

**Functional analysis of the xylanolytic enzyme  
spectrum of the fungus *Myceliophthora  
thermophila* C1**

EPNOE 2011

Wageningen, 30<sup>th</sup> August 2011

Sandra Hinz



# Introduction Dyadic Netherlands

---



## Research Subsidiary of Dyadic Inc, USA

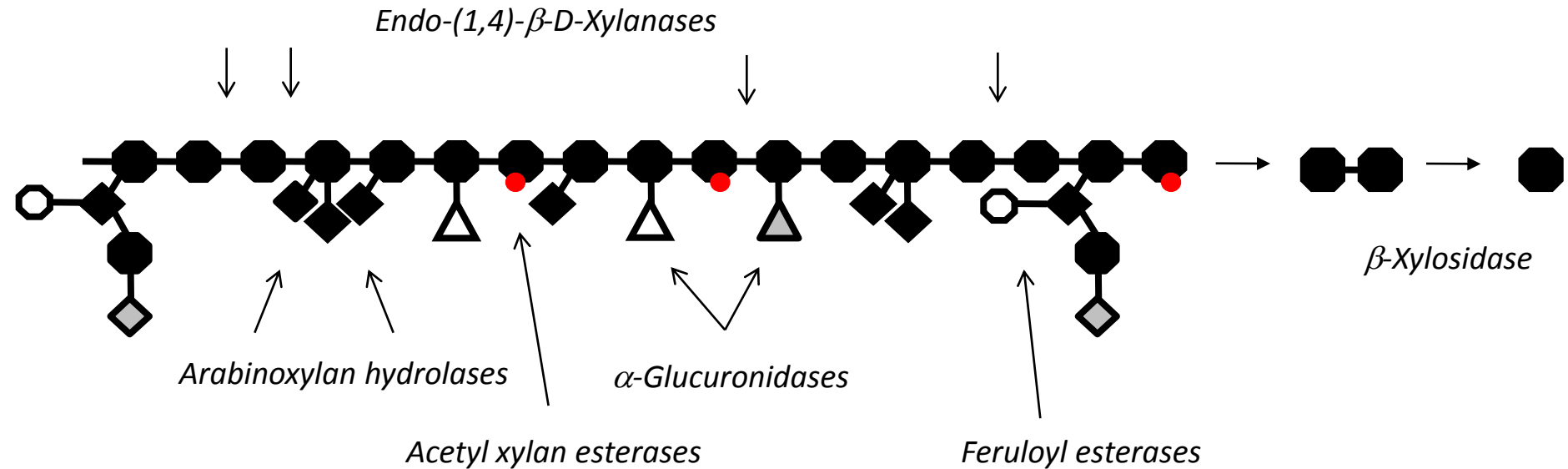
- Dyadic Nederland BV, Wageningen, The Netherlands
- [www.dyadic.nl](http://www.dyadic.nl)

## Focus

- Discovery and development of enzymes
- Fields of application: bioenergy/biorefinery, food/feed, textile and paper and pulp
- Production in *Trichoderma* and *Myceliophthora thermophila* C1 (previously known as *Chrysosporium lucknowense* C1)



# Xylan and the degradation thereof



- There is a great variation in xylan structures
- Variation is dependent on the species, climate, season



# Variation in xylan structure

---

Variation between species:

- **Corn fibre:**

highly branched with: arabinose, glucuronic acid (methylated), ferulic acid, acetic acid, rare side branches

- **Wheat:**

branched with: arabinose, glucuronic acid (methylated), ferulic acid, acetic acid

- **Birch wood:**

less branched with: methylated glucuronic acid, acetic acid

- **Oat spelt:**

mainly linear xylan, small amounts of arabinose



**Variation in xylanases is needed → exploration of C1 xylanases**

our enzymes; nature at work



# C1 potential for xylan degradation

Putative activity	Number of genes in C1
$\beta$ -Glucosidases	7
Endo-glucanases + CBH	13
GH61	26
CDH + CDH-like	9
<b><math>\beta</math>-Xylosidase</b>	<b>5</b>
<b>Xylanase</b>	<b>13</b>
<b>Arabinoxylan hydrolases (AXH)</b>	<b>7</b>
<b>Esterases (AXE + FAE)</b>	<b>13</b>
<b><math>\alpha</math>-Glucuronidases</b>	<b>2</b>
Arabinases + arabinofuranosidases	7

Potential to degrade different xylans

*Based on manual annotation*



# Thirteen xylanases in C1

---

## **GH10 enzymes:**

- Less hindrance by side-groups (smaller oligosaccharides)
- 4 Putative enzymes found in C1 genome

## **GH11 enzymes:**

- Hindered by side-groups (larger oligosaccharides)
- 7 Putative enzymes found in C1 genome

## **GH30 enzymes:**

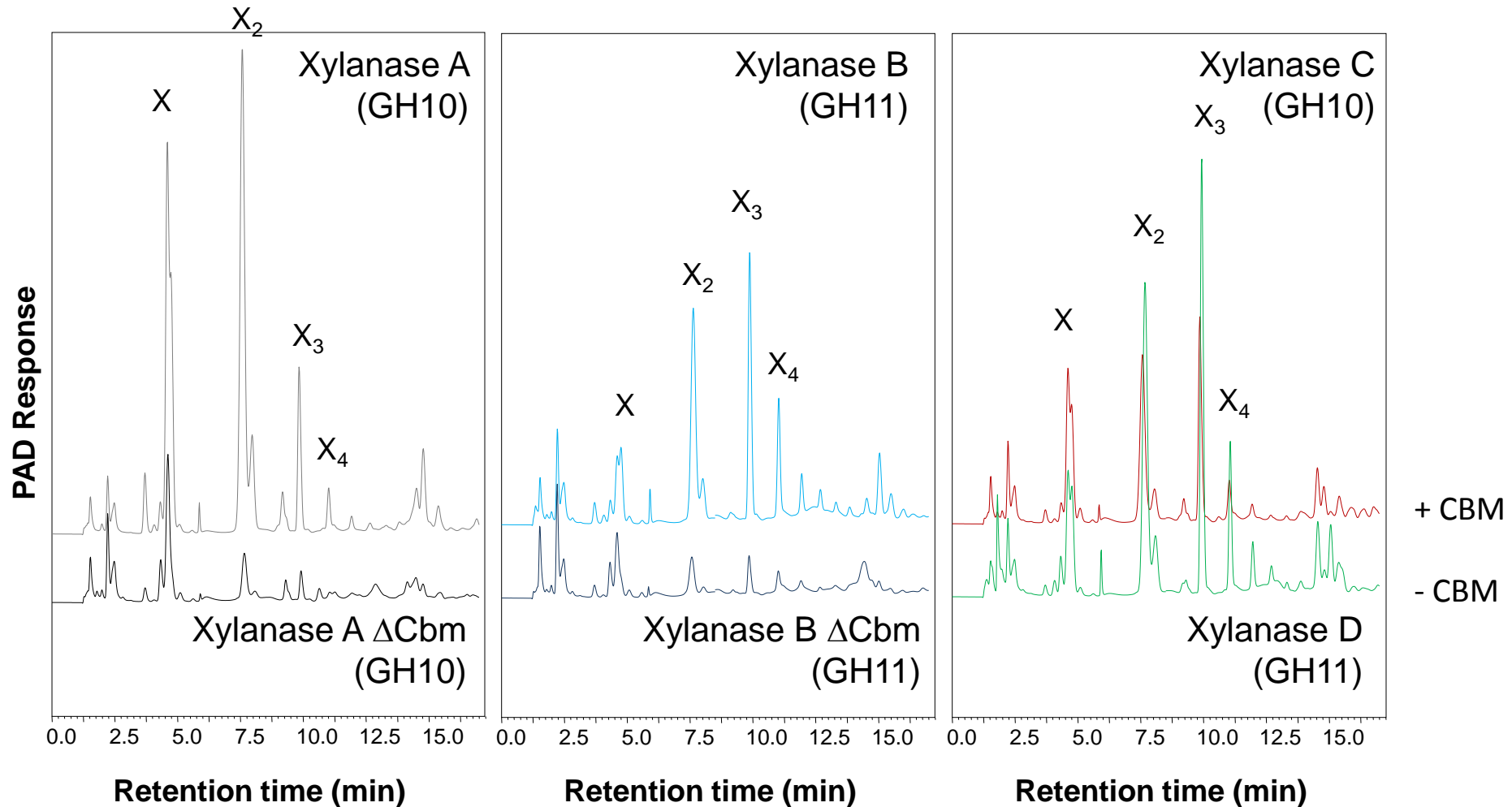
- Cleave closer to GlcA side-group (Glucuronoxylanases)
- Exo-xylanase from *Trichoderma*, acting from reducing end
- 2 putative enzymes found in C1 genome

All 13 xylanase have been produced in a dedicated C1 strain

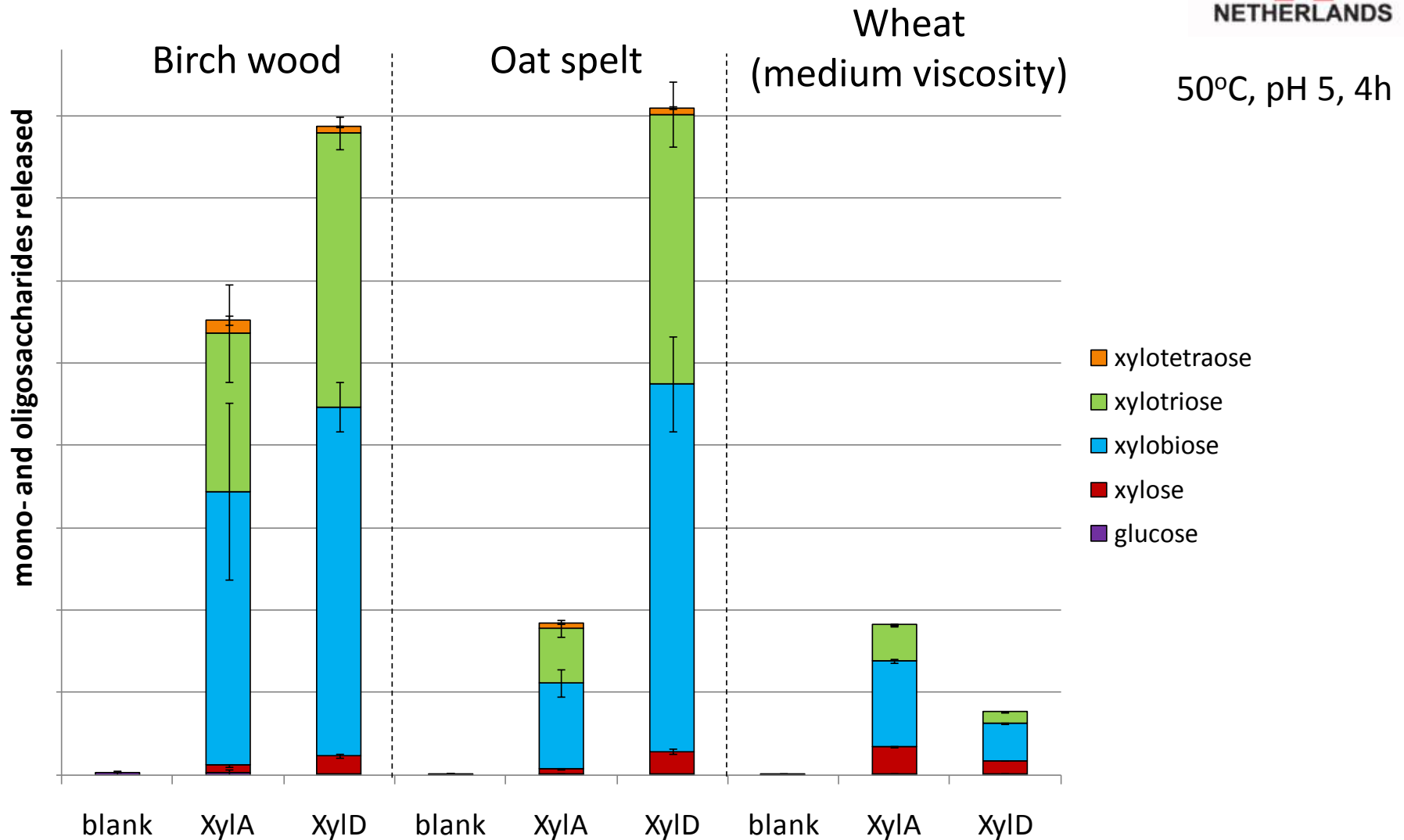


# Activity of xylanases towards wheat straw

Conditions: 24h, pH5.0, 50°C



# Activity towards xylans from different sources

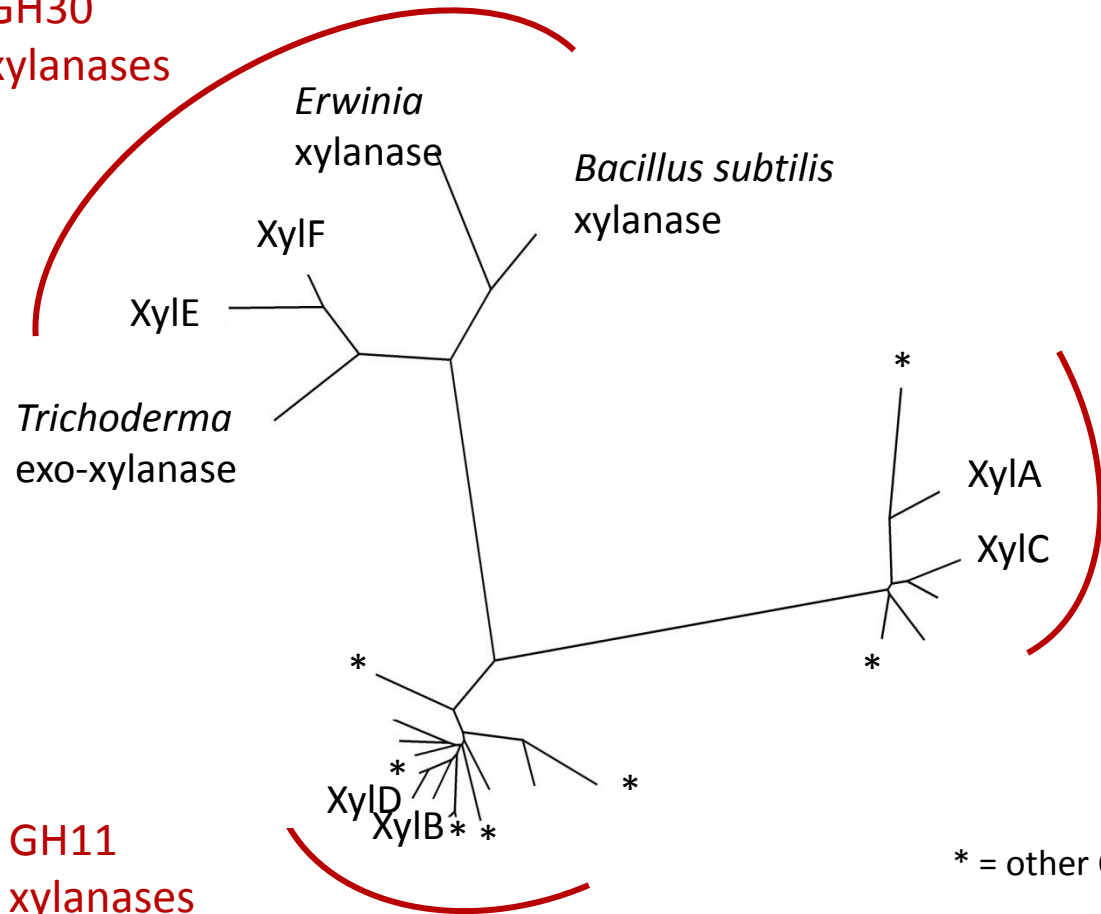


# Two novel C1 xylanases

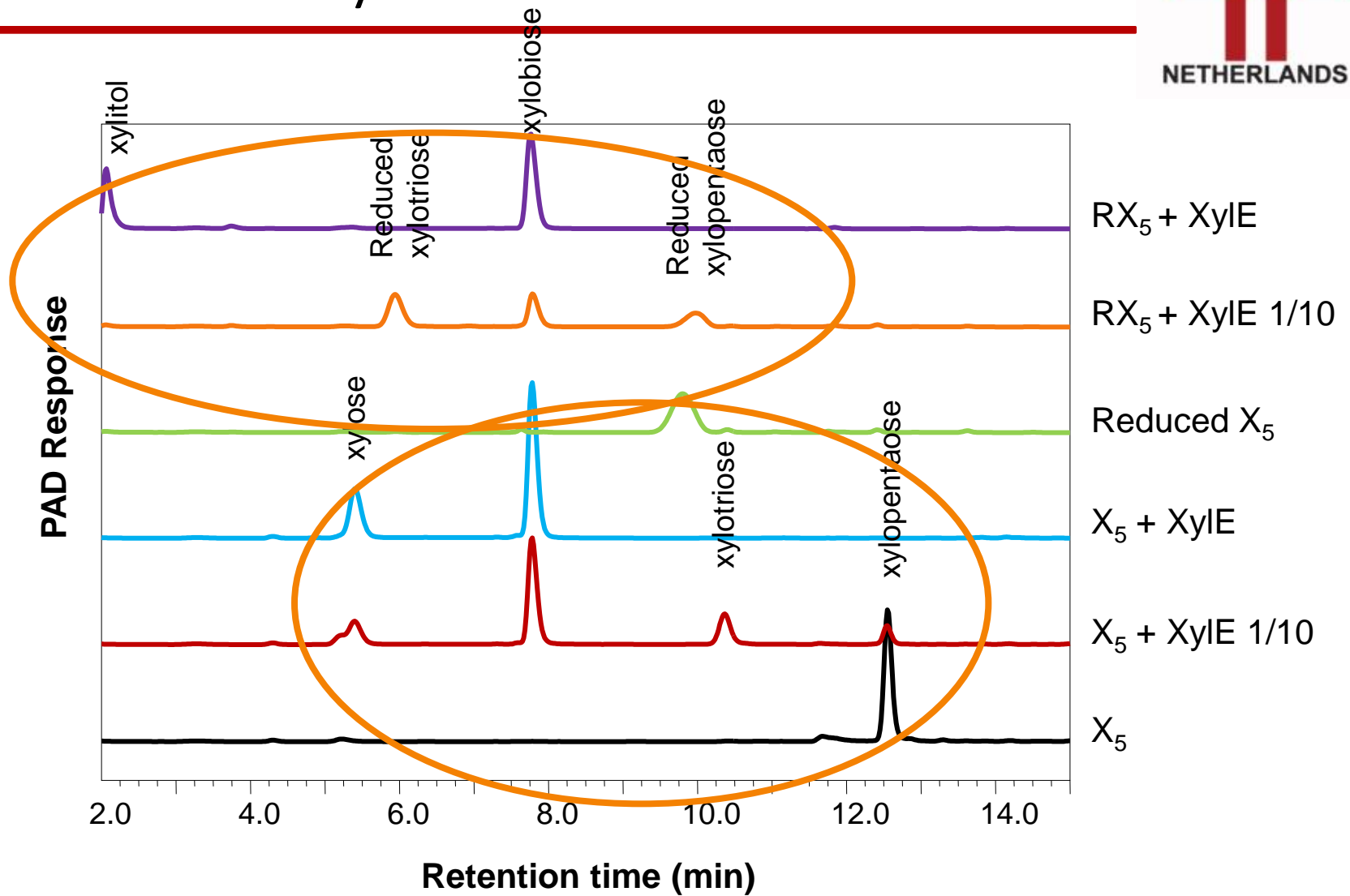
Two novel C1 xylanases have been identified and produced:

- XylE → GH30
- XylF → GH30

GH30  
xylanases



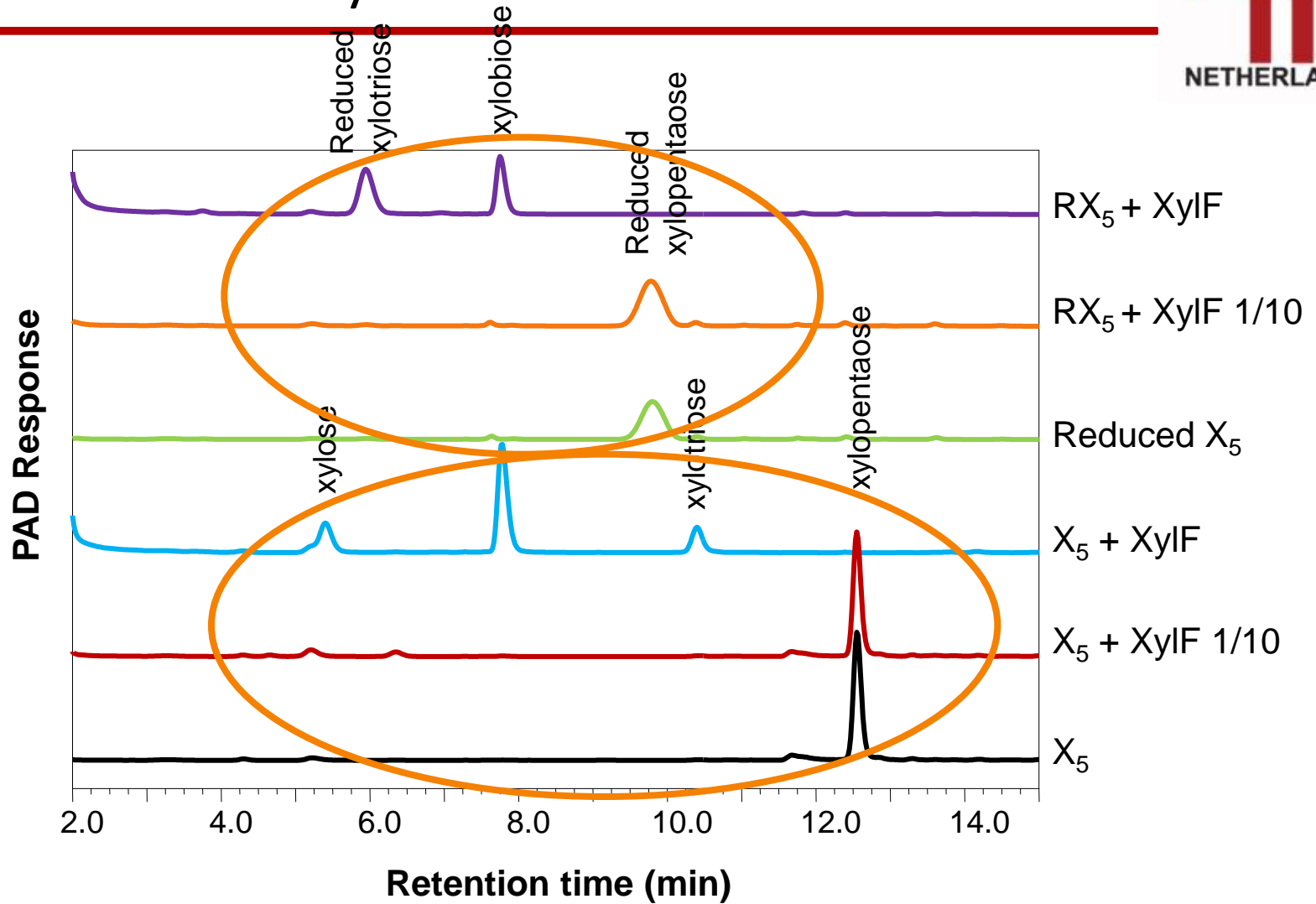
# Mode of action Xylanase E



Release of xylobiose, xylofuranose, xylohexaose



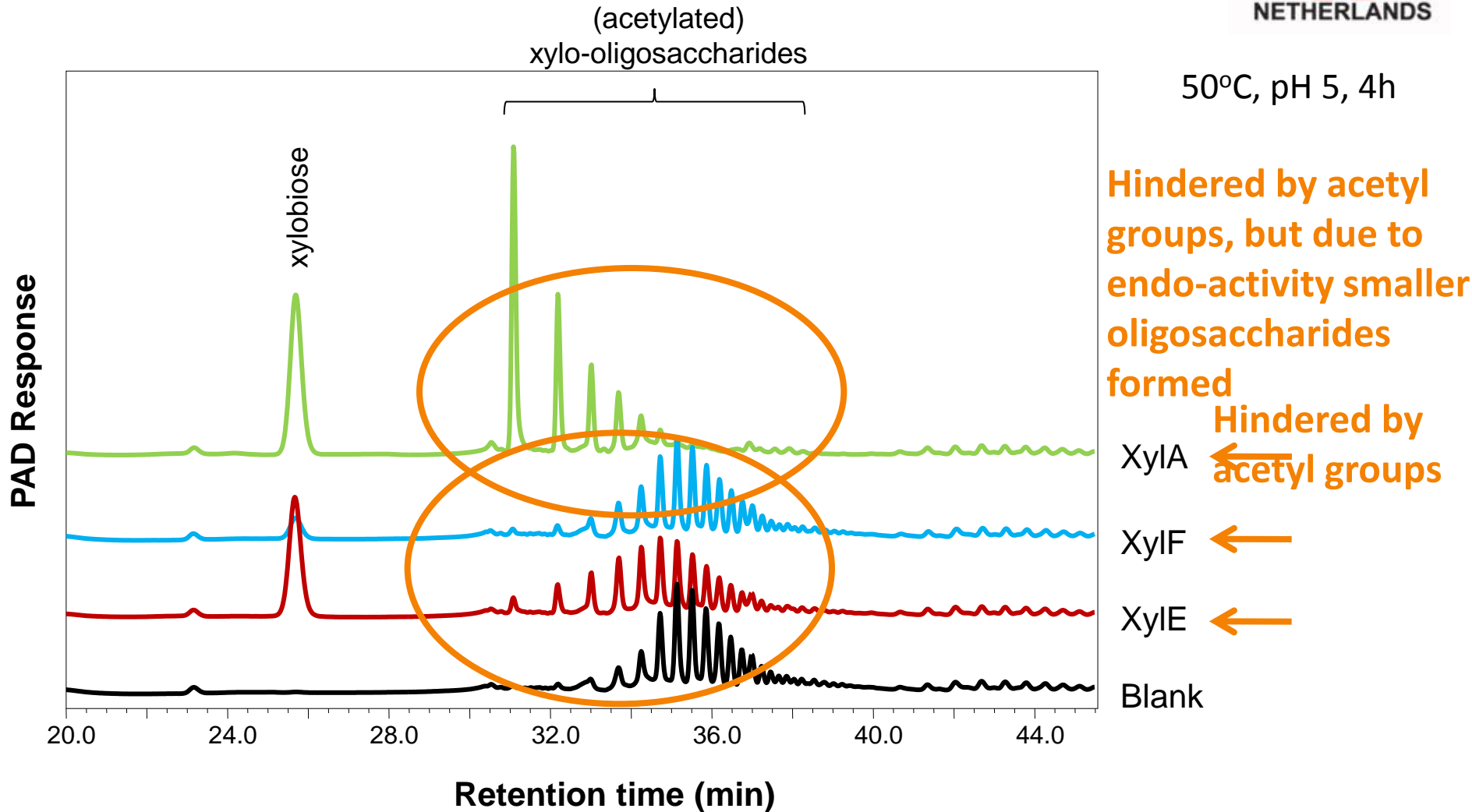
# Mode of action Xylanase F



→ Acts as a xylobiase and xylopentaose

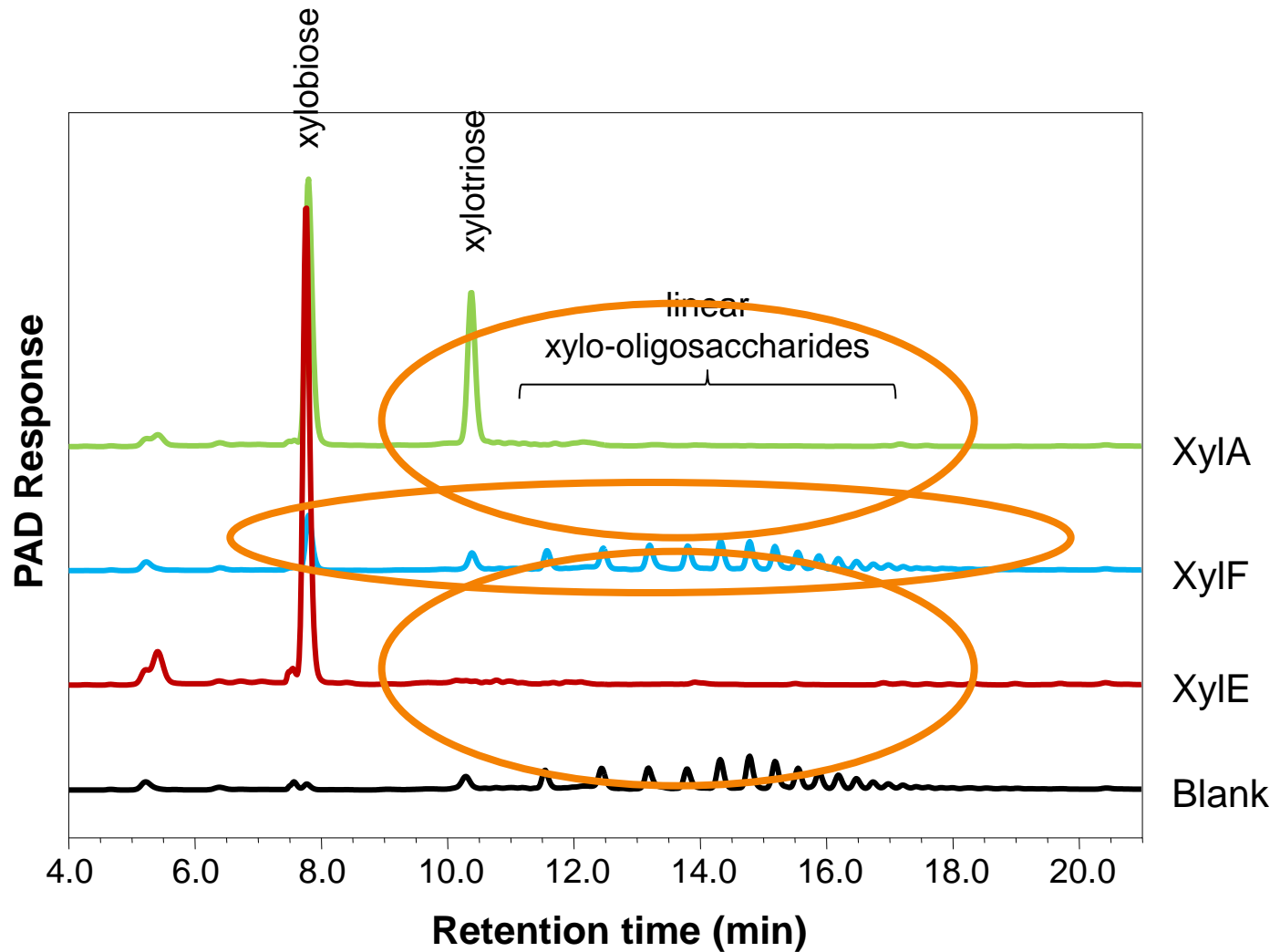


# Activity towards acetylated xylo-oligosaccharides



# Activity towards saponified xylo-oligosaccharides

50°C, pH 5, 4h

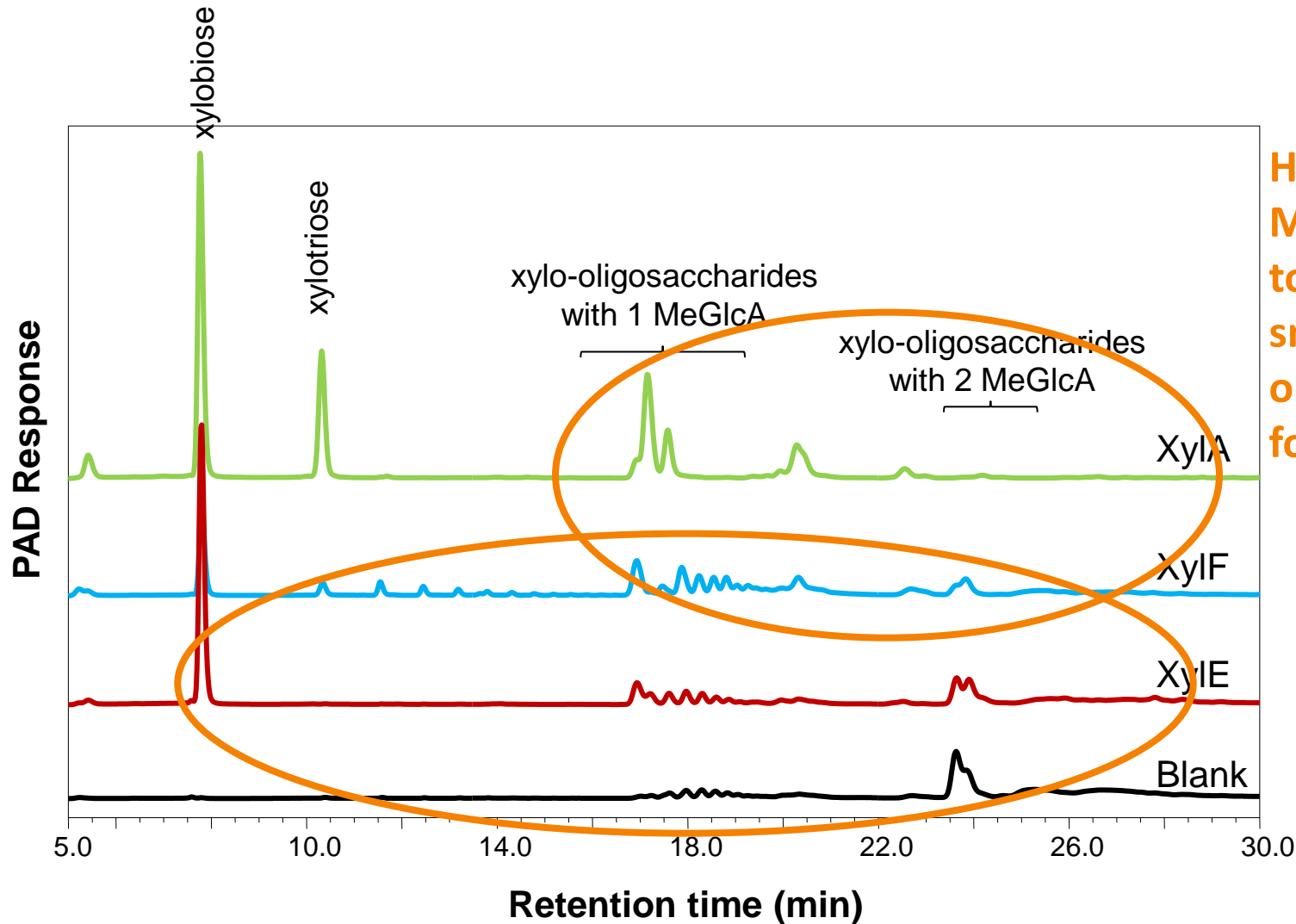


← Enzyme is slower than XylE

← Complete degradation

# Activity towards acid xylo-oligosaccharides

50°C, pH 5, 4h

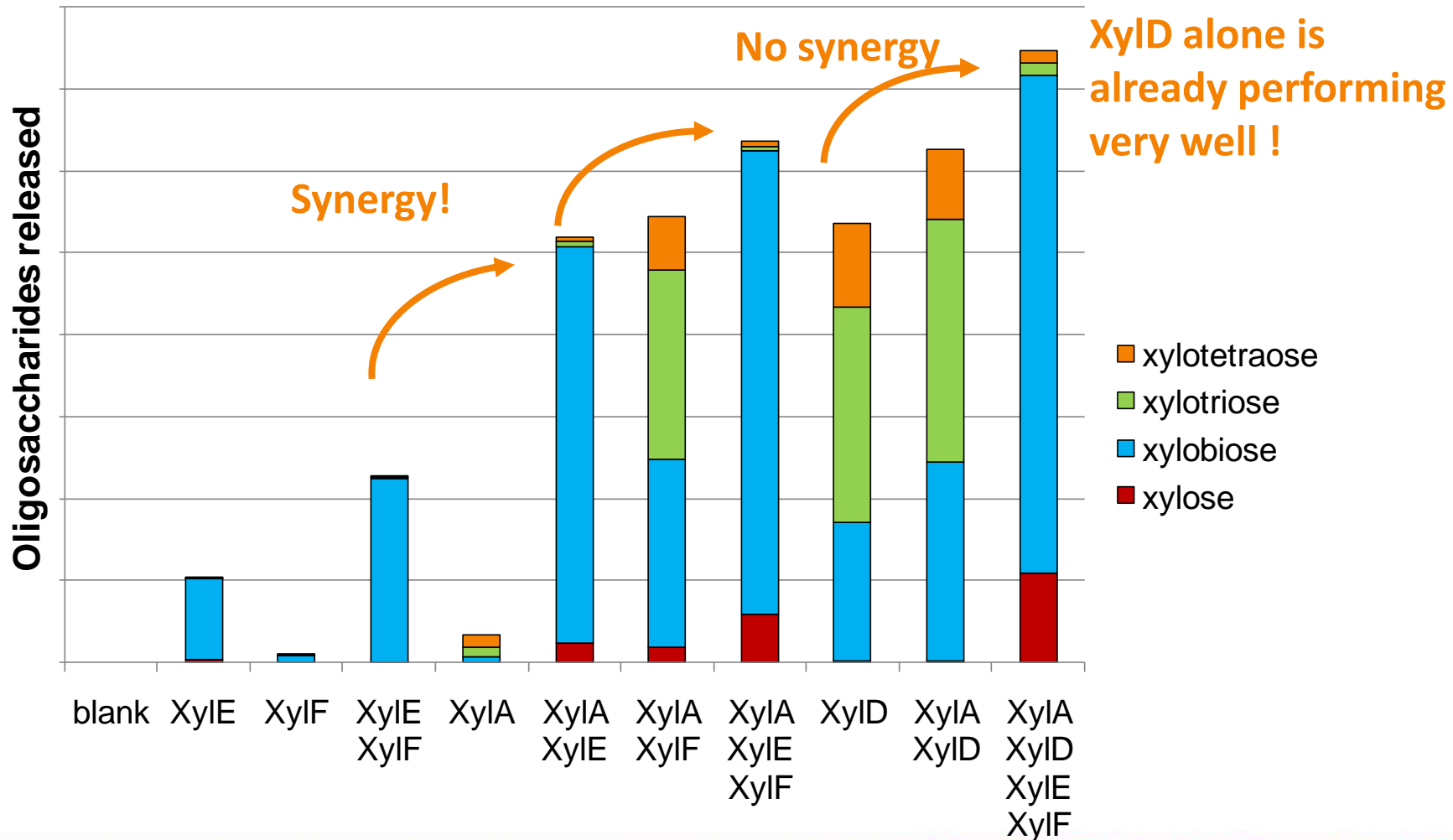


**Hindered by MeGlcA, but due to endo-action smaller oligosaccharides formed**  
← **Hindered by MeGlcA**

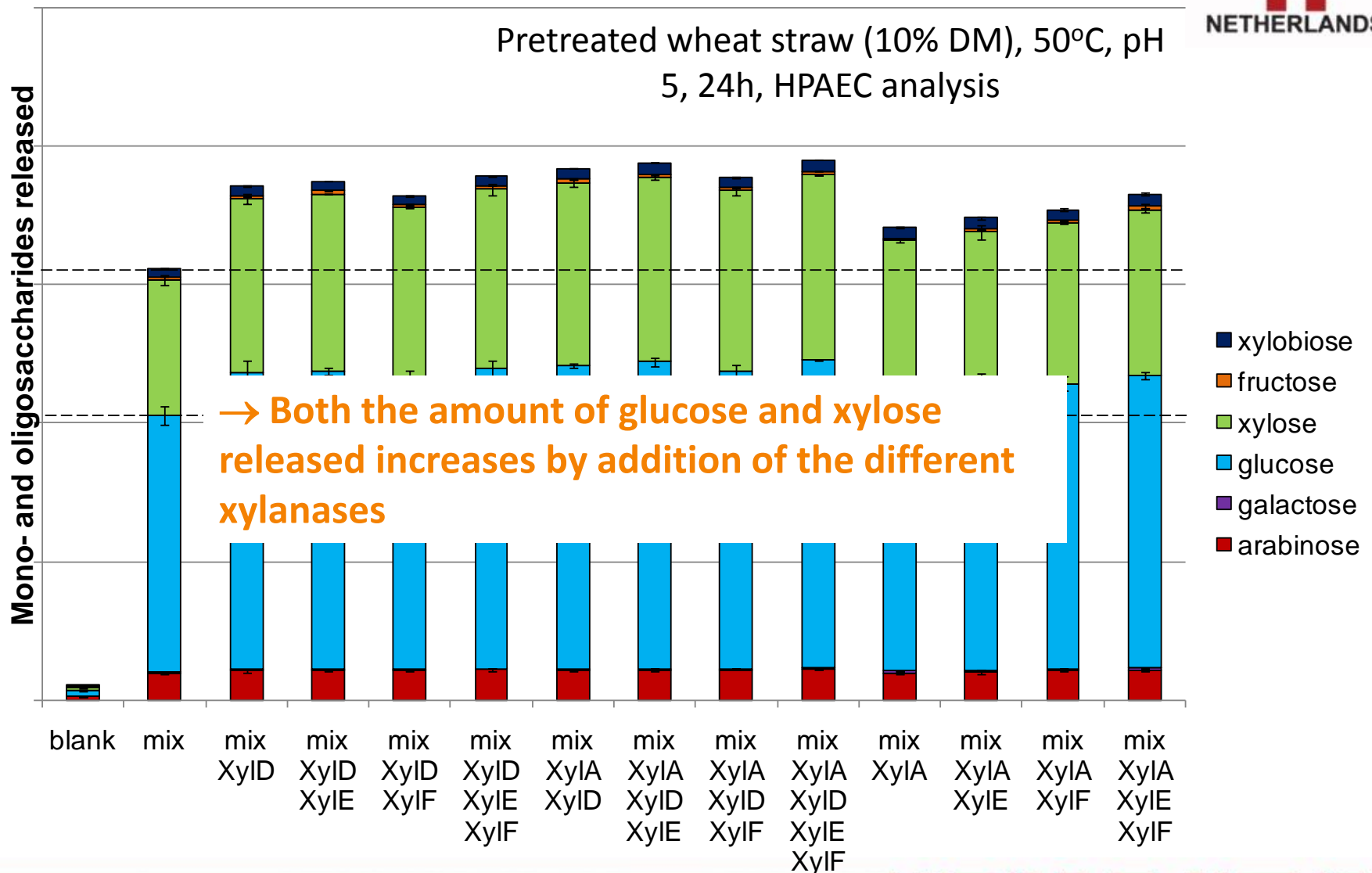


# Synergistic action of the xylanases

Birch wood xylan, 50°C, 1h, HPAEC analysis



# Synergistic action on complex substrates



# Conclusions

---

- C1 has a broad spectrum of xylanases
- XylE and XylF:
  - Exo-xylanase releasing xylobiose
  - Acts from the non-reducing end of the xylan
  - Hindered by acetyl groups and MeGlcA
- Enzymes have synergistic activity together and in combination with other xylanases
- These enzymes help to completely degrade xylans



# Thank you for your attention!

---



## Acknowledgements

### DNL

- Gonny van Muiswinkel
- Sanaz Mokhtari
- Jeffrey Bartels
- Jan Wery

### Consultant

- Jaap Visser

