

# Cellulosic ethanol fermentation development – the next steps towards commercial scale production using xylose fermenting yeast

Kim Olofsson, Ole Sibbesen, Thomas Hvid Andersen and Birgitte Rønnow

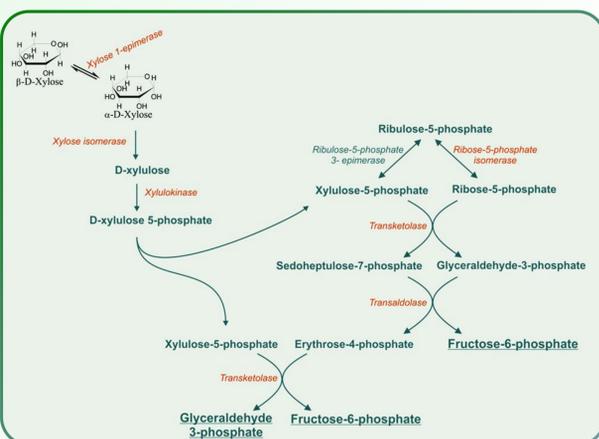
Terranol A/S, c/o Technical University of Denmark, Building 223, DK-2800 Lyngby, Denmark

## The strain

Terranol has developed a robust and rapid xylose fermenting yeast (*Saccharomyces cerevisiae*) for commercial production of lignocellulosic ethanol. By up-regulation of the pentose phosphate pathway, and expression of a bacterial xylose isomerase, as well as a xylose 1-epimerase, the strain utilizes an efficient xylose metabolic pathway. All inserted genes are stably integrated in the genome of the yeast and the strain has undergone extensive evolutionary engineering, resulting in rapid xylose consumption, very low formation of xylitol, as well as high resistance towards biomass derived inhibitors.

## Terranol's commercial strains

- IP exclusively owned by Terranol
- Strain samples and license available through Terranol or Novozymes



## Fermentation technology

Terranol is constantly working on developing fermentation strategies in order to enhance the performance of the yeast strains as much as possible. Moreover, due to different raw materials, pretreatment, process conditions and/or configurations at different ethanol plants it is crucial to be able to run the fermentation in different ways.

By moving from batch fermentations to various controlled fed-batch fermentations, optimizing e.g. sugar concentrations and growth conditions, further improvement of ethanol yields, productivities and titers can be achieved. Consecutive fed-batches or continuous fermentation are further design options. Regardless of process preference, the balance between ethanol yield and ethanol productivity – ethanol production and yeast growth – must be well understood and tuned. Results from a few fermentation examples are shown in the table below.

## About Terranol A/S

- Develops yeast strains/technology for lignocellulosic bioethanol production
- Founded in 2007
- Privately owned
- Located in Copenhagen, Denmark

## Collaborations

Terranol is collaborating with several leading ethanol producers and actors within the field, for example:

- Novozymes
- Abengoa
- Inbicon

In an ongoing project with Inbicon (Dong Energy), fermentation tests of Terranol's strain will be carried out in 250 000 liter demo-scale in Kalundborg, Denmark. Other large scale fermentations are also being made or are under way.

## Examples of fermentation results

Type of hydrolysate	Mode of operation	Total yeast pitch (g/L)	Fermentation time (h)	Overall ethanol yield (%)	Ethanol yield on consumed sugars (%)	Ethanol titer (g/L)
Wheat straw	Batch	1	<48	87	87	53
Wheat straw	Fed-batch	0.5	<48	93	93	54
Corn stover	Fed-batch	0.5	<48	93	93	55
Corn cobs/stover	Fed-batch	0.5	<48	95	95	55

1. Yield of theoretical based on all available glucose and xylose  
2. Yield of theoretical based on consumed glucose and xylose

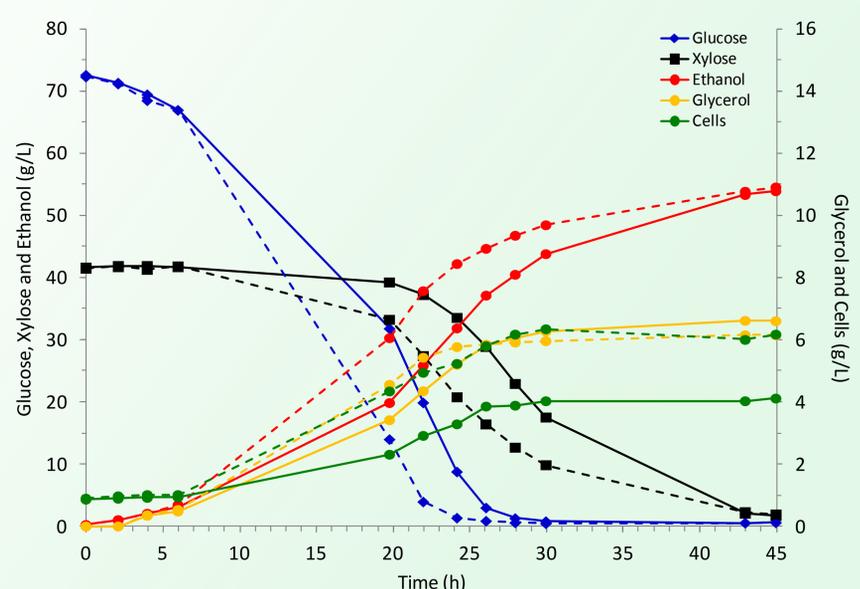
## Traits of Terranol's strain V1

- Low yeast pitch (0.25-1 g dw/L)
- Ethanol yields up to 95 % on xylose and glucose
- Ethanol titers above 7 % (w/w)
- Short fermentation time – less than 48 hours
- Inhibitor tolerant (e.g. furfural and acetic acid)
- Low by-product formation, such as xylitol
- Approved for use in the U.S.

## Improvements with strain cV-40 (including V1 traits)

- Faster glucose fermentation
- Higher growth rates and better propagation performance
- Devoid of antibiotic resistance markers, which makes it easier to get approvals
- To be approved for use in the U.S.

## Comparison of strain V1 and cV-40 in wheat straw hydrolysate



Batch fermentation using strain V1 (solid lines) and strain cV-40 (dotted lines) with 0.9 g/L yeast pitch, 30°C, pH 5.5, 1 g/L urea (no other supplements).

## Acknowledgements

This work was supported by the Danish Energy Authority's Programme for Energy Technology Development and Demonstration (EUPD).