Rapid xylose and glucose fermentation by engineered \textit{S. cerevisiae} for commercial production of cellulosic ethanol

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Summary

By identifying and alleviating a previously unexplored bottleneck in the xylose metabolic pathway, Terranol has developed an industrial \textit{S. cerevisiae} strain for fermentation of xylose at increased rates. In addition to upregulation of several activities of the pentose phosphate pathway, and an efficient bacterial xylose isomerase, the strain expresses a \textit{x}-epimerase. The epimerase catalyzes an otherwise slow conversion between the \textit{x}- and \textit{D}-anomers of D-xylose, of which only the \textit{x}-anomer is a substrate of the xylose isomerase.

All inserted genes are stably integrated in the genome of the yeast and the strain has undergone extensive adaptation by evolutionary engineering. The resultant strain has high resistance towards biomass derived inhibitors, low formation of the by-product xylitol, and ferments both hexose and xylose rapidly in lignocellulosic hydrolysates, e.g. pretreated straw and corn stover.

Terranol’s strain \textit{V1} is ready for commercial ethanol production and can ferment xylose and glucose in lignocellulosic hydrolysates with a resulting ethanol yield above 90% of total sugars and a final ethanol titer above 5% (w/v) in as little as 48 h.

Choosing our entry strain

A robust strain was selected based on
- Good spore viability and transformability
- Resistance towards inhibitors from wheat straw hydrolysate
- Good ethanal productivity and yield in industrial setting

Strain construction

Xylose fermenting \textit{S. cerevisiae} was constructed by
- Identification and expression of a proprietary efficient bacterial xylose isomerase (ca. 1 U/mg yeast protein)
- Expression of xylose-\textit{x} epimerase
- Stable chromosomal integration of heterologous genes and overexpression of pentose phosphate pathway genes
- Optimized combination of yeast promoters in front of pentose phosphate pathway genes
- Evolutionary engineering of strain by serial transfer in xylose medium

Fermentation of lignocellulosic hydrolysates – examples of Terranol’s strain \textit{V1}

Strain \textit{V1} is capable of completely fermenting glucose and xylose in different pretreated lignocellulosic materials within a short period of time. The materials shown here are undiluted hydrolysates obtained after pretreatment and subsequent enzymatic hydrolysis using Cellic CTec2 from Novozymes A/S.

Through development of improved fed strategies, the ethanol yield and productivity can be further increased in fed-batch. By controlling the sugar concentrations during the fermentation, better conditions for co-fermentation of glucose and xylose can be achieved.

In the fed-batch fermentation (examples \textit{B} and \textit{C}) the total sugar concentration is kept constant during the feed phase (highlighted in example \textit{C}), enabling a more favorable concentration level for simultaneous uptake of xylose and glucose in comparison to a batch fermentation.

Complete optimized pathway

D-xylose can be converted to D-xyulose either via the bacterial type pathway – using xylose isomerase – or a fungal type pathway involving xylose reductase and xylitol dehydrogenase. We chose to use the xylose isomerase pathway because it is more direct than the fungal type pathway and does not introduce a co-factor imbalance.

Xylose isomerases prefer the less abundant anomer a-D-xylose over b-D-xylose as substrate. A xylose 1-epimerase was therefore included in the pathway to increase the rate of equalization of the \textit{x}- and \textit{b}-xylose anomers inside the cell. Further assimilation of xylitol takes place via the pentose pathway in yeast. In order to achieve a high flux through this pathway, several endogenous activities have been enhanced.

Fermentation of lignocellulosic hydrolysates – examples of Terranol’s strain \textit{V1}

- \textit{A} Inbicon wheat straw C5/C6 solution
  Batch fermentation 0.75 g dw/t, total yeast pitch, 3 g/L urea, pH 5.5, 30°C

- \textit{B} Fed-batch fermentation with automatic feed control 0.75 g dw/t, total yeast pitch, 3 g/L urea, pH 5.5, 30°C

- \textit{C} NREL corn stover hydrolysate liquid
  Fed-batch fermentation with automatic feed control 1 g dw/t, total yeast pitch, 3 g/L urea, pH 5.5, 30°C

Example Type of hydrolysate Mode of operation Fermentation time (h) Overall ethanol yield (%) Ethanol yield on consumed sugars (%) Ethanol titer (g/L)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>Batch</th>
<th>46</th>
<th>75</th>
<th>86</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Wheat straw Fed-batch with automatic feed control 46</td>
<td>88</td>
<td>92</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Corn stover Fed-batch with automatic feed control 48</td>
<td>95</td>
<td>93</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 1. Fermentation not completed after 46 h.
- 2. Yield of theoretical based on all available glucose and xylose
- 3. Yield of theoretical based on consumed glucose and xylose

Summary of fermentation results with strain \textit{V1}

- A low yeast pitch (0.25-1 g/L) is viable for fermentation of lignocellulosic hydrolysates
- Ethanol titers well above 5% (w/v) can be achieved
- Ethanol yields above 90% of theoretical (on glucose and xylose) can be achieved
- Complete fermentation of all sugars (glucose and xylose) available can be fermented within as little as 48 h.
- The strain quickly converts furfural and other inhibitors
- Low xylitol formation, below 0.5 g/L
- Cell mass and glycerol are the only major fermentation products – apart from ethanol

Acknowledgements

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