

Novozymes Cellic® CTec3 HS

- secure your plant's lowest cost

Cellic® CTec3 HS is a highly efficient cellulase and hemicellulase complex that ensures a cost-efficient conversion of pretreated lignocellulosic materials to fermentable sugars. The improvement in conversion efficiency provided by Cellic® CTec3 HS over previous generations unlocks new opportunities to optimize pretreatment, hydrolysis, and fermentation processes to ultimately secure the lowest total cost of producing cellulosic ethanol for your plant.

Benefits

- **Significant reduction in the total cost of producing ethanol**
- **Great process and substrate versatility**
- **New opportunities to optimize conversion processes, including:**
 - Increasing biomass to sugar conversion
 - Decreasing enzyme dosing
 - Increasing total solids loading
 - Reducing hydrolysis time
 - Reducing the severity of pretreatment

Performance

Cellic® CTec3 HS contains proficient cellulase components boosted by proprietary enzyme activities, including advanced AA9 molecules, improved β -glucosidases as well as a new array of hemicellulase activities, and together, they improve the conversion efficiency of Cellic® CTec3 HS by at least 1.5 times over Cellic® CTec2.

Usage

Novozymes has partnered widely with leading industry players, and together, we have worked with the Cellic® product family to optimize the cellulosic ethanol production process. We have gathered application information using Cellic® CTec3 HS in various process configurations with different types of feedstock and pretreatment processes, and the insights we have gained have helped to generate the general application recommendations contained in this document.

It is recommended to dose Cellic® CTec3 HS according to the level of cellulose contained in the substrate to allow for direct comparisons of enzyme efficiency on various biomass materials, including those from different types of feedstock and pretreatment combinations. The suggested enzyme trial dosing levels for initial investigation of a substrate are between 1% and 6% w/w (g-Cellic® CTec3 HS/g-cellulose). These data points should then be used to generate a dose-response curve (enzyme dosing vs. percentage cellulose conversion). Commercially feasible cellulose conversion depends on many factors, including feedstock cost, type of pretreatment, plant capacity, process yields, and value of co-products.

In general, the lower end of the dosing range recommended above should serve as a target for what could be considered commercially feasible cellulose hydrolysis. Additional testing is recommended to refine the dose response curve and determine the effects of time, solids loading, cellulose conversion, and enzyme dosing. Note that Cellic® CTec3 HS contains a significant amount of sugar, so a reference/blind sample should be included if a high enzyme dose is applied (see note on product formulation below).

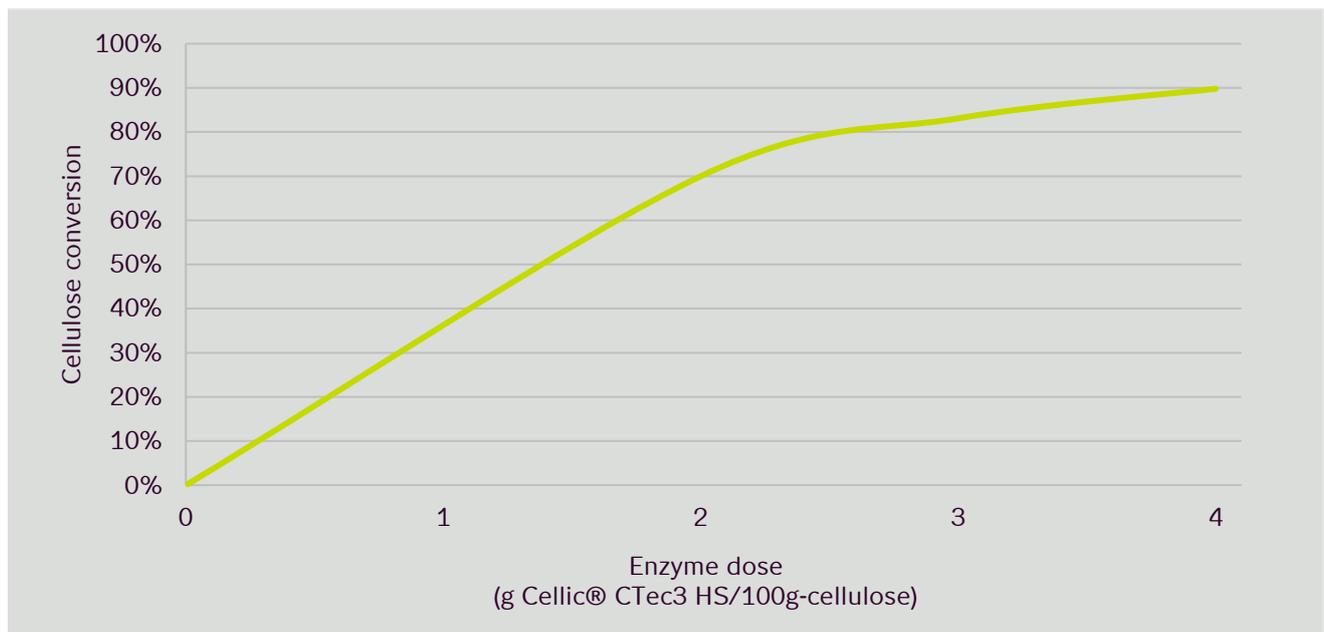


Fig. 1. Enzyme dose response for Cellic® CTec3 HS after 5 days of enzyme hydrolysis at 50°C and pH 5.0 of an unwashed sample of dilute acid-pretreated corn stover (18% total solids loading). If 80% conversion is required, then the recommended dosage for this feedstock under the hydrolysis conditions mentioned above would be ~3% w/w (30g-Cellic® CTec3 HS/kg-cellulose)

Cellic® CTec3 HS is a highly efficient cellulase and hemicellulase complex that works on a variety of substrates, including acid, auto-hydrolyzed, and alkaline pretreated substrates. Frequently, only Cellic® CTec3 HS will be needed. However, if the pretreated feedstock of interest contains an appreciable amount of hemicellulose, it is advisable to combine Cellic® CTec3 HS with other lignocellulytic enzymes to further boost overall conversion yields and potentially reduce the severity of your pretreatment. The enzyme type and dosage recommendation will depend on product, feedstock type, pretreatment technology, and processing conditions. For further technical advice about your specific substrate and targets, please contact your technical representative from Novozymes.

Considerations for enzyme application

Historically, cellulosic enzymes were significantly affected by feedback inhibition resulting from glucose production, which led to ineffective conversion. Later generations of enzymes, including Cellic® CTec3 HS, were developed to eliminate this effect on the enzyme complex. With the introduction of Cellic® CTec3 HS, there are more options available for consideration when selecting optimal process layout and targeting cost-efficient cellulose conversion. These include simultaneous saccharification and fermentation (SSF), separate hydrolysis and fermentation (SHF), and a modified version of these referred to as hybrid hydrolysis and fermentation (HHF).

The hybrid HHF process can also be described as viscosity reduction and pre-saccharification followed by SSF for ethanol production, and this setup can be configured in relation to overall process layout and conversion targets. The impact of hydrolysis and fermentation conditions using Cellic® CTec3 HS on pretreated corn stover is illustrated in figure 2 (enzyme and fermentation kinetics and final ethanol yields will depend on pretreated substrate, fermentation organism, and process configuration).

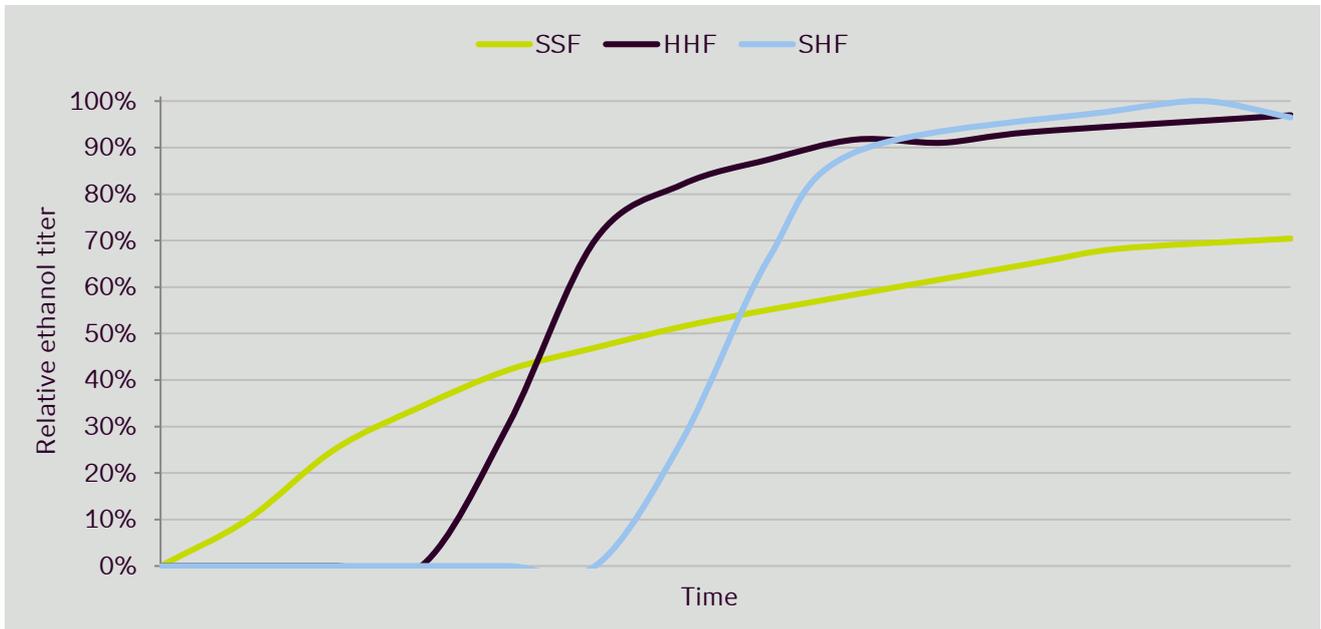


Fig. 2. Ethanol yield under varying hydrolysis conditions using Cellic® CTec3 HS at pH 5.0 and 50 °C on unwashed dilute acid-pretreated corn stover at 18% total solids loading. The yeast was pitched at different times, as indicated by initiation of ethanol production. The ethanol yield will vary depending on the substrate, enzyme dosing, yeast pitch, and hydrolysis configuration. In this example, an SSF configuration does not achieve the same yields as the options that include a dedicated hydrolysis step prior to fermentation. The process time available for hydrolysis and fermentation will dictate the options available

Optimum pH and temperature

Optimal performance of Cellic® CTec3 HS occurs at a temperature range of 50-55°C and at pH 4.75-5.25, as illustrated in figures 3 and 4. The optimal conditions can vary with specific pretreated substrates and process conditions (e.g. solids content and hydrolysis time); however, our recommendations are to conduct hydrolysis within the range of conditions specified above.

Please consult with your Novozymes representative for further guidance on identifying optimal conditions for your specific substrate and process. We recommend that you monitor and control pH and temperature within the ranges suggested above in that stage of your process where enzymes are applied, as major deviations could have an impact on hydrolysis kinetics and final conversion yields. Complex enzyme systems may be irreversibly damaged by high temperature and strong acidic or alkaline conditions. Due to this fact, we strongly recommend that you adjust pH and temperature to proper operational conditions prior to enzyme addition in order to ensure optimal conversion of your pretreated substrate.

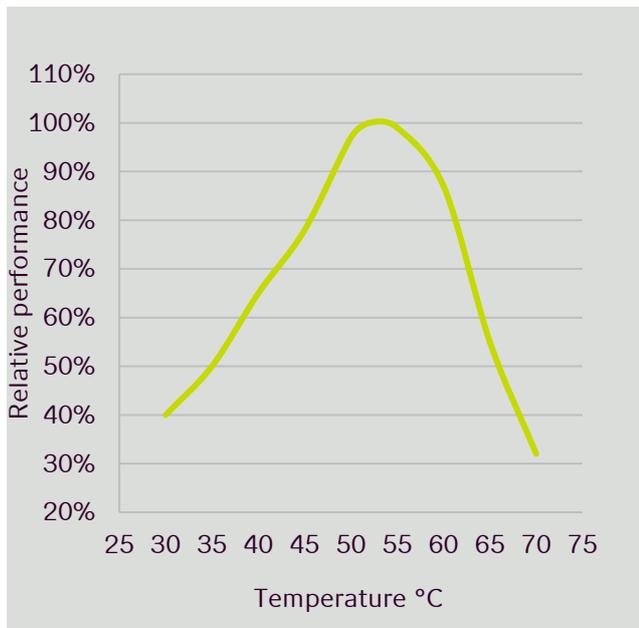


Fig. 3. Relative product performance of Cellic® CTec3 HS with respect to reaction temperature for unwashed acid-pretreated corn stover (PCS) at 18%TS for 3-5 days; pH 5 for all data points

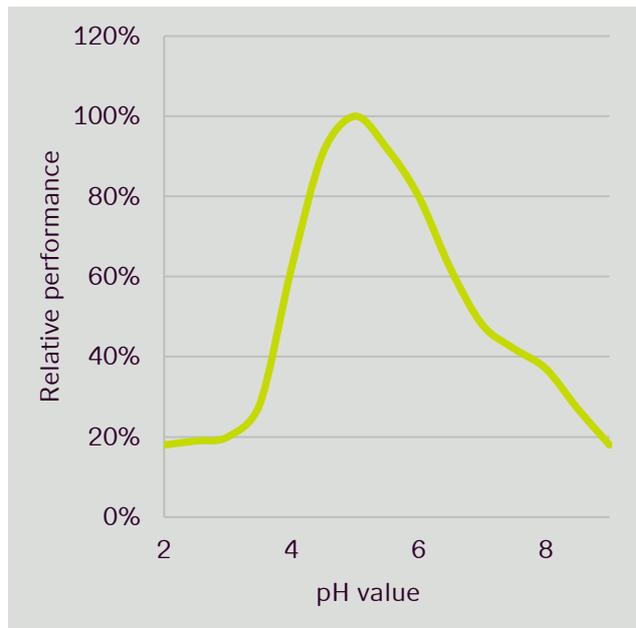


Fig. 4. Relative product performance of Cellic® CTec3 HS with respect to pH of reaction for unwashed acid-pretreated corn stover (PCS) at 18%TS for 3-5 days; temperature 52.5°C for all data points

The product

Biomass substrates are composed of lignin and a number of mutually entangled and chemically bonded carbohydrate polymers that require multiple enzymes working together synergistically for complete hydrolysis. For this reason, the performance of a complex multicomponent enzyme product such as Cellic® CTec3 HS cannot be evaluated based on the activity of a single enzyme or using single polymer substrate such as filter paper, Avicel or carboxymethyl cellulose (CMC). Therefore, Novozymes has developed a performance activity assay based on acid-pretreated corn stover for our internal quality control, so we can deliver high performance products to our customers in a consistent manner. The method uses a fluorescence enhancement marker and measures the extent of cellulose hydrolysis relative to a standard enzyme concentration for quantification of Biomass Hydrolysis Units - BHU(2)-HS. Cellic® CTec3 HS contains chemical formulations for improved storage stability, including glucose and/or sucrose. These compounds may affect the interpretation of hydrolysis data analysis when Cellic® CTec3 HS is dosed at high levels.

There is more information about the products available at [Novozymes Market](#).

Safety, handling and storage

For best product performance, Cellic® CTec3 HS should be stored at cool temperatures in closed containers protected from sunlight. The product has been formulated for optimal storage stability; however, enzymes gradually lose their activity over time. The recommended storage conditions are 0-10°C (32-50°F). Prolonged storage time and/or adverse conditions such as higher temperature may lead to a higher dosage requirement.

Safety, handling, and storage guidelines are provided with all products. Please contact your Novozymes representative for this information.

Additional information

Our committed expert technical and commercial teams will be pleased to help you commercialize cellulosic ethanol production while always keeping your specific goals in mind. For further information, please contact your Novozymes representative. Join us on the path to commercializing cellulosic ethanol!

About Novozymes

Novozymes is the world leader in biological solutions. Together with customers, partners and the global community, we improve industrial performance while preserving the planet's resources and helping build better lives. As the world's largest provider of enzyme and microbial technologies, our bioinnovation enables higher agricultural yields, low-temperature washing, energy-efficient production, renewable fuel and many other benefits that we rely on today and in the future. We call it Rethink Tomorrow.

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