Designing an SSCF process for bioethanol production from lignocellulosic substrates by co-fermentation of xylose and glucose

Kim Olofsson*, Magnus Wiman and Gunnar Lidén

Department of Chemical Engineering
Lund University
Sweden

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* *kim.olofsson@chemeng.lth.se*

Background

Lignocellulosic materials, from e.g. forest industry and agriculture, are potentially interesting feedstocks for ethanol production.

To meet desired overall ethanol yields, it is important to use both hexoses and pentoses.

Saccharomyces cerevisiae does not naturally ferment pentoses.

Genetically modified *S. cerevisiae* strains can ferment xylose, but requires a favorable ratio between xylose and glucose.
TMB3400* was used in these studies.

* Wahlbom et al. (2003) *Generation of the improved recombinant xylose-utilizing Saccharomyces cerevisiae TMB 3400 by random mutagenesis and physiological comparison with Pichia stipitis CBS 6054*. FEMS Yeast Res 3: 319-326

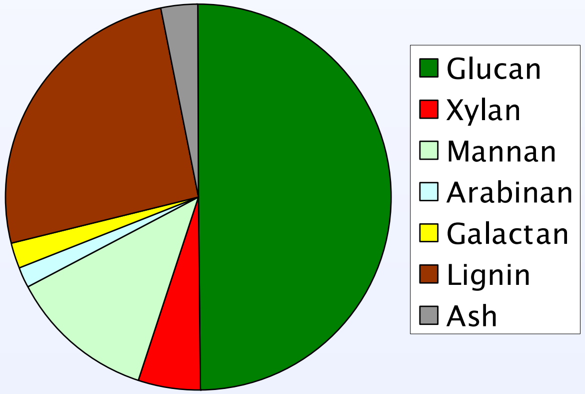
Background

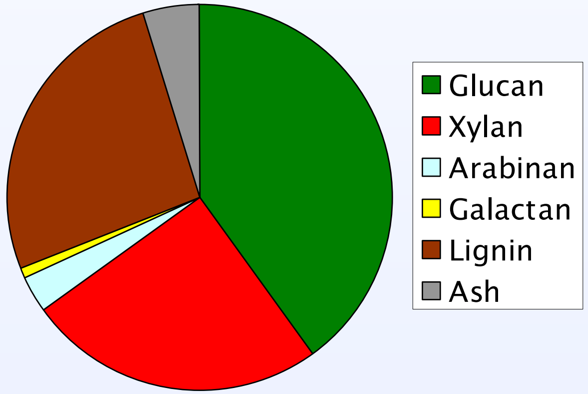
Spruce

Low ratio between xylose and glucose makes total xylose conversion challenging.

Wheat straw

Relatively good ratio between xylose and glucose. However, large amounts of xylose makes total xylose conversion challenging.





A suitable process design for simultaneous saccharification and co-fermentation (SSCF) is needed for sufficient xylose utilization.

SSCF

Efficient xylose fermentation needs high xylose/glucose ratio,
i.e. low (but non-zero) glucose concentration in SSCF

After steam pretreatment (with SO_2) of the material:
Nearly all xylose in liquid fraction,
and most of the glucose in fibers as glucan

⇓

Model for
yeast sugar uptake rate vs *enzyme sugar release rate*
in SSCF

SSCF of spruce with enzyme feed

When do we start, and what feed rate is appropriate?

Hydrolysis experiments

$$v_{hydrolysis}(t) = f(enz, glu, \alpha)$$

SSCF with glucose feed (without enzymes)

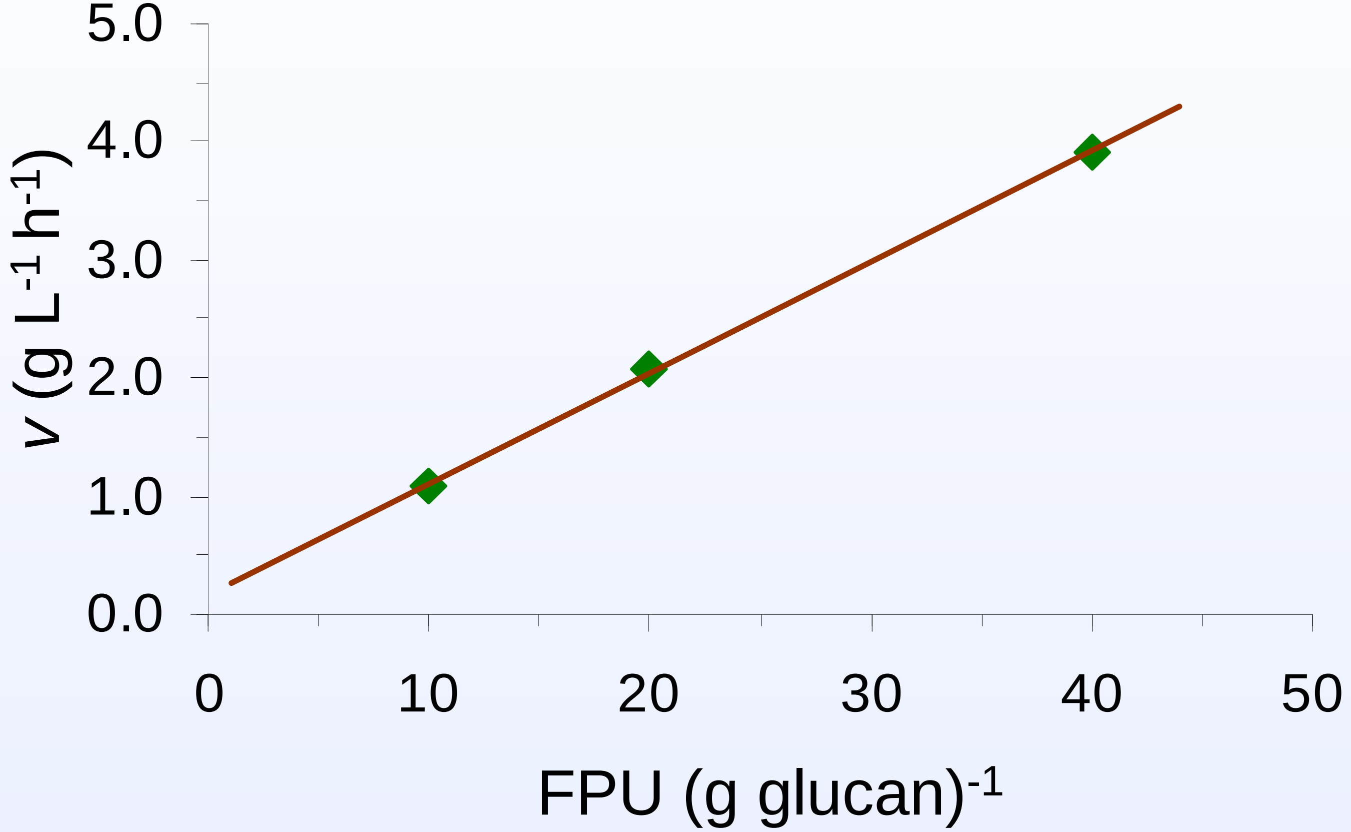
$$v_{glu\_uptake} = v_{glu\_feed}$$

⇘ ⇙

Designing SSCF with enzyme feed

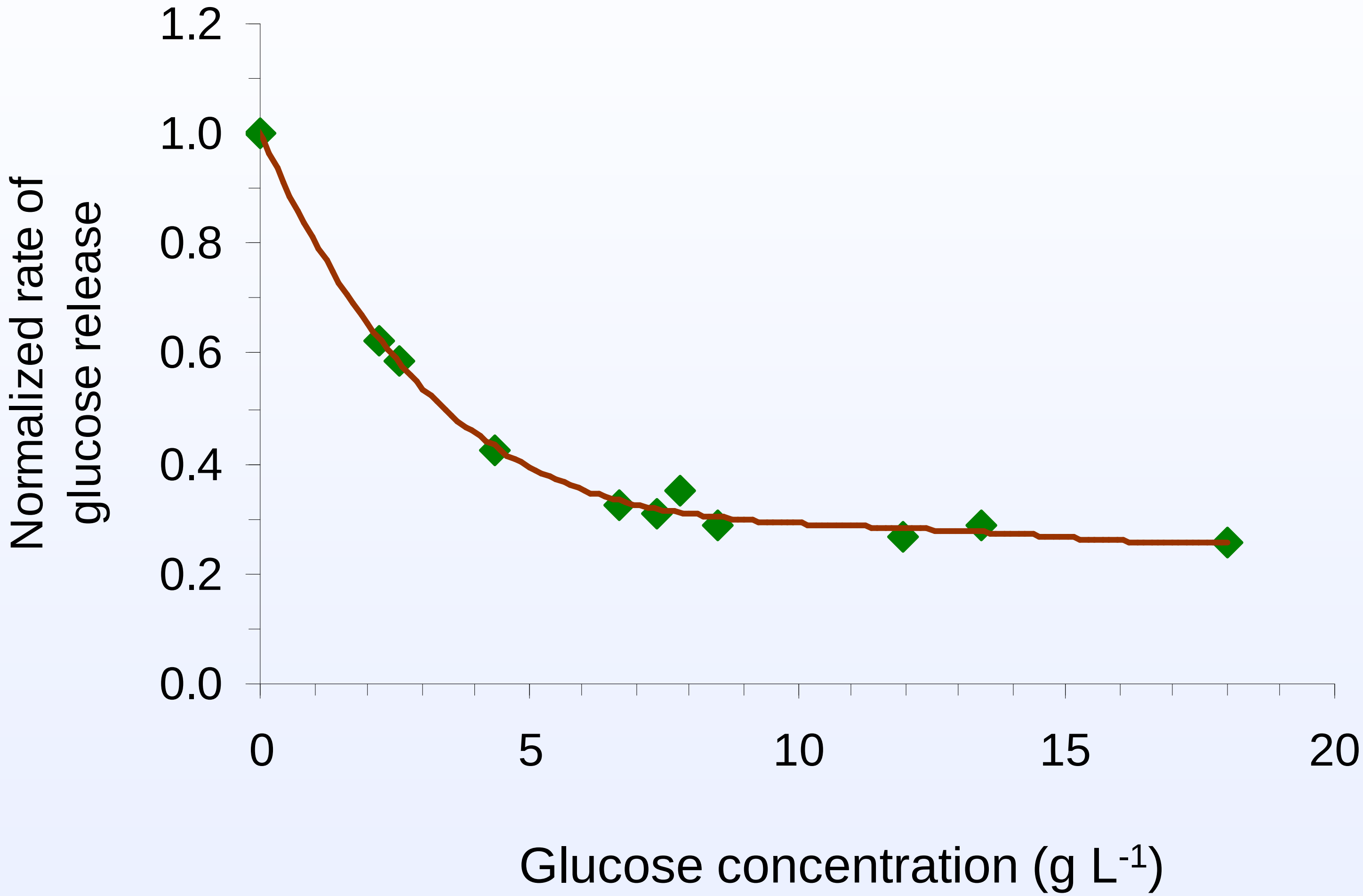
$$v_{hydrolysis} = v_{glu\_uptake}$$

Hydrolysis



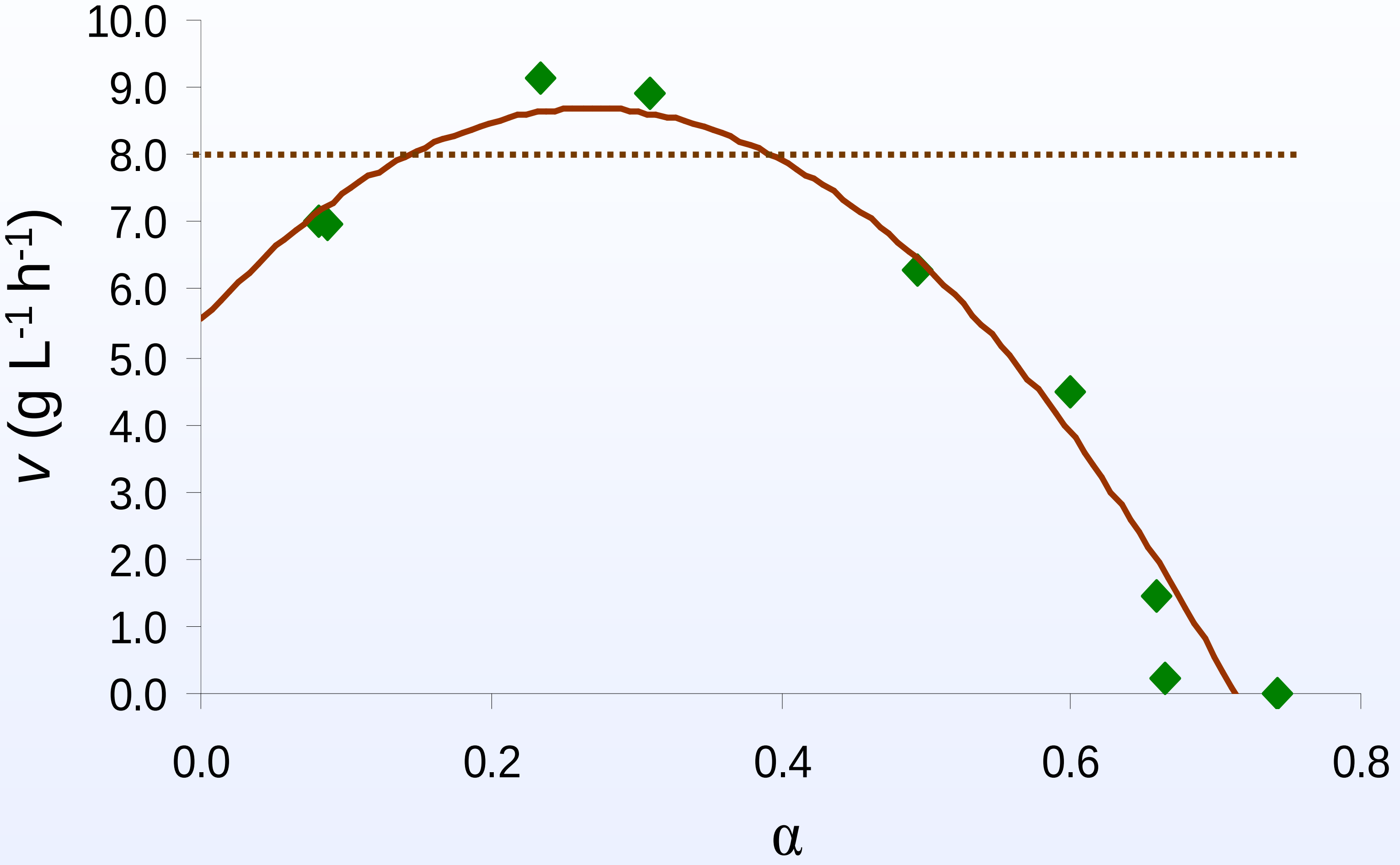
Rate of glucose release as a function of enzyme load

Hydrolysis



Normalized rate of glucose release as a function of the glucose conc.

Hydrolysis



Rate of glucose release as a function of the fractional degradation (α)

Sugar uptake

Mimicked SSCF

Aim

- Determine the maximum glucose uptake rate by the yeast in the real medium (hydrolysate)

Method

- Controlled glucose feed instead of enzymes

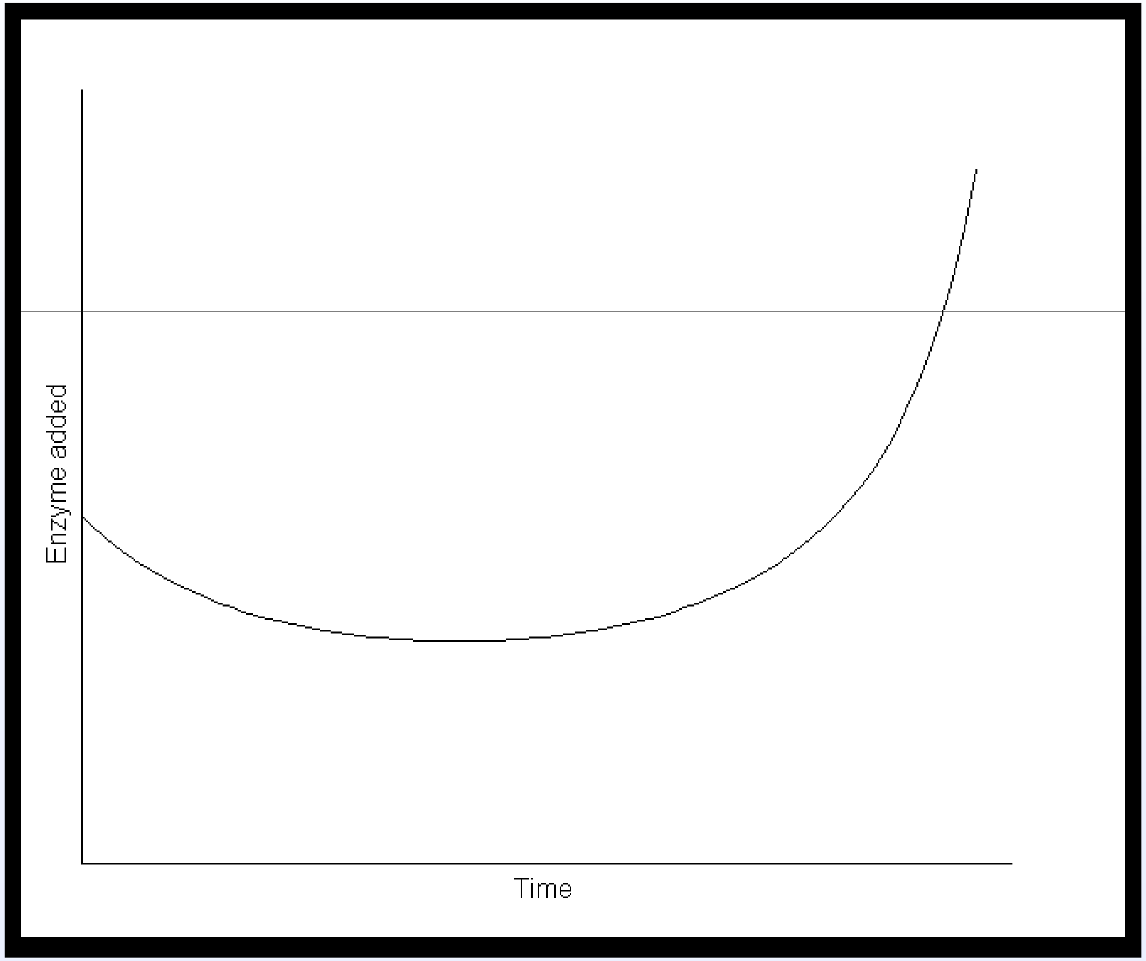
Result

- Maximum glucose uptake rate in SSCF was 2.5 g $L^{-1}h^{-1}$
- 2.0 g L^{-1} h^{-1} seemed most feasible for SSCF

Designing the SSCF

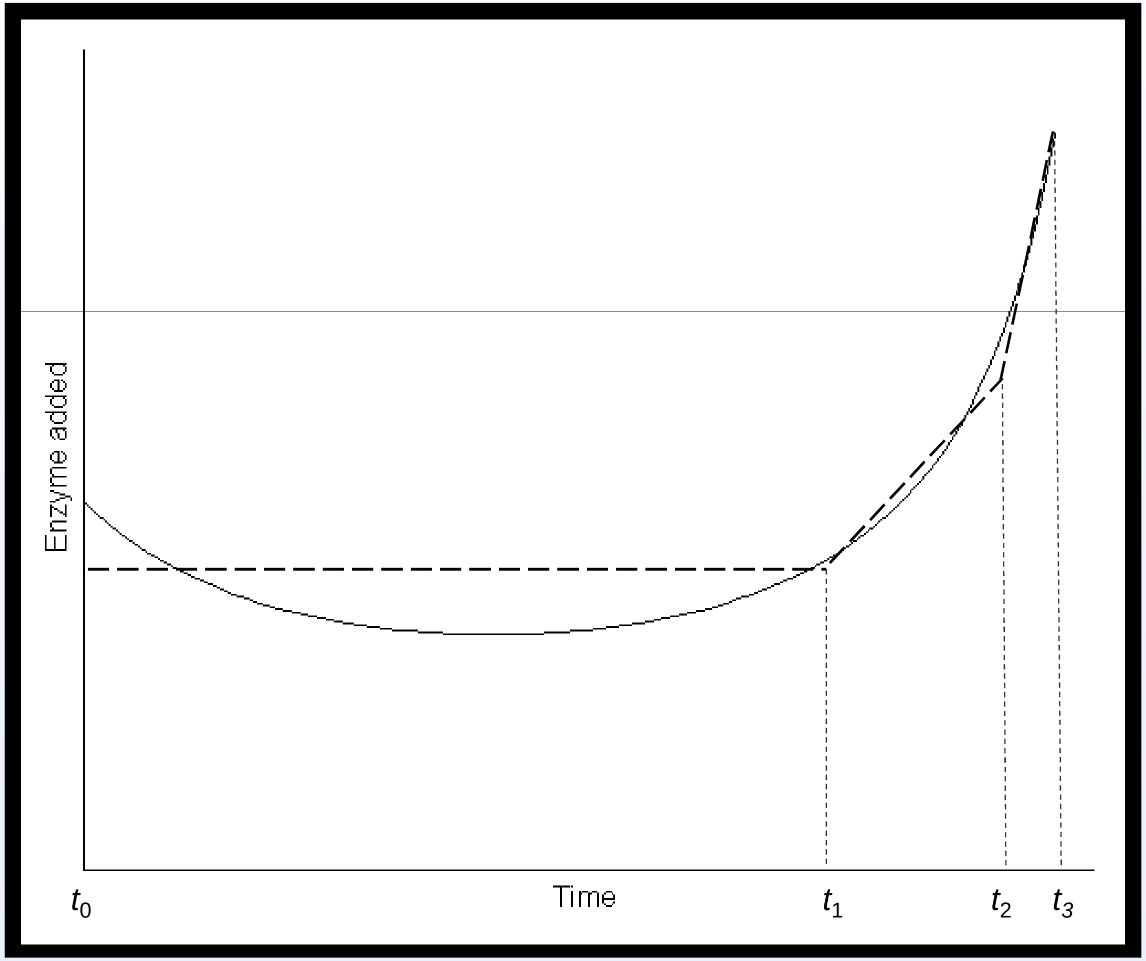
Creating a mathematical model for the SSCF

combining the results from hydrolysis and sugar uptake rate investigations



Designing the SSCF

Creating a mathematical model for the SSCF

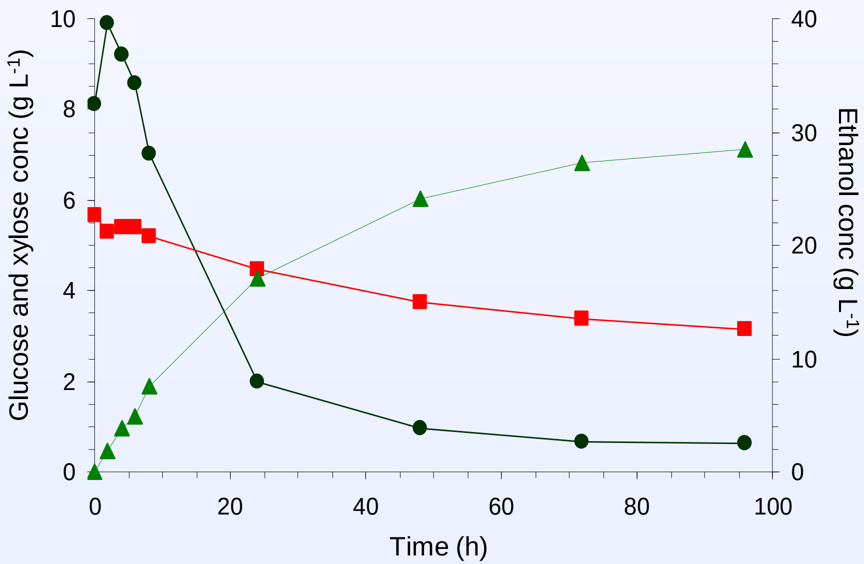


Results - SSCF of spruce

Batch experiment (ref)

Xylose uptake: 44%

- Ethanol yield: 0.34 g g^{-1} on total sugars (66% of max theoretical yield)
- Final ethanol conc: 28.5 g L^{-1}

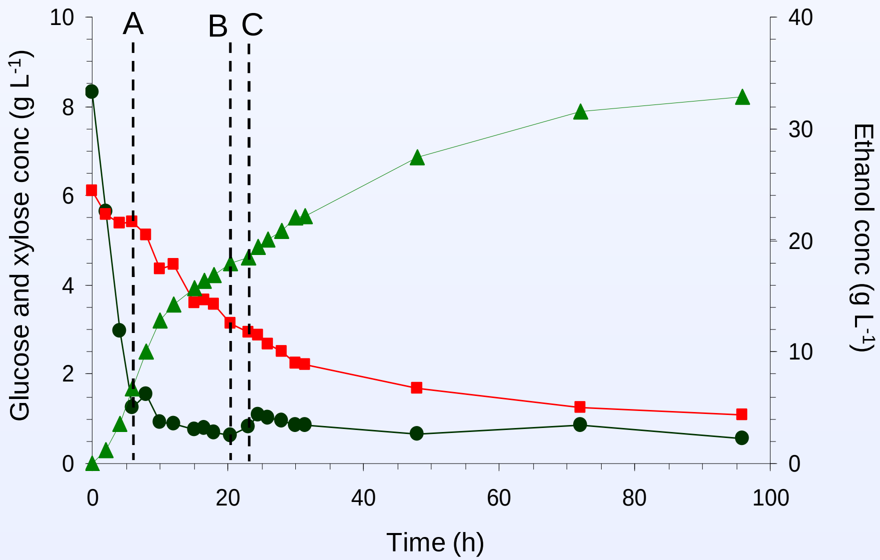


SSCF with enzyme feed

glucose release rate of 2.0 g L^{-1} h^{-1}

Xylose uptake: 82%

- Ethanol yield: 0.39 g g^{-1} on total sugars (77% of max theoretical yield)
- Final ethanol conc: 32.9 g L^{-1}



Measured concentrations during SSF of spruce with 8% WIS showing glucose (●), xylose (■) and ethanol (▲)

SSCF of wheat straw

Fed-batch operation lowers the glucose concentration in SSCF in comparison with batch and hence improves xylose utilization *

Still, there is a large amount of xylose left after fed-batch SSCF…

Is it possible to further improve xylose uptake in fed-batch mode?

↓

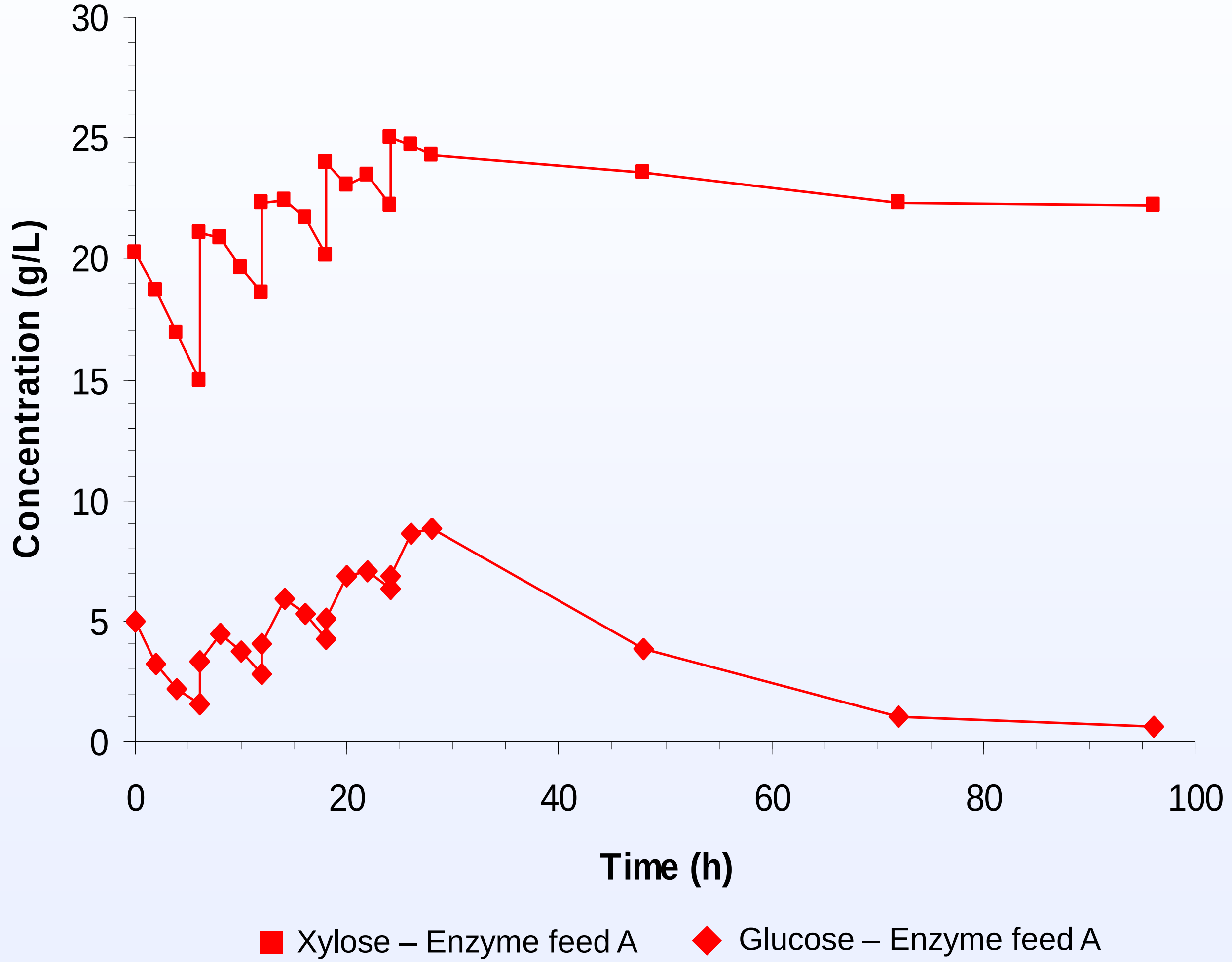
Enzyme feed along with substrate feed

* Olofsson et al. *Designing simultaneous saccharification and fermentation for improved xylose conversion by a recombinant strain of Saccharomyces cerevisiae* Journal of Biotechnology 134 (2008) 112–120

Results - SSCF of wheat straw

Fed-batch SSCF
8-11% WIS

Enzyme feed A
Xylose uptake: 38%
Ethanol yield: 61%
(*close to ref fed-batch*)

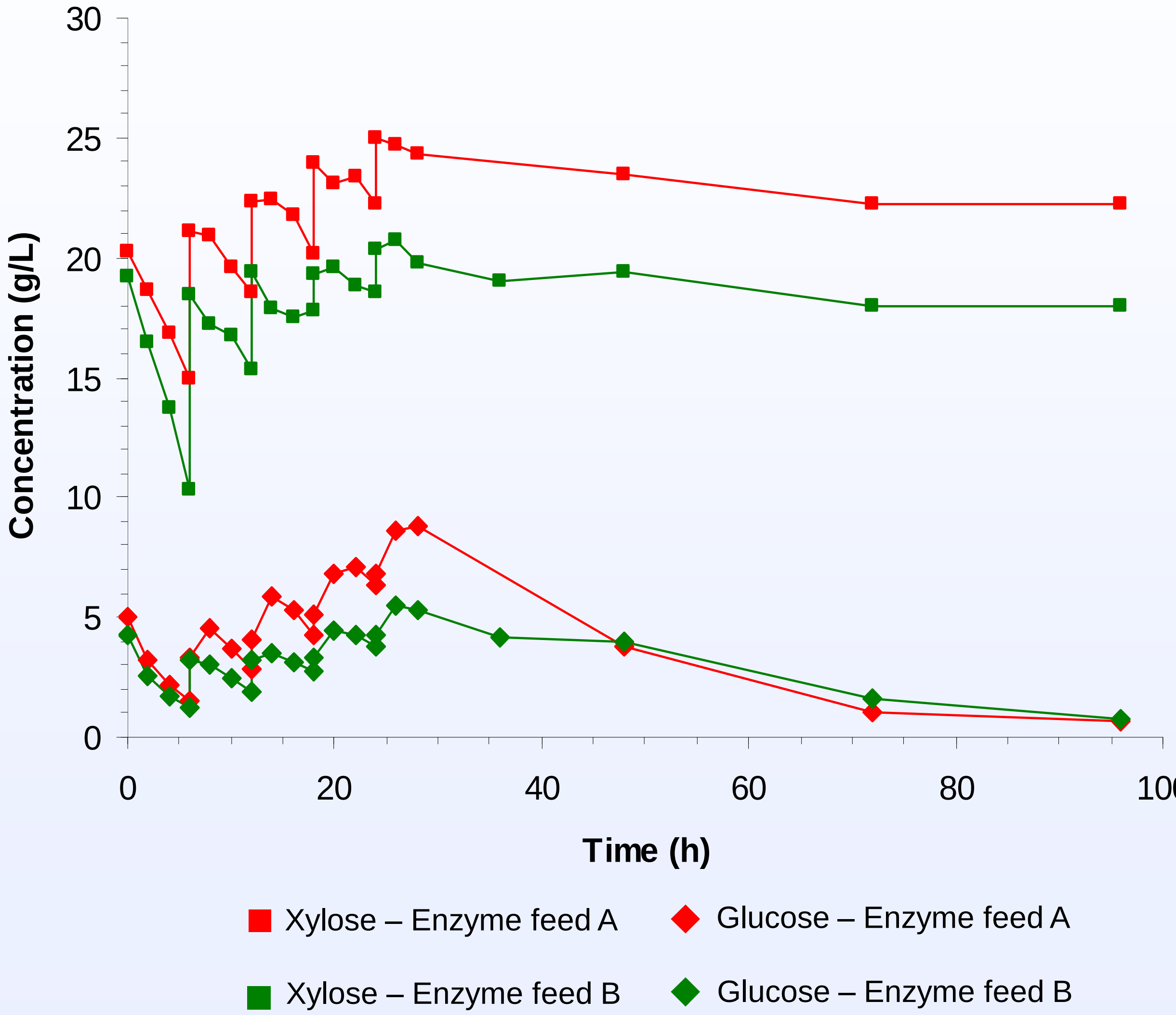


Results - SSCF of wheat straw

Fed-batch SSCF
8-11% WIS

Enzyme feed A
Xylose uptake: 38%
Ethanol yield: 61%
(*close to ref fed-batch*)

Enzyme feed B
Xylose uptake: 44%
Ethanol yield: 68%



Summary

A controlled enzyme feed improves co-fermentation in SSCF.

The model for glucose release and uptake proved to be very useful for calculating the enzyme feed profile.

Spruce (batch with enzyme feed)

- The total xylose consumption in SSCF of spruce could be doubled in comparison to regular batch SSCF (from 44% to 82%).
- The total ethanol yield was improved (from 66% to 77%).

Wheat straw (fed-batch with enzyme feed)

- The total ethanol yield in fed-batch SSCF of spruce could be increased from 59% to 68% by employing enzyme feed.

This concept may be used in the design of many different process set-ups for enhanced sugar utilization.

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