# Technology offer: Cellulase Saver

*The most available resource on land for the production of sustainable biofuels and biobased chemicals is lignocellulose. It is the main constituent of almost all plants and consists of cellulose, hemi-cellulose and lignin. After a pretreatment step, cellulose can be broken down to yield sugars by adding (cellulase) enzymes (this is called "enzymatic hydrolysis"). The sugars can be used to produce sustainable biofuels and chemicals. The cost of enzymes form a large portion of the total production costs of biofuels (e.g. circa $ 0.18-0.39 per liter for ethanol produced from cornstover note). ECN has developed a method that substantially reduces the amount of enzymes needed for enzymatic hydrolysis of lignocellulose. This brings affordable second generation biofuels and chemicals a step closer to reality.*

*Keywords: 2nd generation biofuels, biobased chemicals, enzymes, cellulase, cellulose, lignin, proteins, herbaceous biomass, organosolv, steam explosion, hydrothermal pretreatment, diluted acid hydrolysis.*

## Description

- Cellulase Saver is a method developed by ECN to significantly reduce the amount of enzymes needed for the hydrolysis of cellulose.
- Principle: proteins are extracted from biomass before pretreatment and are re-added during the enzymatic hydrolysis step together with the cellulase enzymes. These proteins occupy free sites on (residual) lignin, preventing costly cellulase enzymes to bind there. This enhances the efficient use of cellulase enzymes.
- The protein extraction process is simple and can be integrated at low cost, resulting in a high ROI.

## New and innovative aspects

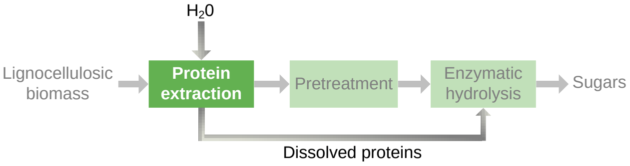
- ECN was the first to discover (and patent) that:
  - Enzyme activity during enzymatic hydrolysis of cellulose is significantly enhanced when a protein-rich aqueous extract is added during enzymatic hydrolysis; and
  - This extract can be prepared by washing the feedstock with water and filtering the mixture.

## Main advantages of its use

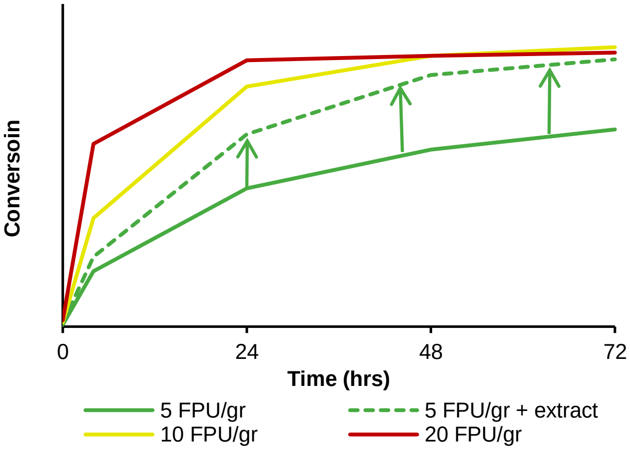
- Reduction of required enzyme dose with approx. 25-50% for effective conversion of pretreated lignocellulosic biomass into sugars.
- Alternatively, while maintaining the usual enzyme dose residence time can be reduced drastically.
- The method is independent of the pretreatment technology and can be integrated at low cost.

## Specifications

- Process described in figure 1.
- Aqueous protein extraction performed <75°C, degradation of proteins occurs at temperatures typically applied for pretreatment process.
- Observed enzyme reduction (fig.2):
  - Hydrotherm. pretreated wheat straw: ~25%.
  - Organosolv pretreated wheat straw: ~33%.
  - Organosolv pretreated rice straw: ~50%.
- Effective for lignocellulosic biomass containing (residual) proteins, so particular in herbaceous biomass such as wheat and straw. Principle also works for woody biomass, but this requires external sources of proteins.




▲ *Fig. 1: Process for Cellulase Saver. Highlighted are the new process steps*




▲ *Fig. 2: Enzymatic hydrolysis of organosolv-pretreated rice straw at different enzyme doses using a commercial cellulase. Enzyme dose required for maximum glucose yield: 10 FPU/gr. Addition of extract reduces required enzyme dose to 5 FPU/gr.*

## Potential applications

- Integration with pretreatment technologies to create 2nd generation biofuels and biobased chemicals from lignocellulose such as: steam explosion, organosolv, diluted acid hydrolysis and hydrothermal pretreatment.

## State of development

- Proof-of-principle succesfully demonstrated.

## Transaction type and partner profile

- License agreement or spin-off
- ECN would like to meet with companies interested in assessing the potential reduction in enzyme dose in their specific biomass pretreatment process.

## Publications and IP

- Dutch patent granted, PCT patent filed. WO2014098589 (click to download).

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