Business from technology

Development of processes for the conversion of xylose to xylitol using high formic acid containing hydrolysates

Marilyn Wiebe
VTT Technical Research Centre of Finland

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BIO-COMMODITY REFINING

24 partners - 13 countries - 5 SMEs 4 MNI

biocore

SEVENTH FRAMEWORK PROGRAMME

a 4-year EU project
Cost : 20 274 484 €
EU contribution : 13 920 238 €

Coordination : INRA M. O'Donohue

biocore

Varied biomass → **Fractionation** → Intermediates → **Chemistry Biotechnology**

- Hemicellulose
- Cellulose
- Lignin

Final products

- 2nd generation fuels
  - Ethanol
- Thermoplastics
  - PVC, polyolefins, polyurethanes, polyesters
- Resins/Adhesives
- Food additives
- Detergents
- Wood panels

Application sectors


Energy


Materials


Packaging


Building


Adhesives and paints

LEARN TO USE EVERY DROP

Oil refining provides a paradigm

- All oil fractions are valorized
- Non-energetic products generate the highest revenues
- Both fuel and chemicals are produced

Biorefining should use every 'drop'

- A cellulose to fuel concept is insufficient
- Pentose sugars and lignins must be valorized
- Higher value products must be derived from biomass

TECHNOLOGIES FOR THE BIOECONOMY

Biotechnology will be a key driver (Suschem report)

- Energy efficiency
- Lowered environmental impact
- High catalytic diversity

Chemistry will continue to play a pivotal role

- Proven technologies and processes
- Cleaner reactions inspired by REACH and principles of green chemistry

  (**R**egistration, **E**valuation, **A**uthorisation and **R**estriction of **C**hemical substances)

Integrated processes using both biotechnology and chemistry will become frequent

- Smart integration will be critical for efficient biorefinery processes

OPTIMIZED BIOMASS FRACTIONATION

CIMV Organosolv

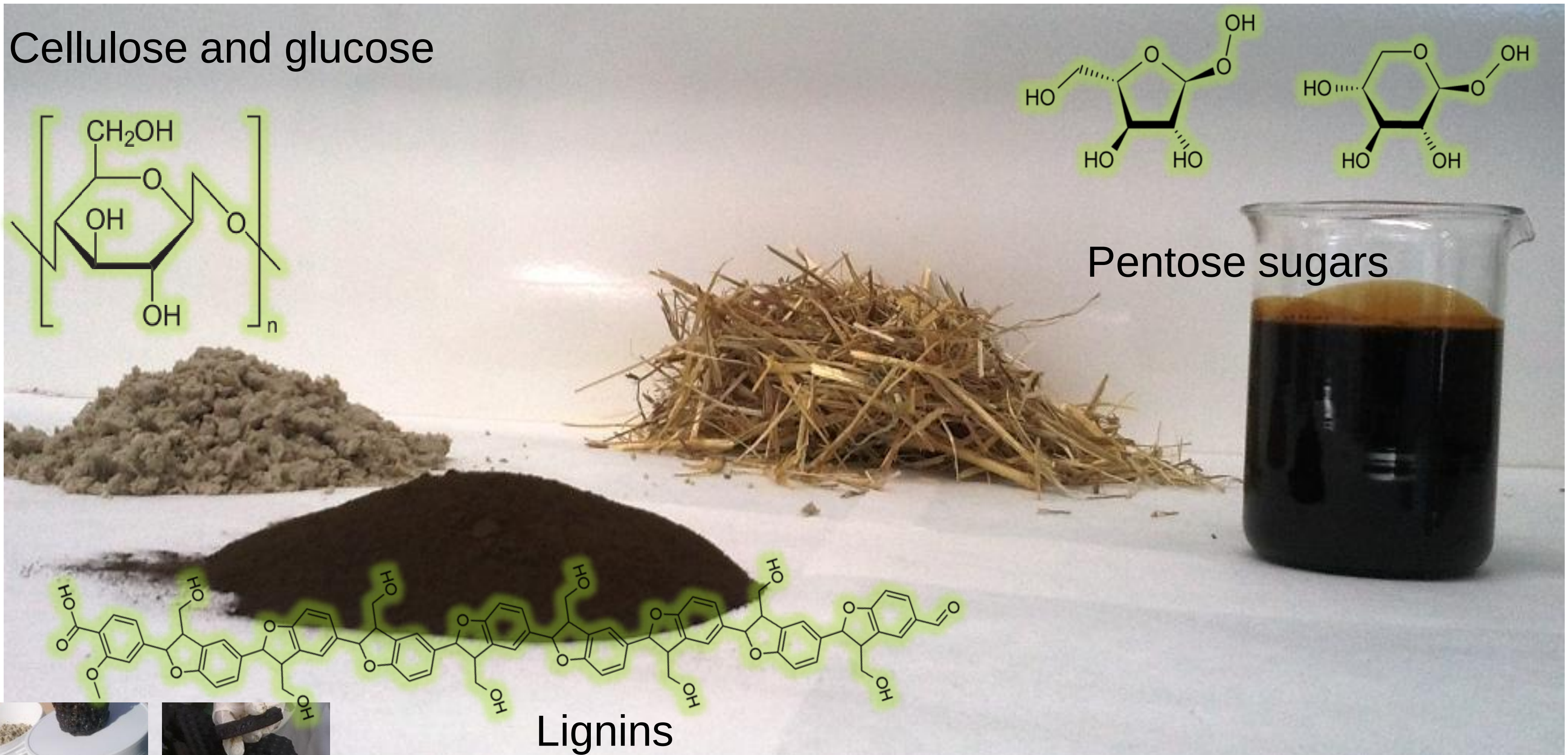
- Uses a **formic : acetic** acid solvent system (generation of peracids)
  - Dissolves lignin and hemicelluloses
- Multi-biomass
  - Hardwood
    - SRC woods (with bark)
  - Cereal co-products
    - Wheat straw
    - Rice straw
    - Maize cane
  - Dedicated energy crops

The pilot plant

- 100 kg/h biomass
- In operation since 2006
  - >50 runs completed

CIMV = Compagnie Industrielle de la Matière Végétale

THREE PLATFORM INTERMEDIATES

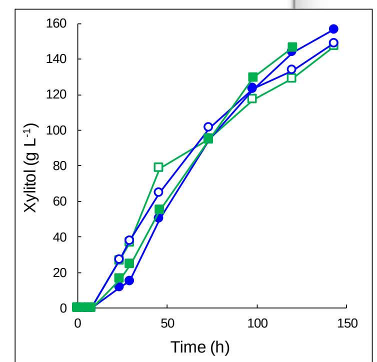


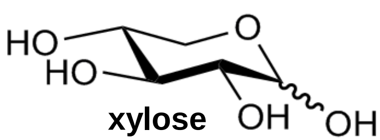
Biotechnological production of xylitol and xylonate

CIMV organosolv

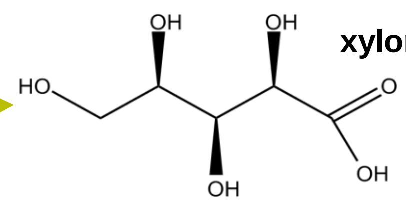
Yeast catalyzed conversion

- Using pure xylose up to 150 g xylitol L^{-1} at high productivity (yield 0.73 g xylitol/ g xylose consumed)
- up to 170 g/L of xylonate
- Pentose syrup refining is still a challenge

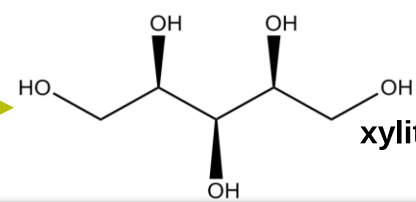




xylose



xylonic acid



xylitol

Pentose fraction – composition

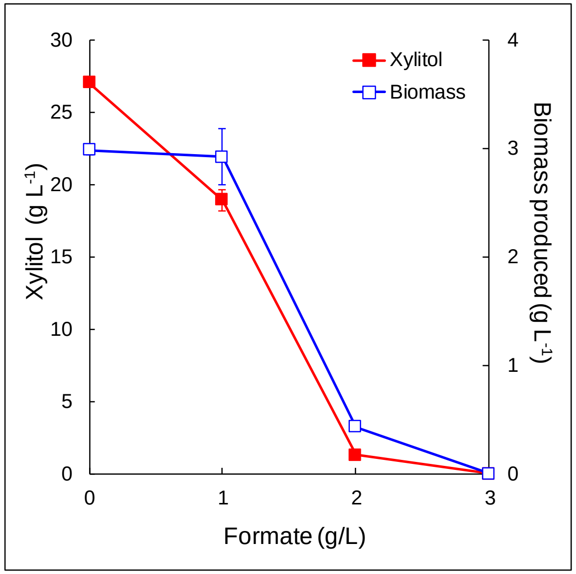
- The CIMV process generates a pentose fraction which contains a high concentration of monomeric xylose (~50% total xylose).
- Formic and acetic acids are recycled, but the concentration in the C5 hydrolysate remains relatively high. Reducing the formic acid concentration without also reducing the xylose concentration may be prohibitively expensive.
  - Steam-stripping
  - Ion exchange
- Hydrolysis of oligomeric xylose residues could double the xylose concentration.

Formic acid is toxic to yeast

The pentose hydrolysate contains 22 – 100 g formic acid l^{-1}

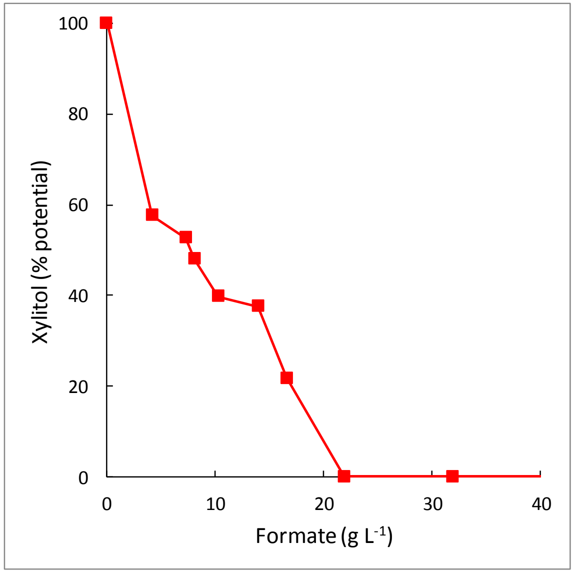
- *S. cerevisiae* B67002 tolerates < 3 g formic acid l^{-1} if unbuffered
- *S. cerevisiae* B67002 tolerates 15-20 g formic acid l^{-1} when buffered

unbuffered



Recombinant *S. cerevisiae* was grown in unbuffered YPDX medium (50 g D-xylose l^{-1} and 20 g D-glucose l^{-1}) with the addition of formic acid, in 250 ml flasks (200 rpm, 30°C).

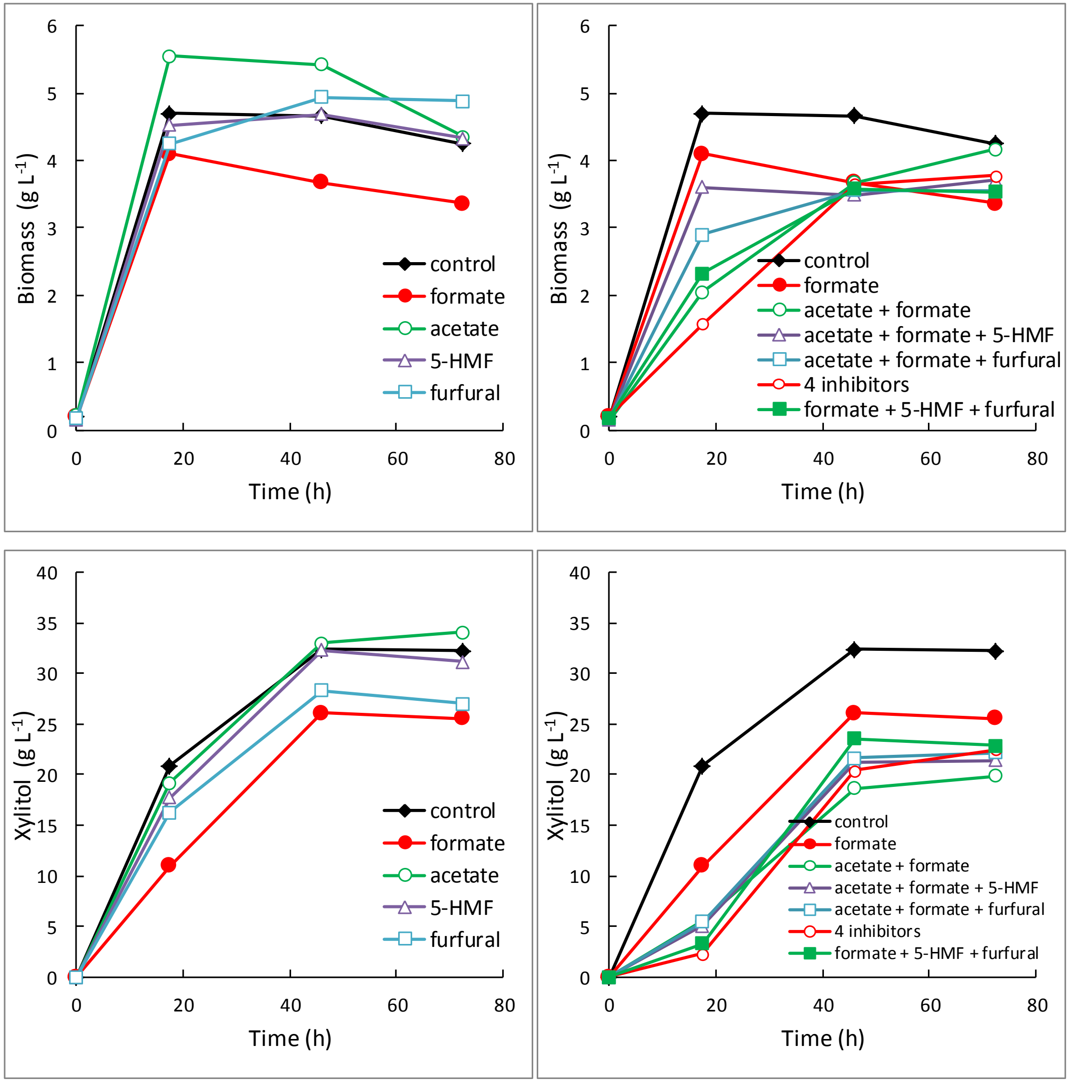
buffered (pH 5.5-6.5)



Recombinant *S. cerevisiae* was grown in $CaCO_3$ buffered complex medium (20 g D-glucose l^{-1}, variable D-xylose) with the addition of formic acid (and acetic acid), in 250 ml flasks (200 rpm, 30°C).

- Reducing the formic acid content to < 5% is challenging, expensive and the D-xylose content may become diluted
- **Can the formic/acetic acid organosolv process provide C5 hydrolysate for biotechnological conversion?**

Formic acid is the inhibitor in the pentose fraction, but not the only one



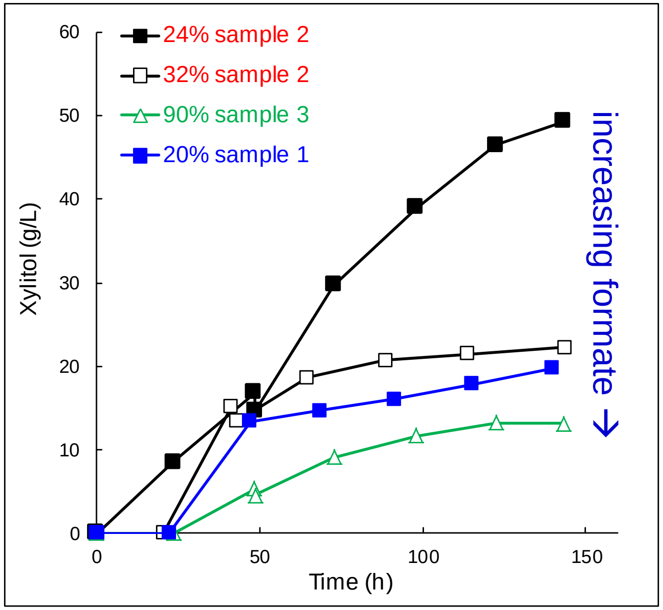
- Formate reduced biomass and xylitol production more than any other single inhibitor.
- Synergistic effects were seen when multiple inhibitors were present, particularly affecting biomass production.
- *S. cerevisiae* should be able to grow and produce xylitol in diluted CIMV pentose hydrolysate.

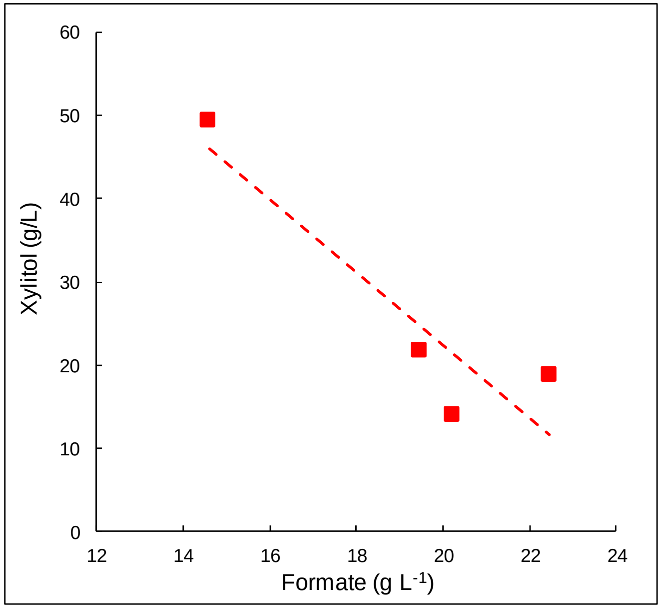
Recombinant *S. cerevisiae* was grown in $CaCO_3$ buffered YP medium (~35 g D-xylose l^{-1} and 18 g D-glucose l^{-1}) with addition of ~18 g formate l^{-1}, 8 g acteate l^{-1}, 1 g 5-HMF l^{-1}, or 1 g furfural l^{-1}, in 250 ml flasks at 30°C, 200 rpm.

Production of xylitol from CIMV pentose fraction – batch cultures

- The production of xylitol from CIMV pentose hydrolysate is dependent on the formic acid content and extent to which it has been diluted.
  - diluted hydrolysates were supplemented with xylose to prevent xylose limitation, testing the capacity for xylitol production with similar total xylose concentrations

- 24% dilution is too low to provide sufficient xylose for a productive process.



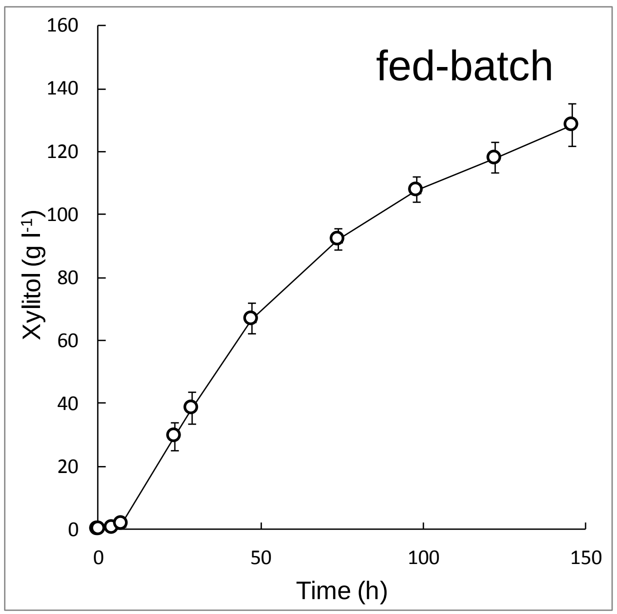


Recombinant *S. cerevisiae* was grown in CIMV pentose fractions supplemented with D-xylose and D-glucose to give initial concentrations ~50 and 20 g L^{-1}, respectively, plus yeast extract and peptone to provide vitamins and nitrogen (later shown to be unnecessary). Additional D-xylose was provided after ~48 h.

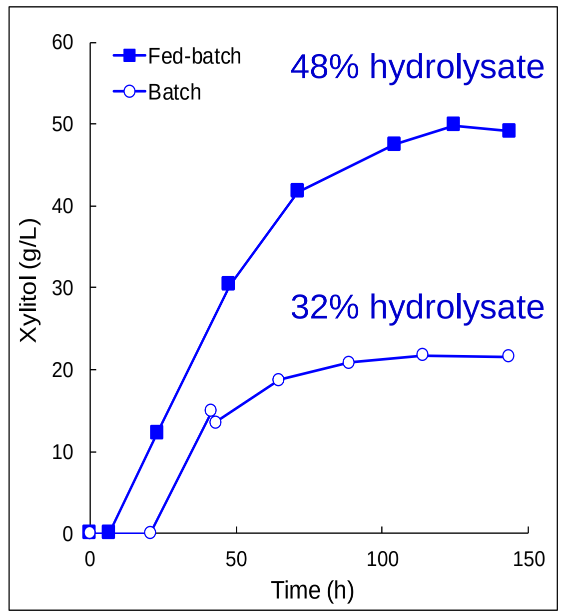
Production of xylitol from CIMV pentose fraction – fed-batch cultures

- Fed-batch processes are very effective for production of xylitol from xylose.
- Providing hydrolysate as a concentrated source of xylose in the feed resulted in higher utilisation of xylose from the hydrolysate, more xylitol production and tolerance of higher concentrations of hydrolysate

YP xylose



hydrolysate



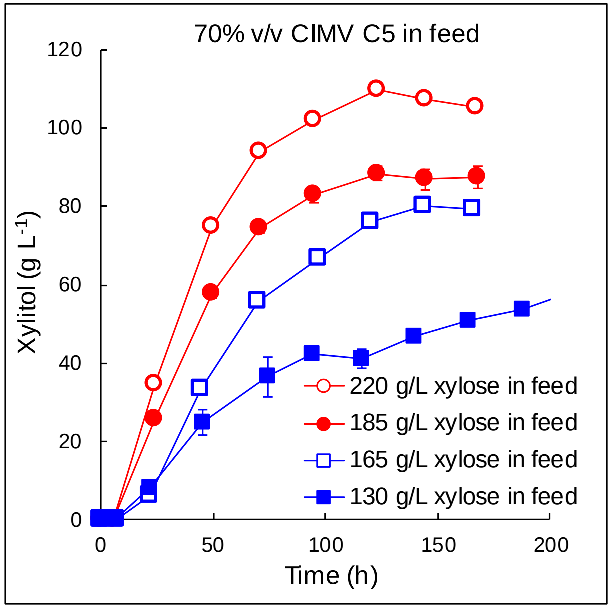
fed-batch

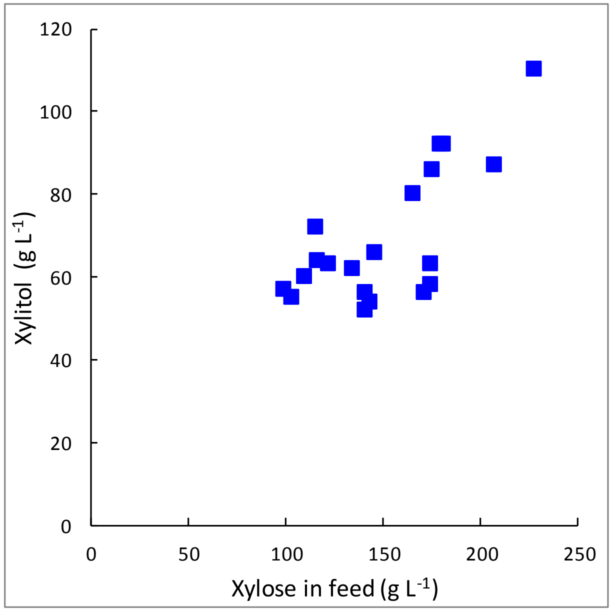
batch

Recombinant *S. cerevisiae* was grown in (left) YP medium fed with 300 g xylose l^{-1} and 90 g glucose l^{-1}, or (right) CIMV pentose fraction supplemented with D-xylose and D-glucose to give initial concentrations ~50 and 20 g L^{-1}, respectively, plus yeast extract and peptone to provide vitamins and nitrogen (32% hydrolysate) or fed with 70% (v/v) hydrolysate supplemented to provide a total xylose content of 140 g D-xylose l^{-1} and 40 g D-glucose l^{-1} (48% hydrolysate).

Production of xylitol from CIMV pentose fraction – fed-batch cultures

- For fed-batch to be efficient the feed must contain a very high concentration of the substrate.
  - Biomass hydrolysates typically do not contain high enough concentrations of xylose to provide sufficient substrate for fed batch.
  - If all the oligomeric xylose in CIMV pentose fraction were available, concentrations of xylose as high as 200 g l^{-1} could be provided – this is adequate for feeding.
- Xylitol production was largely dependent on the total xylose provided, regardless of the concentration of hydrolysate in the feed (40 to 90%),
  - however xylitol production generally stopped after the concentration in the reactor was >50%.





Recombinant *S. cerevisiae* was grown in defined medium (or ½YP) containing 20 g glucose l^{-1}. Feed contained (right) 40 to 100% CIMV pentose fraction supplemented with D-xylose (100 – 225 g l^{-1})and D-glucose (0.4 x xylose), NH^{4+} and vitamins.

Production of xylitol from CIMV pentose fraction – fed-batch cultures

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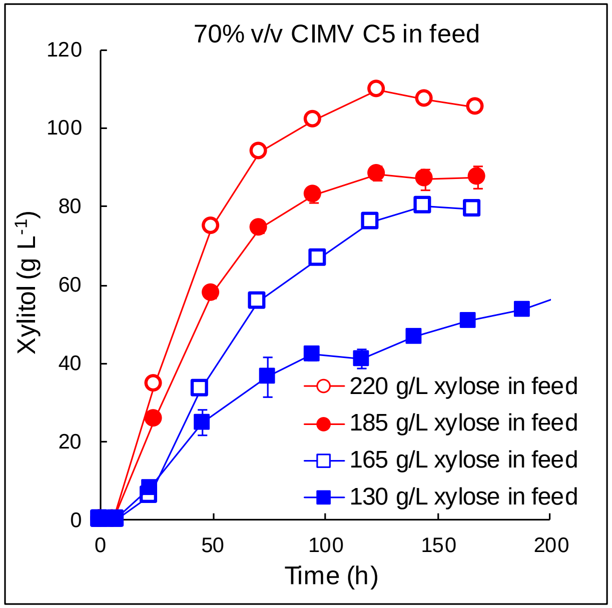
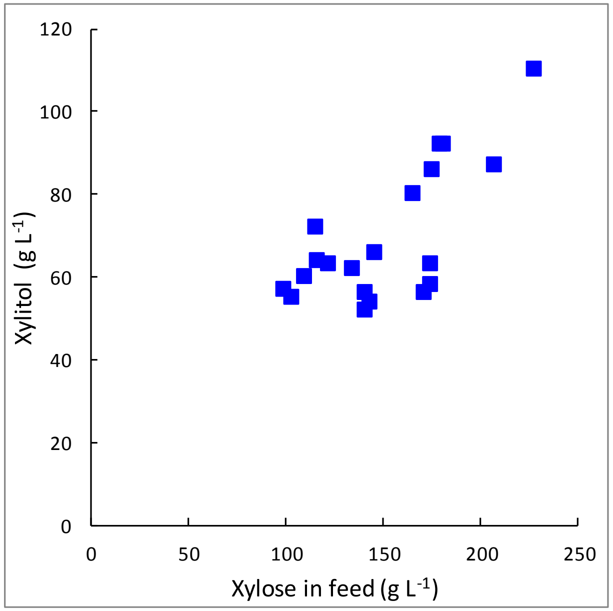
- The *S. cerevisiae* strain produced:
  - >100 g xylitol l^{-1}
  - at 0.9-1.0 g l^{-1} h^{-1}
  - and yield 0.9 g xylitol [g xylose]$^{-1}$
- when fed 70% hydrolysate with a total 225 g xylose l^{-1}.



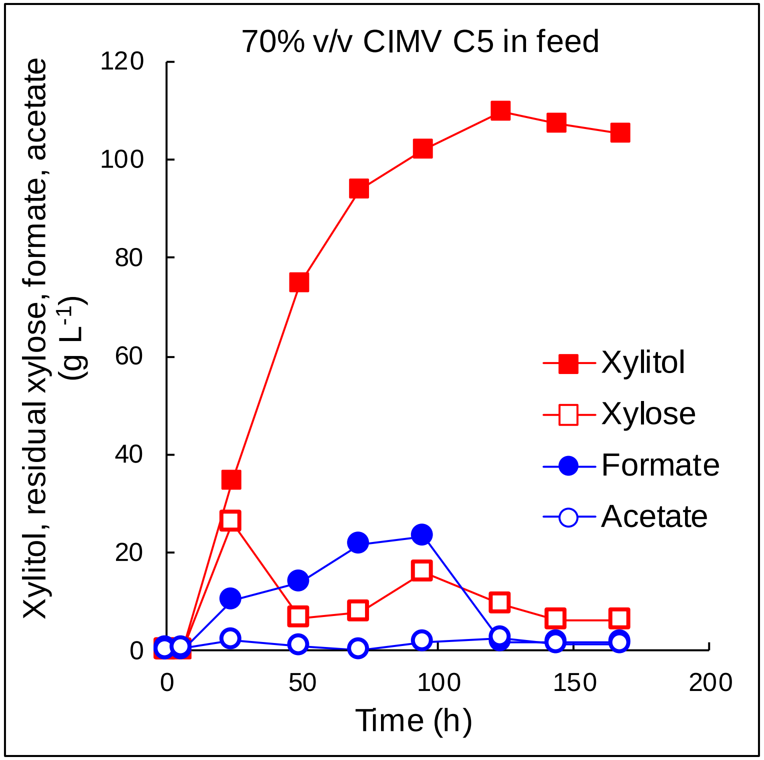


Recombinant *S. cerevisiae* was grown in defined medium (or ½YP) containing 20 g glucose l^{-1}. Feed contained (right) 40 to 100% CIMV pentose fraction supplemented with D-xylose (100 – 225 g l^{-1})and D-glucose (0.4 x xylose), NH^{4+} and vitamins.

Advantages and Challenges

- The concentration of xylose is high in the organosolv pentose fraction – additional concentration is not needed after formic acid stripping to ~5% (if all xylose available)
- In fed-batch cultivation the initial production rates are high and induction of formate dehydrogenase and acetate metabolism reduces the concentration of these inhibitors.

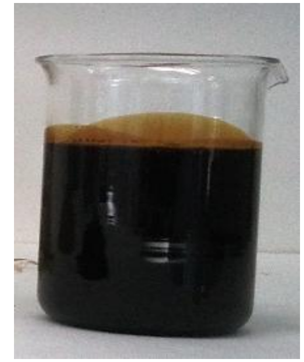
- The hydrolysate is diluted in the feeding process.
- Xylosidases with high activity on short xylose oligomers in the pentose hydrolysate are needed.
- C6 oligomers in the hydrolysate should supply the co-substrate.
- The inhibitors still in the hydrolysate should be reduced by ~30%.
- Acetate may be released when oligomers are hydrolysed and may become toxic.

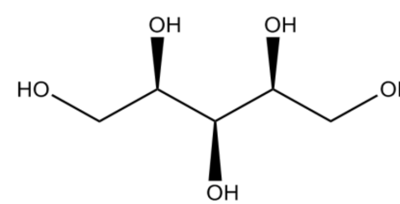


Recombinant *S. cerevisiae* was grown in defined medium containing 20 g glucose l^{-1}. Feed contained 70% CIMV pentose fraction supplemented with D-xylose (to 225 g l^{-1})and D-glucose (to ~70 g l^{-1}), NH^{4+} and vitamins.

Conclusions

- The C5 fraction generated by the CIMV formic/acetic acid organsolv process generates a hydrolysate with a **high concentration of xylose** which can be converted to xylitol biotechnologically in a fed-batch process.
- **Steam-stripping** to remove formic and acetic acids for recycling is sufficient treatment for the fed-batch process.
- Refining of the C5 fraction is still in progress
  - release of oligomeric xylose
  - further reduction of inhibitors
- Hydrolysate tolerant **recombinant *S. cerevisiae*** is suitable for xylitol production
  - High yields
  - High tolerance
  - No organic N or other expensive supplements are required for the process





> 100 g L^{-1} xylitol possible in the fed-batch process.

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Scientific

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Thank you for your attention!