

Industrial verification of pentose fermenting yeast

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Goal

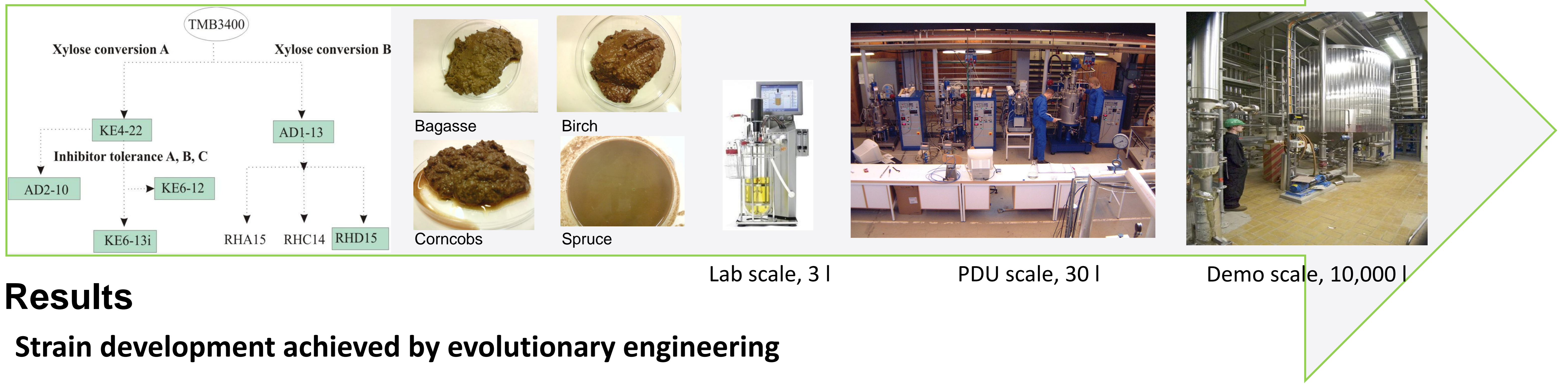
Transfer the technology of pentose fermentation from lab to demonstration or industrial scale

Aims

Increase the yield from pentoses to 0.34-0.38 g/g
 Increase the ethanol yield to about 40% through the fermentation of pentoses

Work methodology

1. Development of yeast strains
2. Raw material Production
3. Development of improved process technology & validation



Results

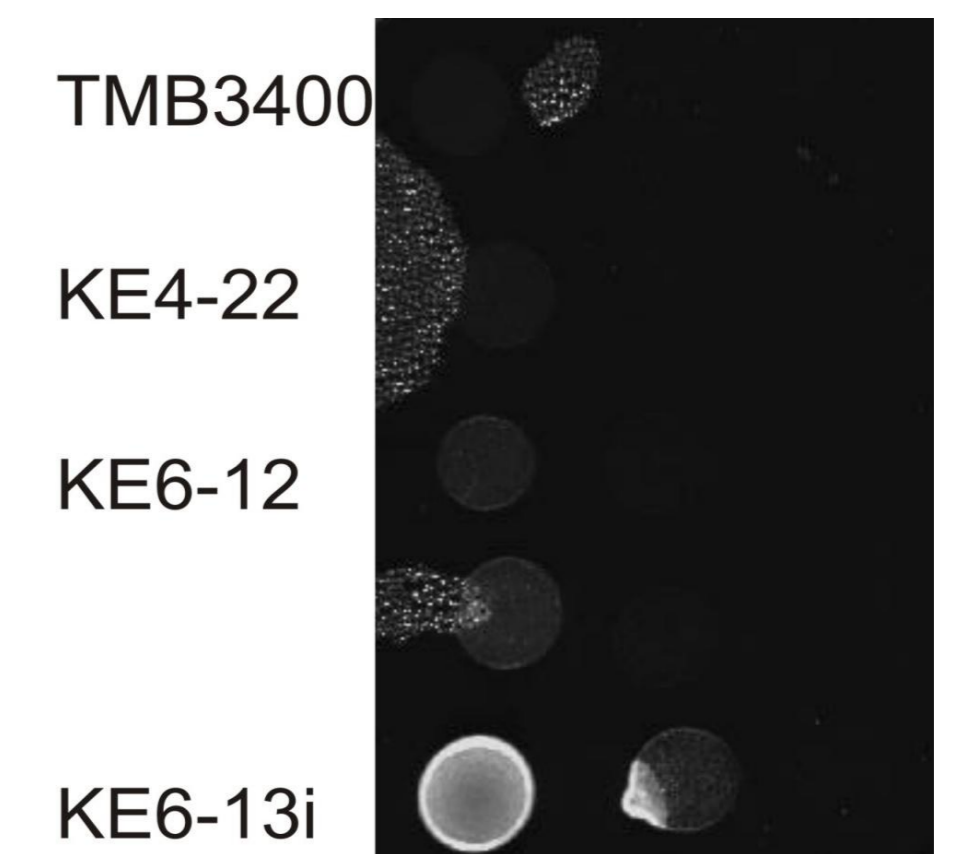
Strain development achieved by evolutionary engineering

Table: Characterization of improved strains in anaerobic shake flasks with hydrolysate of corncobs. Data indicated are relative to their parent strain; RHD-15 from AD1-13 and AD2-10 and KE6-12 from KE4-22

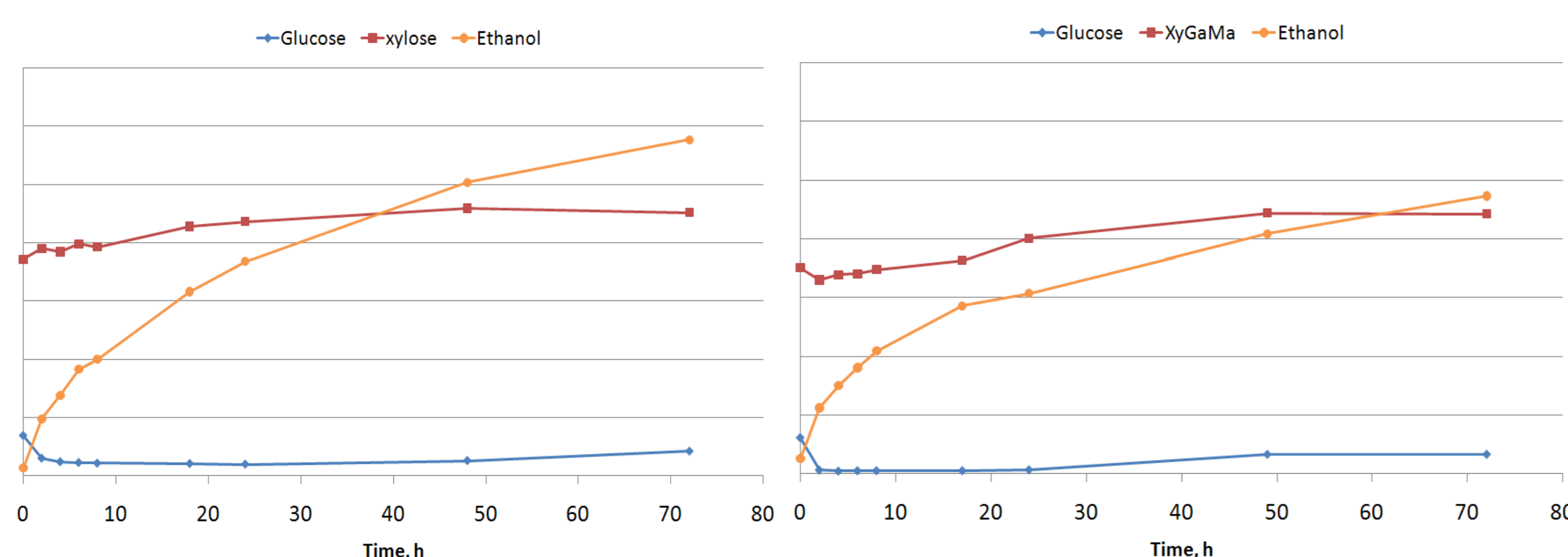
Strains	Ethanol yield (g/g total available sugars)	Final ethanol concentration after 96 h (g/l)	Xylose consumed after 96 h (%)	Xylitol yield (g/g consumed xylose)
RHD-15	+67%	+67%	+224%	-55%
AD2-10	+50%	+48%	+148%	+5%
KE6-12	+50%	+48%	+142%	-24%

Inhibitor tolerance

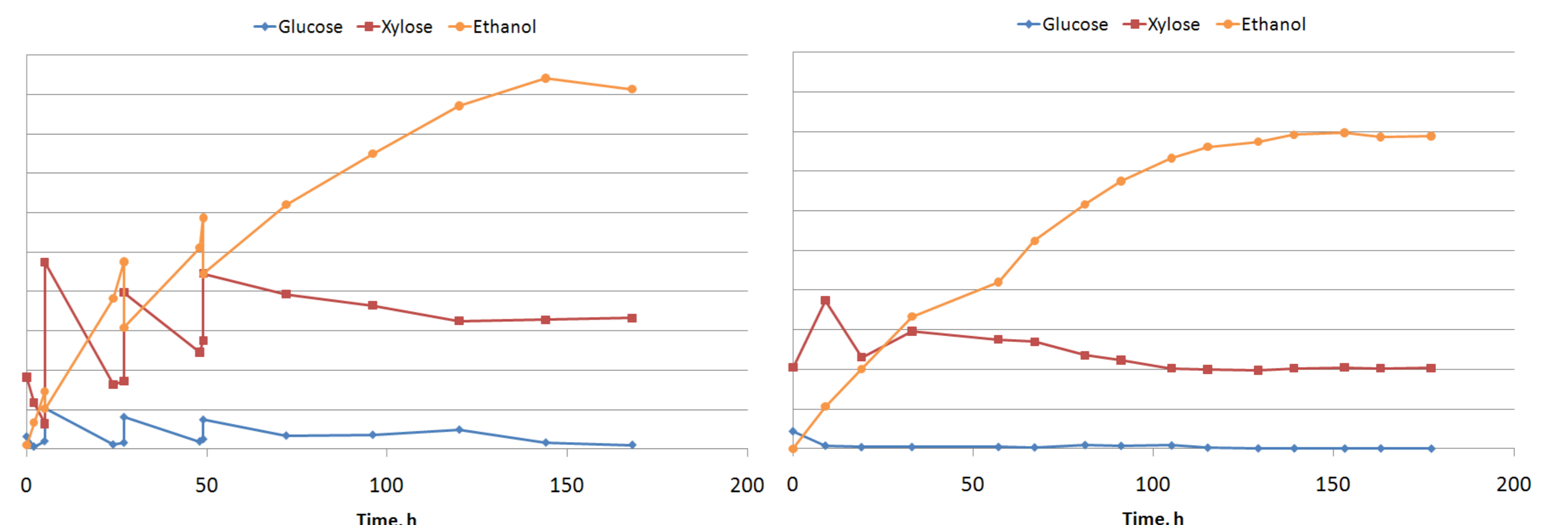
Drop test on YPD inhibitor plate comparing evolved strains with TMB3400 reveals improvement in inhibitor tolerance



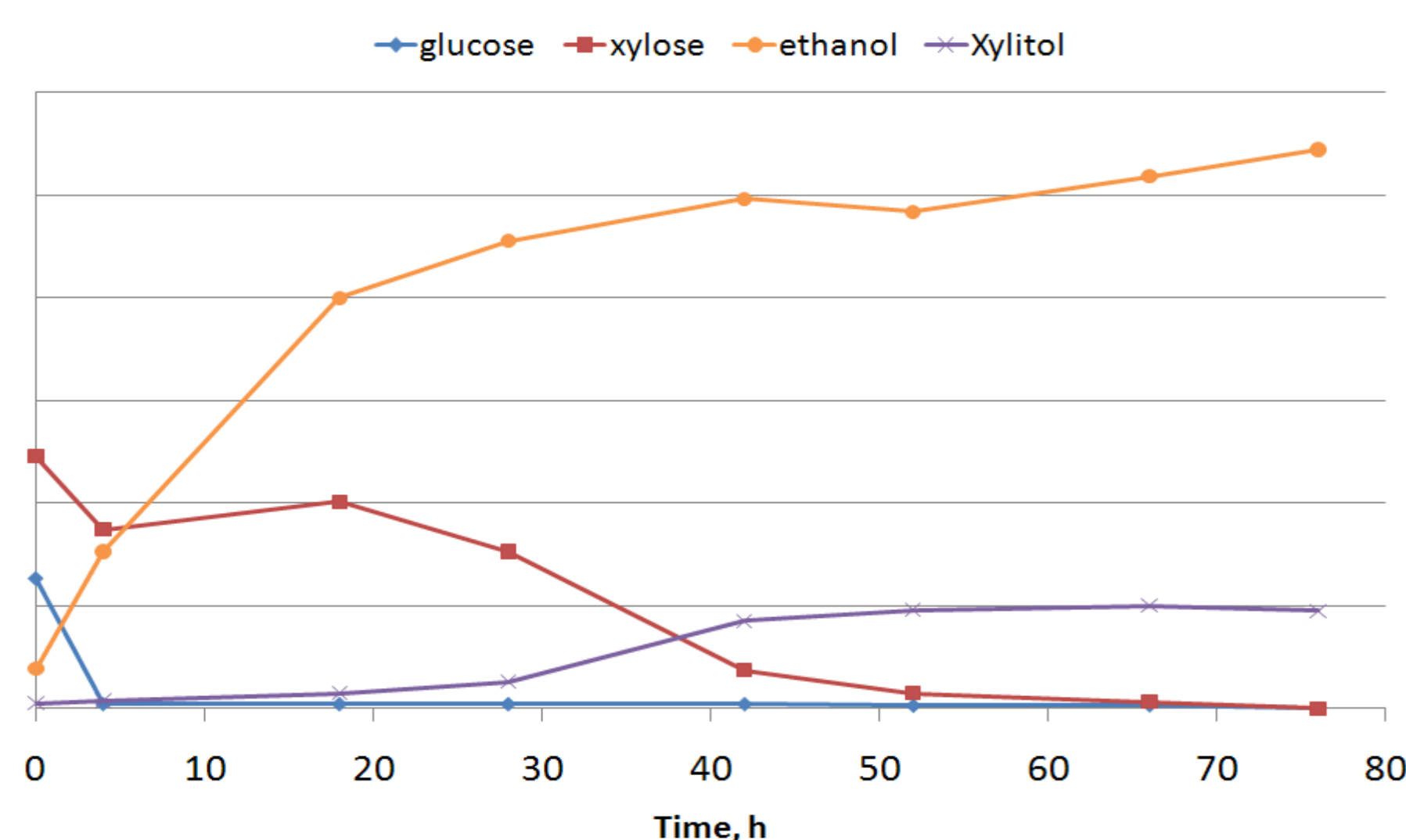
Technology transfer



From lab to PDU scale: TMB3400 in simultaneous saccharification and fermentation tests at 35 °C with bagasse slurry at 7.5% WIS, 3 g dry weight of yeast per liter and 5 FPU / g WIS-cellulose degrading enzymes. Left: lab scale (1.5 l), right: PDU scale (20 l).



From PDU to demo scale: KE6-12 in simultaneous saccharification and fermentation experiments at 35 °C with corncobs slurry at 10% WIS, 5 g dry weight of yeast per liter. Slurry feed occurred during 48 h and the enzyme added in five portions, which gave a total of 15 FPU / g WIS-cellulose degrading enzymes. Left: PDU scale (20 l), right: demo scale (10,000 l).



Demo scale: complete xylose utilization from KE6-12 in the fermentation of liquid phase of pretreated corn cob material equivalent to 6% WIS. The liquid phase was fed during initial 24 h.

Conclusion

- Improved xylose conversion capacity, less by-product formation, inhibitor and temperature tolerance were successfully achieved during strain development
- SSF and fermentation experiments were highly reproducible at lab, PDU and demo scale
- In the demo experiments, an ethanol yield of 0.44 g/g xylose was obtained which was clearly higher than set in the goals
- The modification of process configurations and feeding modes could lead to simultaneous high yield on xylose and high final ethanol concentration.