formation (such as "beer stone")
Low pH tolerance	Lowers ion exchange cost and reduces caustic use	Allows more backset utilization and reduces caustic use and salt formation
Viscosity reduction	Usually sufficient at 10 DE	Quicker is better
Ultimate viscosity	Not as critical	Impacts heat exchanger and fermentation efficiency
Survivability (past liquefaction)	Undesirable (creates panose)	Desirable (improves ultimate conversion in fermentation)
Saccharide profile of liquefact	Important for peak dextrose	Relatively unimportant
Hydrolysis of partially bound starch	Not an issue	Can greatly increase ethanol yield

Table 1. The different concerns of wet- and dry-millers.

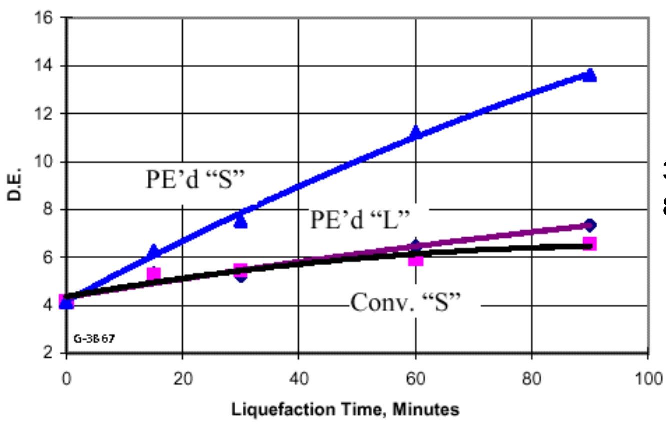
Termamyl SC for the fuel ethanol-producing industry

Termamyl SC is a major step towards addressing the needs of the long-ignored fuel ethanol industry. Using similar protein engineering methodology to that employed in the development of the protein-engineered *Bacillus licheniformis*, Termamyl LC, Novozymes research scientists in Denmark have created a new alpha-amylase specifically for the dry-milling industry. Termamyl SC is a protein-engineered enzyme derived from *Bacillus stearothermophilus* and expressed in *Bacillus licheniformis*. While these two enzymes were made via similar techniques, the enzymes perform quite differently. Following is a review of how Termanyl SC performs relative to key parameters found in whole-grain conversion to ethanol.

Calcium

It is well known that conventional thermostable alpha-amylases require certain metals, notably calcium, for stability. Calcium ions help keep the protein domains comprising the enzyme in the proper spatial configuration for activity as a biocatalyst. Calcium levels of 20-50 PPM were typically needed to stabilize conventional "wild type" alpha-amylases from *Bacillus stearothermophilus* or *licheniformis* in purified starch slurries.

However, whole-grain mashes contain high levels of lipids, proteins, phenols and phytins, all of which complex the calcium. These constituents tend to destabilize conventional alpha-amylases, leading to high enzyme usage or inadequate liquefaction. Figure 1 compares the performance of conventional *B. stearothermophilus* and protein-engineered *B. licheniformis* enzymes with protein-engineered *B. stearothermophilus* in whole-grain mashes under typical industrial conditions.



31% Dry Solids, pH 5.6, 85ºC, 0.6 kg/MT (d.s.b.)

Fig. 1. Protein-engineered types "S", "L" and conventional "S" at industrial conditions.

Lower pH

Decreased operating pH is an important benefit to the dry-miller. Being able to liquefy at pH values lower than those found in the wet-milled starch industry is a double advantage for the whole-grain processor. First, most processors recycle backset from fermentation, which lowers the pH of the mash from the start. When mash pH is raised to accommodate an alpha-amylase, it requires even more acid to lower again just prior to saccharification. This pH adjustment requires one more unit operation. Secondly, this pH shifting results in higher salt concentration, decreasing the efficiency of yeast during fermentation. Less pH adjustment means lower chemical costs for this operation and less time to do this. Figure 2 shows that varying the pH in the range of 5.7 to 5.0 has no significant effect on the performance of our new alpha-amylase. Figure 3 illustrates that only about half as many kilograms of the new *B. stearothermophilus* enzyme is needed, even at lower pH.

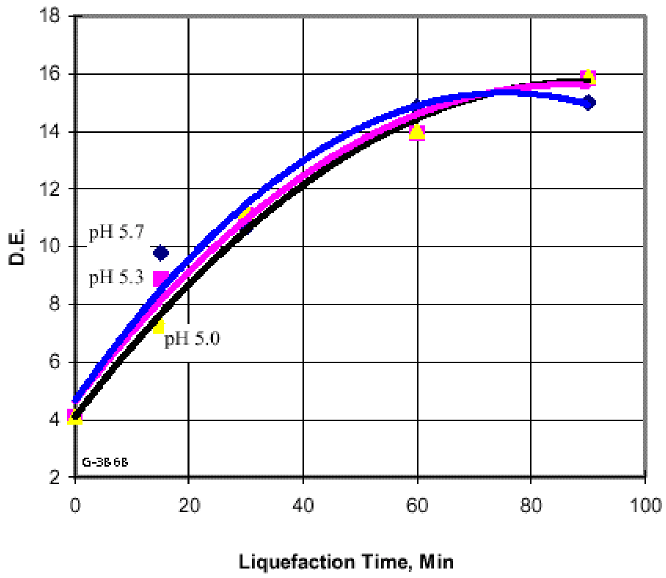
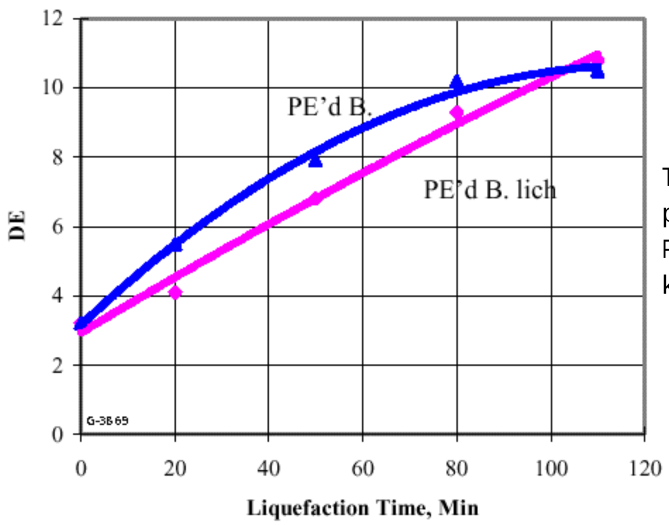


Fig. 2. pH stability of new PE'd *B. stearothermophilus*.



Termamyl SC 0.30 kg/ton pH 5.25
PE'd *B. lich*, alpha 0.54 kg/ton, pH 5.55

Fig. 3. At 55% of the dose and 0.3 units lower pH, PE'd *B. stearothermophilus* alpha-amylase is approximately equal to PE'd *B. licheniformis*.

Calcium oxalate

Calcium oxalate formation in beer is a serious problem due to the time required to clean the refractory and insulating scale from the process pipes and heat exchangers. Oxalate scale also diminishes the efficiency of the heat exchangers, raising energy costs. There are two practical ways to reduce calcium oxalate precipitation in whole-grain processing. One is to decrease the calcium in the system. The second is to lower the pH, so the oxalate is not formed. A protein-engineered *B. stearothermophilus* alpha-amylase helps in both cases. It allows ethanol producers to eliminate the addition of calcium, due to its low calcium requirement. Additionally, it can operate as low as pH 5.0; a full pH unit lower than most liquefactions are run at today. This lower pH tolerance allows the user to eliminate pH adjustment, or add more acidic backset to the mash. Either change is good for the dry-miller's production economy.

Viscosity reduction

Whole-grain processors need an enzyme that rapidly reduces mash viscosity. Lower viscosity improves heat transfer efficiency in the heat exchangers, and it allows the plant to process higher dry solids levels. Only a few years ago, dry-millers used to operate below 30% dry solids. 35% dry solids is common today, with some dry-millers running at 38%. The energy reduction in steam heating of the mash, vacuum cooling of the cooked liquefact and wort cooling prior to fermentation is significant. The action pattern of our protein-engineered alpha-amylase from *B. stearothermophilus* decreases viscosity more quickly than other enzymes (see Figure 4).

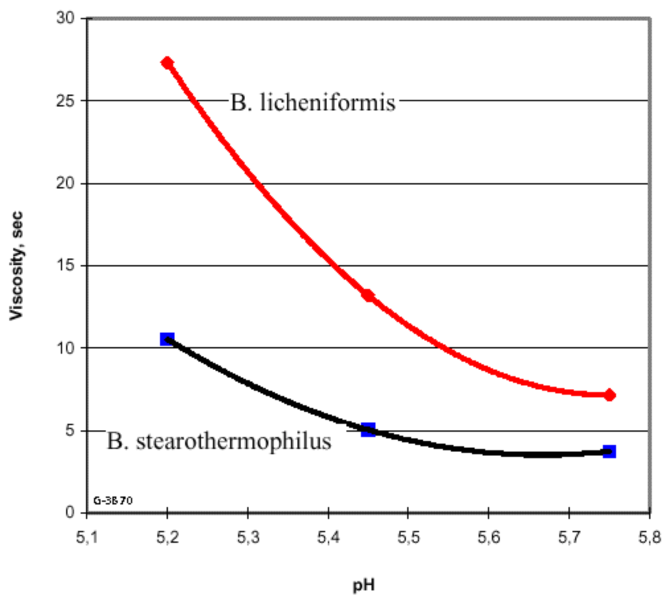


Fig. 4. Viscosity reduction at 90 minutes for PE'd *B. licheniformis* and *B. stearothermophilus*.

Some other viscosity-derived benefits for some plants include:

- Improved heat exchanger efficiency;
- Better heat transfer in the fermenter;
- More rapid release of dissolved carbon dioxide in the fermenter; and
- The ability to run higher solids.

Consistent breakdown of starch

Breakdown of the gelatinized starch to soluble oligosaccharides must produce a consistent product. When viscosity is high, there is often poor mixing that results in some mash flow "short-circuiting" directly to the outlet, while other mash remains in the system for a longer time. This results in long-chain oligosaccharides going into Simultaneous Saccharification and Fermentation (SSF) that can retrograde into non-fermentable carbohydrates. Novozymes's new protein-engineered alpha-amylase from *B. stearothermophilus* allows more ideal mixing due to its rapid viscosity reduction. Further, its improved stability allows it to survive the liquefaction process to continue its action in saccharification and/or fermentation ensuring maximum ethanol yields.

Another aspect of getting greater viscosity reduction in the mash is that jet cookers invariably run smoother. A smoother running jet cooker contributes greatly to ease of control of the process and ensures consistency of liquefaction.

Dose reduction

The activiation energy difference in the protein-engineered *B. stearothermophilus* alpha-amylase also allows a significant dose reduction, as can be seen in Figure 5. Reduced dosage provides the dry-miller with reduced costs for transportation, handling and less paperwork. While not a technical advantage, it is appreciated by the cost-conscious fuel ethanol industry.

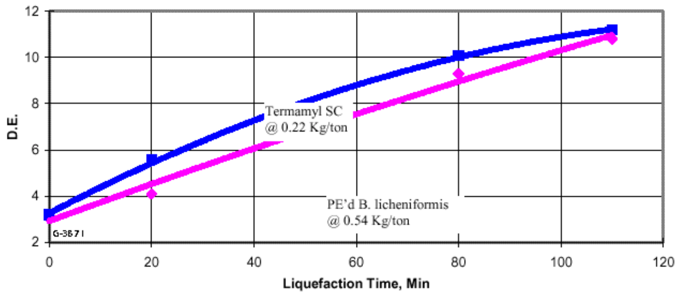


Fig. 5. At pH 5.6 the Termamyl SC dose can be reduced by 60% compared with PE'd *B. licheniformis* enzymes.

Fermentation yield

Presented thus far is a new, robust, alpha-amylase that requires less accommodation by the user. However, the most important aspect of liquefaction is how well the resulting product ferments. A product that fulfills all of the "up-front" dreams, but fails to produce the proper amount of ethanol is not optimal. Some exciting fermentation yield results are being seen in the plant trials with Termamyl SC.

In the first two commercial trials, we observed ethanol levels in the beer that are 0.4-0.5% higher than those obtained with protein-engineered *B. licheniformis* or wild-type *B. stearothermophilus* enzymes. These increases (90% confidence interval that there is 0.4% higher ethanol by Student-T test) were brought to Novozymes' attention by the technical management of these plants. Novozymes continues to examine the composition of the residual carbohydrates in the beer, in order to determine whether this is the result of less reversion (as previously mentioned), or more thorough liquefaction of some of the more difficult starch. Regardless of the mechanism, it is a significant improvement for the whole-grain processor.

Current status

Termamyl SC has quickly become a success story for both Novozymes and our customers. No other product has ever allowed the ethanol producer to increase production levels and decrease energy consumption as this novel enzyme does.

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