Impact of water activity on the effectiveness of high solids enzymatic hydrolysis of lignocellulose

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Enzymatic hydrolysis at high solids conc

- VHG fermentation is today's standard in the fuel ethanol industry = 12-15% ethanol is common
- For lignocellulosic feedstocks this would require 40+ % initial solids
- Technical challenging to operate processes at solids concentrations above 15% DM biomass e.g. mixing problems during hydrolysis
- High solids concentration is important for process economics:
  - Higher sugar and ethanol concentrations mean less energy for distillation
  - Reduced water usage and less water for waste treatment
  - Reduced CAPEX, higher plant productivity

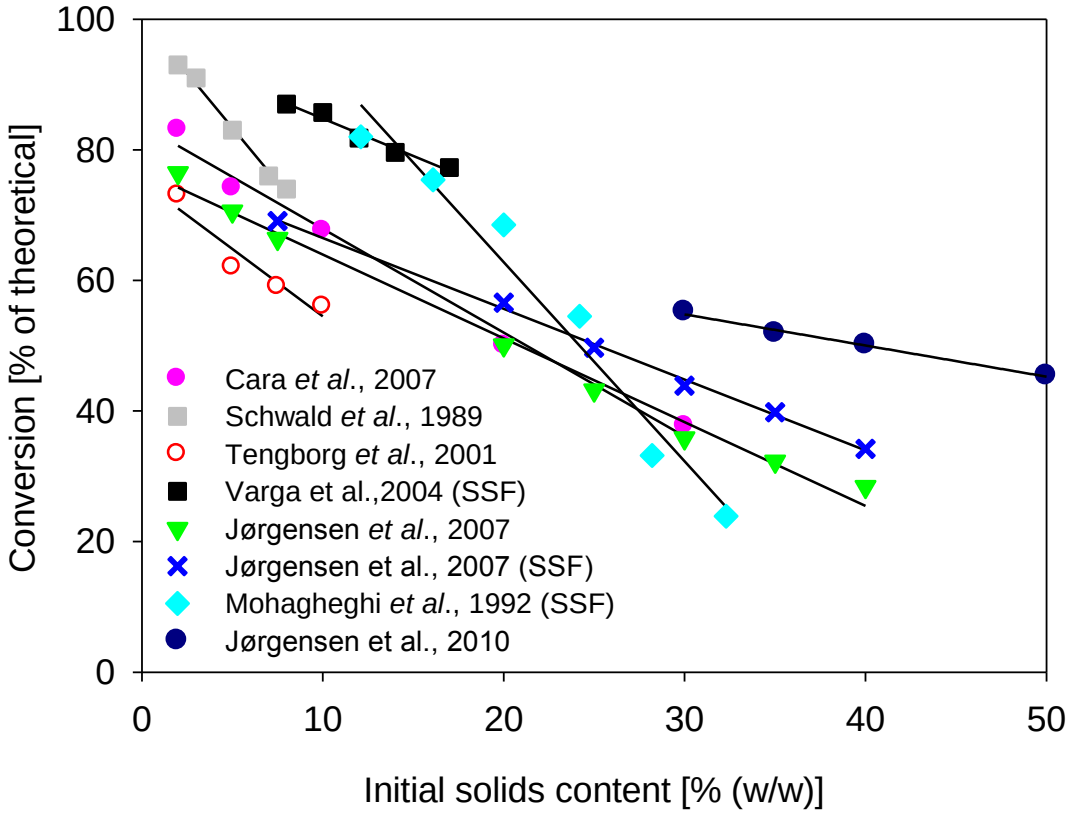
The barrier broken – free fall mixing

Pretreated wheat straw 30 % DM
Final ethanol concentrations up to 8 % (w/w) or 10% (v/v)

Jørgensen *et al.*, (2007) Liquefaction of lignocellulose at high solids-concentrations. *Biotechnol. Bioeng.* 96(5):862-870.

Upscaling and proof of technology

It works BUT it could be better!



Decreasing conversion at increasing initial solids content

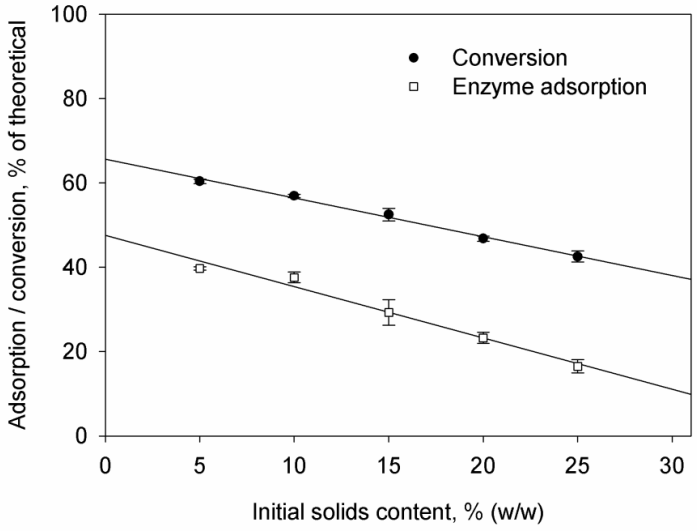
Observed on various feedstocks in hydrolysis but also SSF

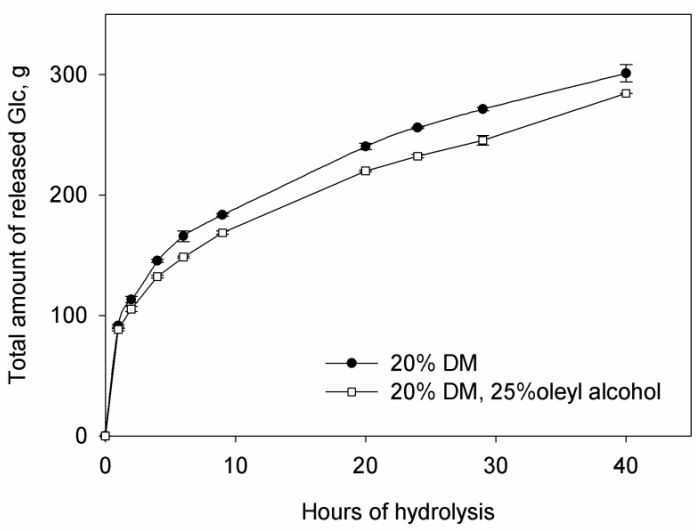
The solids effect

Factors limiting conversion:

- Product inhibition by glucose and cellobiose already during early stage of hydrolysis/liquefaction
- Inhibition by other sugars (classical inhibition)
- Inhibiton by ethanol in SSF
- Stress of yeast due to high initial concentration of sugar and inhibitors originating from pretreatment
- Mass transfer limitations
- Effect of reduced (free) water

Initial study of factors that limits conversion





- Product inhibition does play a significant role
- The increasing concentration of sugars seems to affect enzyme adsoption
- Partial substituting water with oleyl alcohol revealed that water does play an role during hydrolysis – both as substrate and as solvent

Kristensen *et al.,* (2009) Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. *Biotechnol. Biofuels 2:1, 11*.

Water activity

- Water activity is a measure of energy state of the water in a system
- Activity is relative to that of pure water
  - $a_w = p(aq)/p^*(\ell)$
  - Pure water has activity 1
  - Addition of a solute to water (ℓ) always lowers its thermodynamic activity
- Water activity much used in the food industry as a indicator of stability of food towards microbial growth – low a_w depresses growth
- Factors that impact (depress) a_w in a system:
  - Colligative effects of dissolved species (e.g. salt or sugar) - dipole-dipole, ionic, and hydrogen bonds
  - Capillary effects - changes in the hydrogen bonding between water molecules
  - Surface interactions with chemical groups on undissolved ingredients - dipole-dipole forces, ionic bonds , van der Waals forces (hydrophobic bonds), and hydrogen bonds

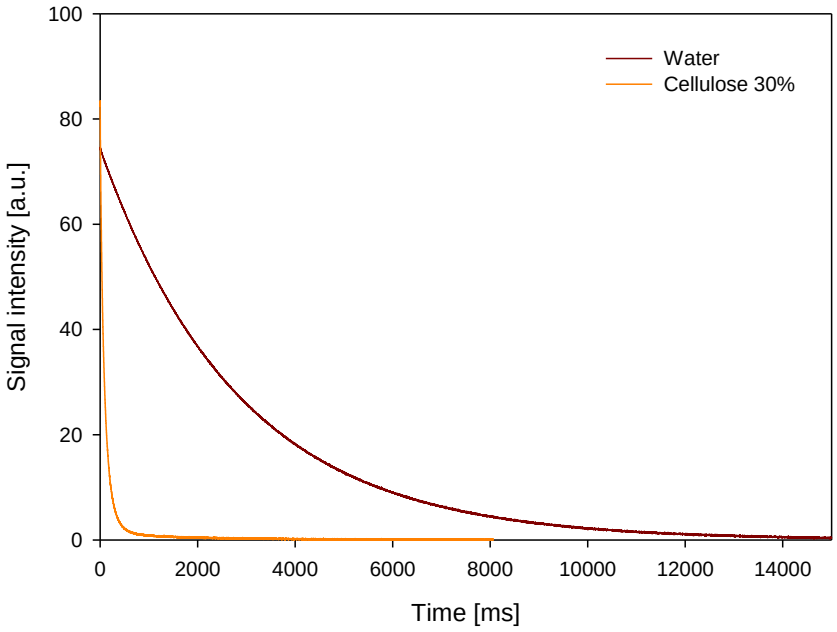
LF-TD-NMR to study the state of water

- Low Field Time Domain NMR
- Probes the states of water in the sample by measuring proton relaxation
- All protons observed at the same resonance frequency (20 MHz) and discriminated by variations in relaxation time
- The *T2* (spin-spin) relaxation time of the hydrogen nuclei depends on how free it is to move, which is determined by the environment
- Longer relaxation times means more free water
- States: tightly bound, loosely bound, and free

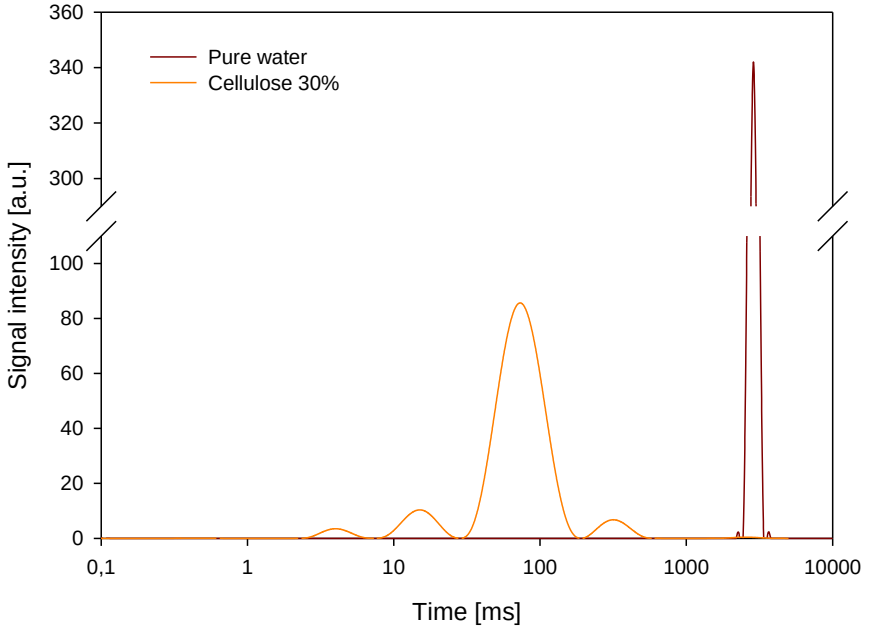
Felby *et al.*, (2008) Cellulose–water interactions during enzymatic hydrolysis as studied by time domain NMR. *Cellulose* 15:703-710.

LF-TD-NMR of water and cellulose

T_2 Relaxation curve (CPMG)



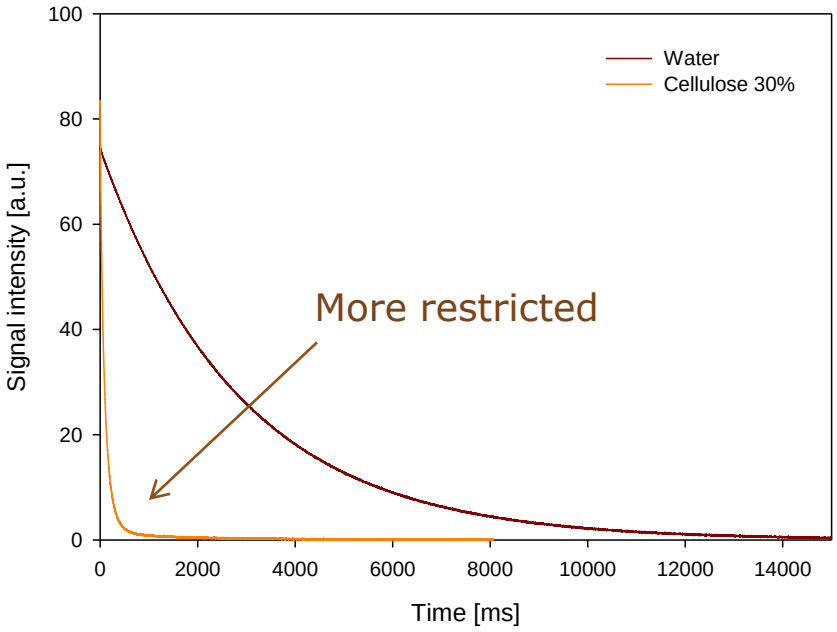
T_2 distribution (CONTIN)



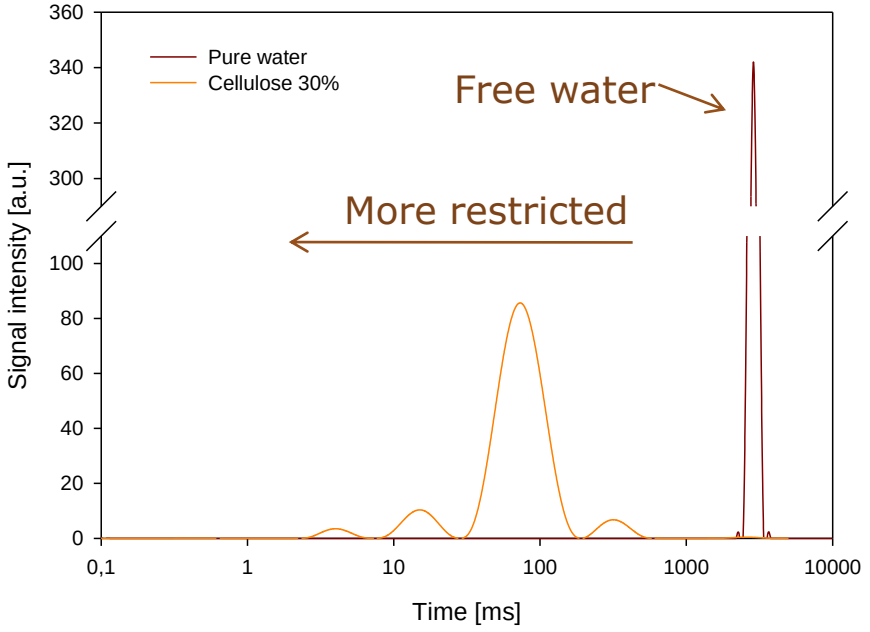
NMR of pure water and a 30% slurry of water and cellulose
Measure transverse (spin-spin) relaxation time (T2) using the Carr-Purcell-Meiboom-Gill (CPMG) sequence and CONTIN T2 distribution profiles

States of water

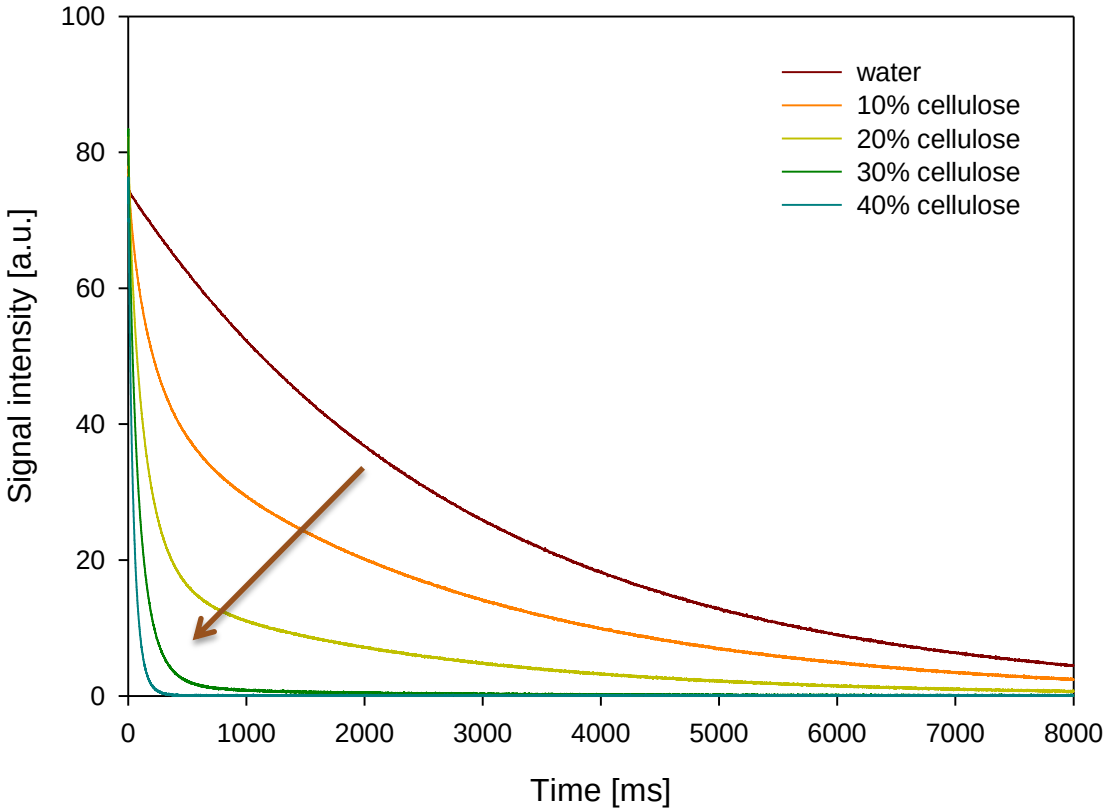
T_2 Relaxation curve (CPMG)



T_2 distribution (CONTIN)

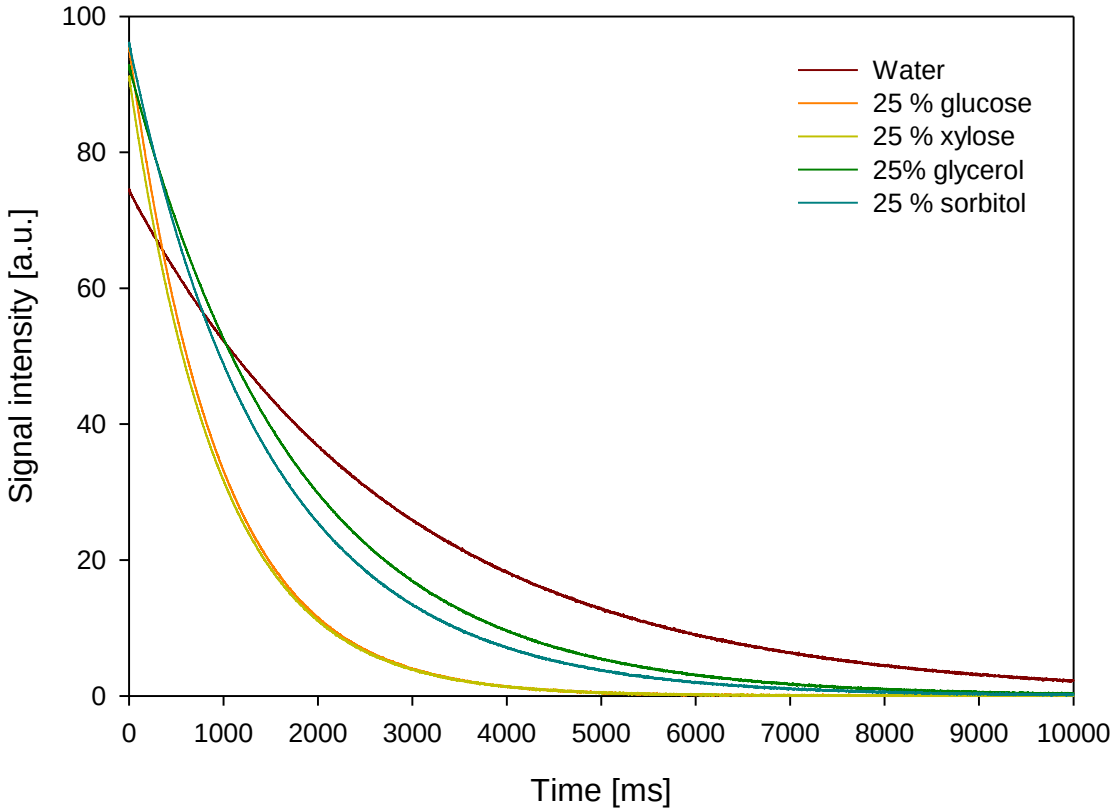


Cellulose-water system

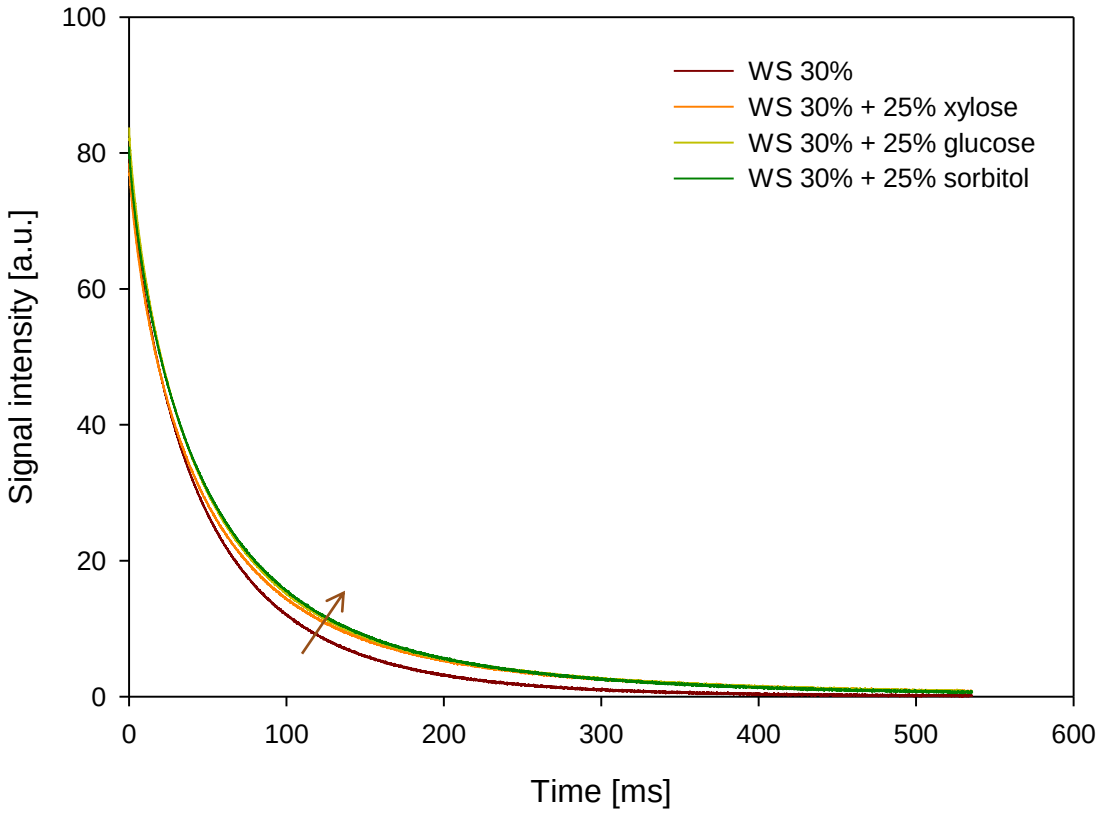


Water more restricted as the solids content increase

Sugars-water system

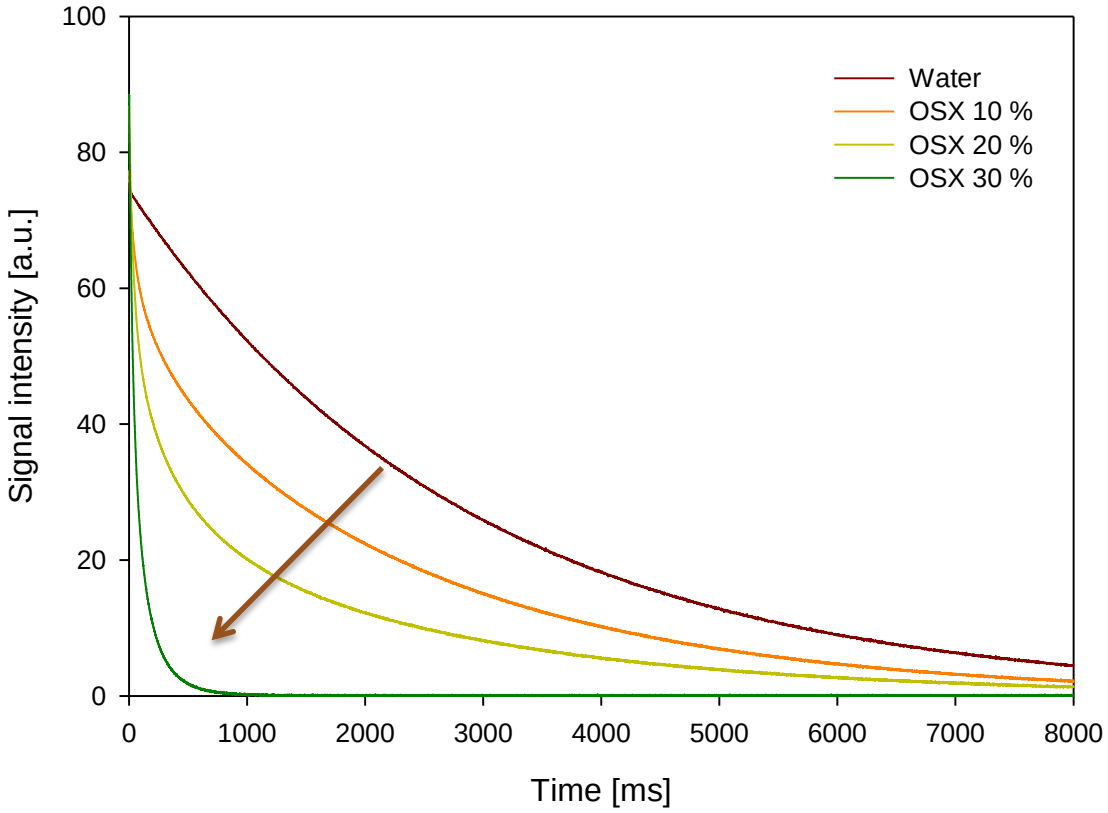


Effect of soluble sugars in WS system

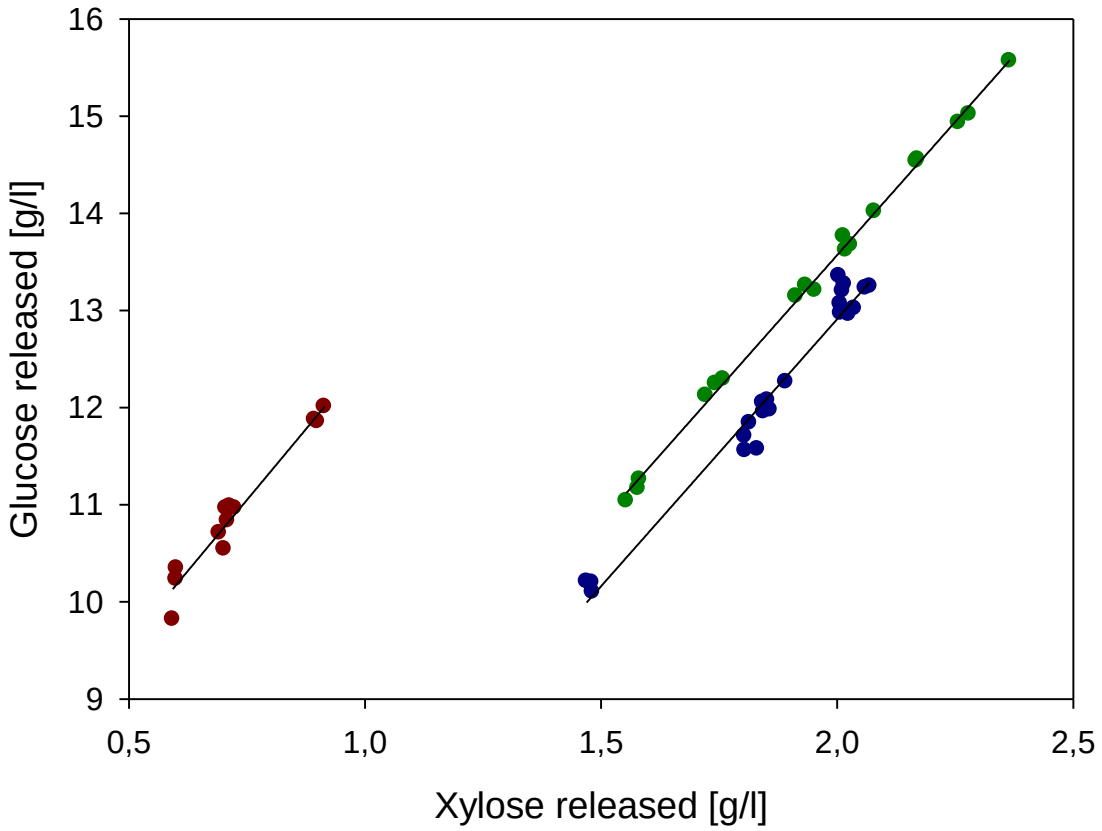


Addition of soluble sugars to system of wheat straw results in some water pools getting less constrained

Xylan-water system

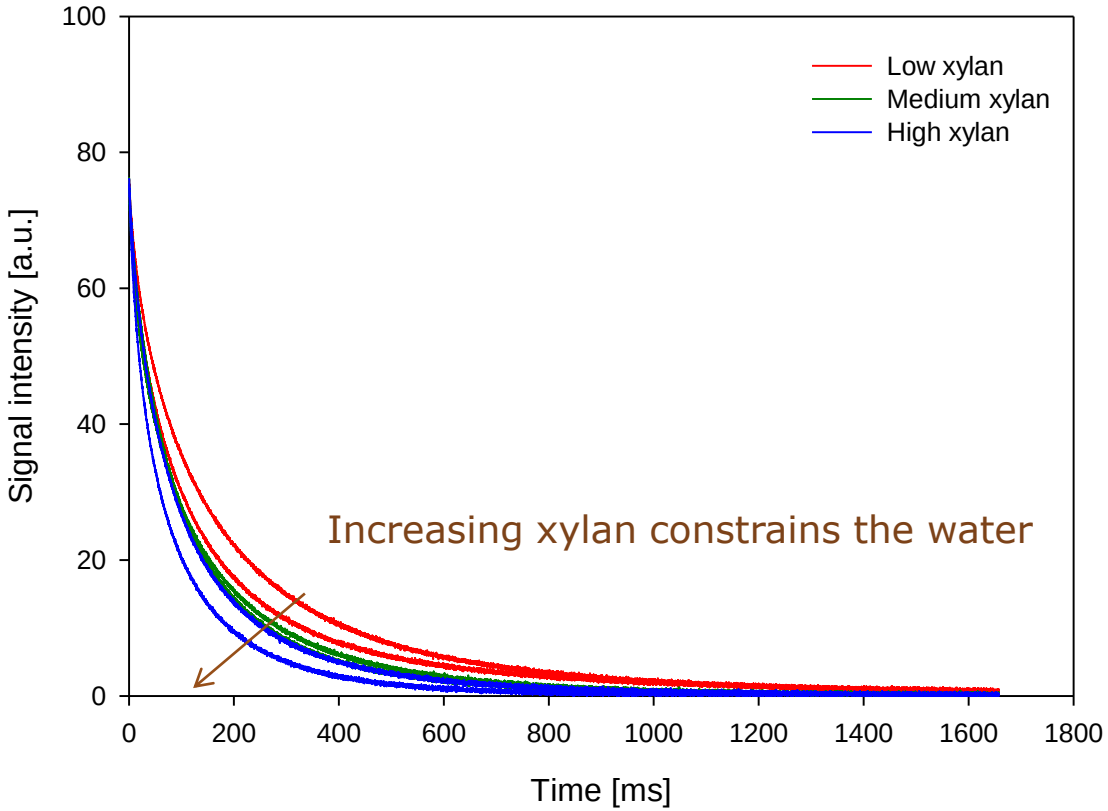


Correlation between cellulose and xylan conversion



Inbicon pretreated wheat straw
Red – low xylan, different xylanase preparations
Green – medium xylan, increasing loading of a xylanase preparation
Blue – medium xylan, different combinations of xylanase and acetyl xylan esterase

Effect of xylan in pretreated biomass



Pretreated wheat straw from Inbicon
Low xylan – 4.0-4.1%
Medium xylan – 7.9-8.2%
High xylan – 13.1-13.4%

Observations

- Adding any solute lowers a_w and NMR data show that water gets more constrained
- There is a correlation between decreased a_w and lower conversion rates
- Insoluble solids such as cellulose do not lower a_w but NMR data do show more constrained water
- Distribution of water pools depend on amount of added solute and which type
- Some sugars/sugar polymers seem to restrict water, e.g. xylan, more than cellulose

Hypothesis

- When water gets more constrained the availability of water at the cellulose surface is altered
- At low water to substrate levels water solubles may (may) pull substrate bound water away from surface of cellulose
- Surface water changes may effect enzyme (cellulase) functionality e.g. the binding of enzyme to the surface
- Previous interpretation of sugars, sugar alcohols and ethanol to be inhibitors may be explained by their ability to bind water and indirectly reduce the enzyme performance

Conclusion

- The function of the cellulase system is affected by the state of water in the system
- At high solids concentration the presence of solutes (soluble sugars, sugar polymers, sugar alcohols and ethanol) can depress the conversion rate by constraining the water and pulling surface bound water away from cellulose
- Water is definitely important for the enzyme performance (especially for enzymes working on a insoluble substrate)
- LF-TD NMR promising technique that combined with other (traditional) analysis methods can expand our understanding of how the enzyme system is working

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Thank your for your attention

Acknowledgement

DONG energy

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Ministry of Science Technology and Innovation

Inbicon

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Top-level Research Initiative

eUDP
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