Microbial stability, fermentation and propagation consistency are crucial to ensure the creation of a robust and stable strain

De-risking fermentation

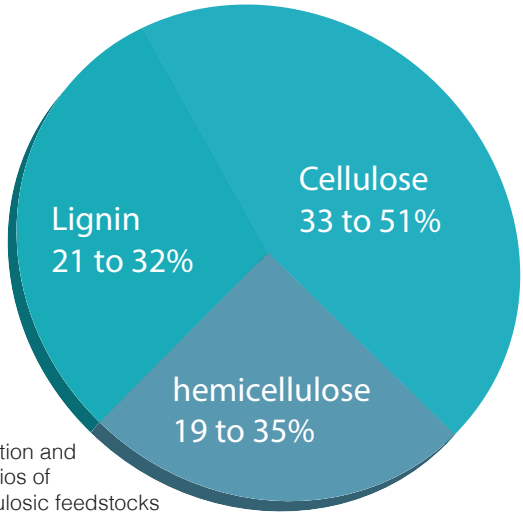
2014 was the year of second generation ethanol with the openings of several plants in North and South America. A number of other multi-partner global projects have also been announced or are currently under construction by major actors of the cellulosic ethanol scene. In the US alone, the E2 advanced biofuel market report published in January 2015, states cellulosic ethanol capacity in 2014 was 58 million gallons, with predictions suggesting it will increase to between 182 million and 215 million gallons by 2017.

In the first stages of the development of cellulosic ethanol plants, the main challenge for Leaf Technologies (Lesaffre's business unit dedicated to ethanol and bio-based chemicals industries) when developing its bio-engineered yeasts was to de-risk the fermentation. Now that the industry is installing and producers are deploying their technology, the focus of yeast producers is to improve the fermentation process to optimise producer's productivity and profitability.

Adaptive yeast strains

One of the main challenges of second generation ethanol fermentation is the variety of feedstocks used. From crop residues, to dedicated energy crops, municipal solid waste or even wood, all these substrates after their conversion into hydrolysates will give different C5-C6 sugar ratios.

In addition to these variations in sugars, the hydrolysates also have different toxicity levels that biocatalysors will have to adapt to.



Composition and sugar ratios of lignocellulosic feedstocks

The challenge for the yeast producer is to develop a robust yeast, able to co-ferment C5 and C6 sugars while resisting toxics, to improve both the efficiency and the consistency of the fermentation.

The construction of Lesaffre's first C5-C6 yeast started by the over expression of five genes in the pentose phosphate pathway and the integration of the Clostridium phytofermentans Xylose Isomerase (XI) gene, which was acquired from Butalco. During the first stages of the strain development, Lesaffre researchers discovered and patented that the addition of a Xylitol Dehydrogenase activity allowed an optimised XI activity. The finding enabled the production of strains that could ferment the xylose deriving from any yeast strain. This was not the case before, where only a limited number of strains efficiently expressed XI genes.

It was then possible to manipulate and evolve any yeast strain. Lesaffre then carried out several cycles of directed evolution to improve the performance of the strain. The resultant yeast strain was commercialised under the name CelluX 1 and was the first C5-C6 co-fermenting yeast available under dry form on the market. Using yeast under dry form enables cellulosic ethanol producers to carry out short propagations before pitching the fermenter as it is the case in most first generation production processes.

Successive stages of molecular biology and classical genetics were used by Lesaffre scientists to further improve CelluX 1. A selected strain highly resistant to toxic compounds derived from second generation hydrolysates was crossed with CelluX 1. The resultant hybrid was subjected to successive mass sporulation and hybridisation steps in order to get maximum genetic diversity from the initial hybrid and in a very short period of time. This strategy of genetic diversity creation was possible thanks to the genetic manipulations that had been done to create CelluX 1: the genes identified as essential for the effective xylose consumption had all been integrated close to a sexual locus. This patented construction enables the entire cellular population obtained to have the capacity to consume xylose and additionally avoids the dilution of the capacity of xylose consumption during the process of generating genetic diversity.

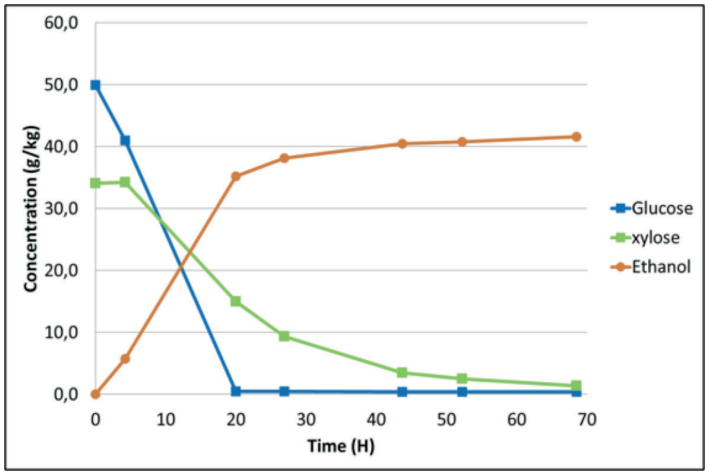
The subsequent cellular population was then evaluated according to screening parameters, allowing the selection of a robust and performing strain. Among these criteria, the absence of complex nutritional requirements, the capacity to be industrially produced and dried, and the rapid consumption of xylose in industrial conditions were particularly considered.

These R&D works have led to the third generation of CelluX which is available today on the market. CelluX 3 can consume more xylose in a shorter period of time and is more resistant to toxics than generations one and two.

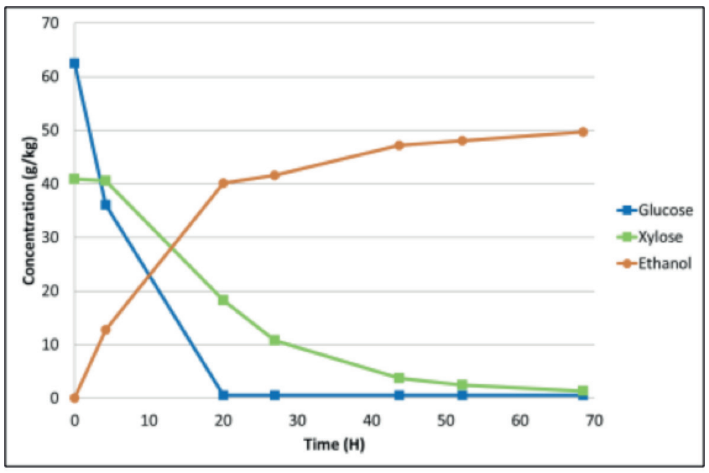
Fermentation trials

Fermentation results on the Proesa technology materials as shown in the graphs overleaf were obtained after propagation of active dry yeast using the same materials. For propagation and fermentation, only mineral nitrogen was added, temperature was regulated at 32°C and there was no pH regulation.

In these basic conditions, CelluX 3 was able to consume all glucose and xylose sugars in less than 72 hours with a sugar to ethanol conversion yield equivalent



Fermentation results on Proesa technology – low dry solids material – temperature: 32°C, pH: 5.0



Fermentation results on Proesa technology – medium dry solids material – temperature: 32°C, pH: 5.5

to the theoretical maximum yield taking the cellular growth and the glycerol produced into account.

From these basic fermentation conditions, the propagation and fermentation processes then need to be adjusted and optimised case by case (depending on the raw material, the lignocellulose biomass pretreatment process and the equipment) to develop a strong process.

Moreover, dry yeast and one-step propagation encourage microbial stability, fermentation and propagation consistency, which are key components in the robustness and the stability of the process.

Conclusion

As the cellulosic ethanol industry continues evolving with new projects coming online in the following months and installed producers ramping up to full capacity production, Leaf Technologies' attention is on further improving bioengineered strains to meet producers' needs and improve economics. ●

For more information:
This article was written by Jean-Michel Bavouzet, Ph.D. senior scientist and team leader at Lesaffre International R&D and Sophie Castelain, marketing and communications manager at Leaf Technologies.
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