

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

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Introduction Dyadic Netherlands



Research Subsidiary of Dyadic Inc, USA

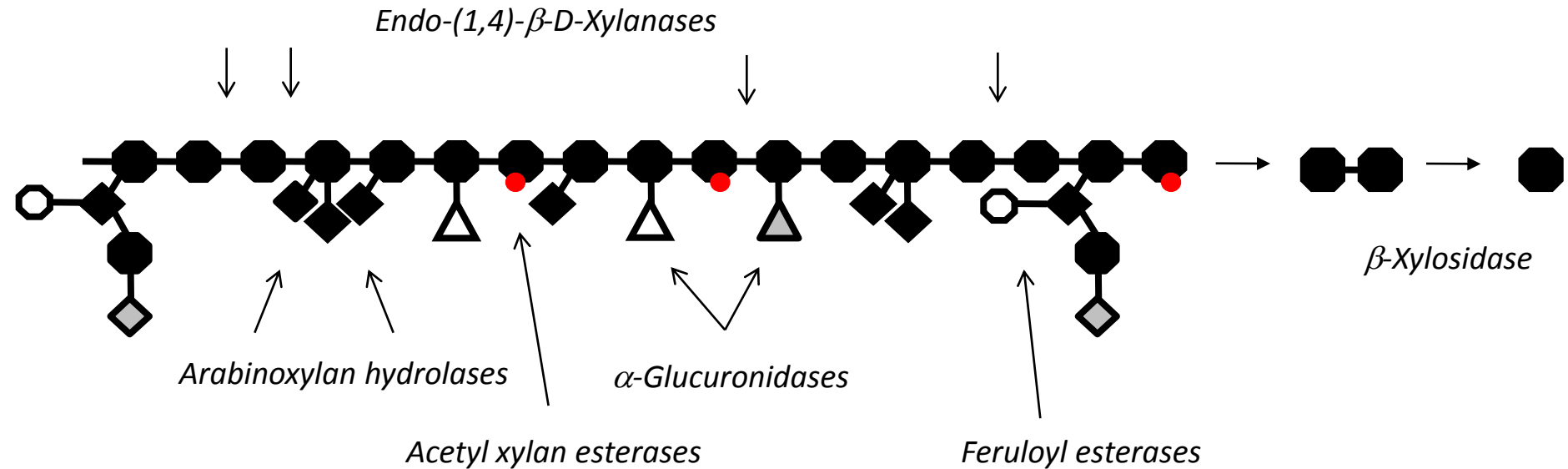
- Dyadic Nederland BV, Wageningen, The Netherlands
- www.dyadic.nl

Focus

- Discovery and development of enzymes
- Fields of application: bioenergy/biorefenergy, 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
β -Glucosidases	7
Endo-glucanases + CBH	13
GH61	26
CDH + CDH-like	9
β-Xylosidase	5
Xylanase	13
Arabinoxylan hydrolases (AXH)	7
Esterases (AXE + FAE)	13
α-Glucuronidases	2
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

GH5/GH30 enzymes:

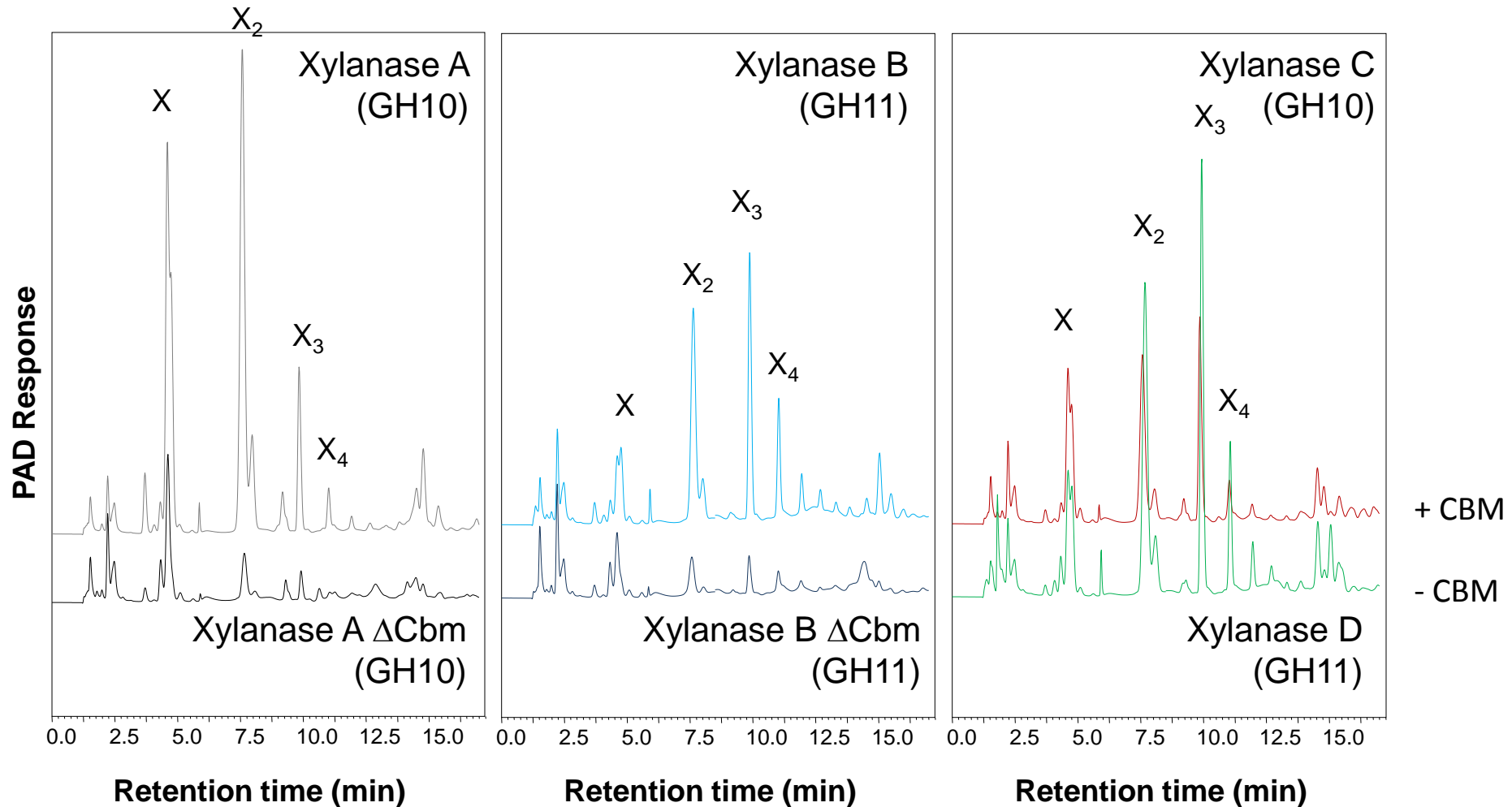
- Cleave closer to GlcA side-group
- Exo-xylanase from *Trichoderma*
- 2 putative enzymes found in C1 genome

All 13 xylanase have been produced in a dedicated C1 strain

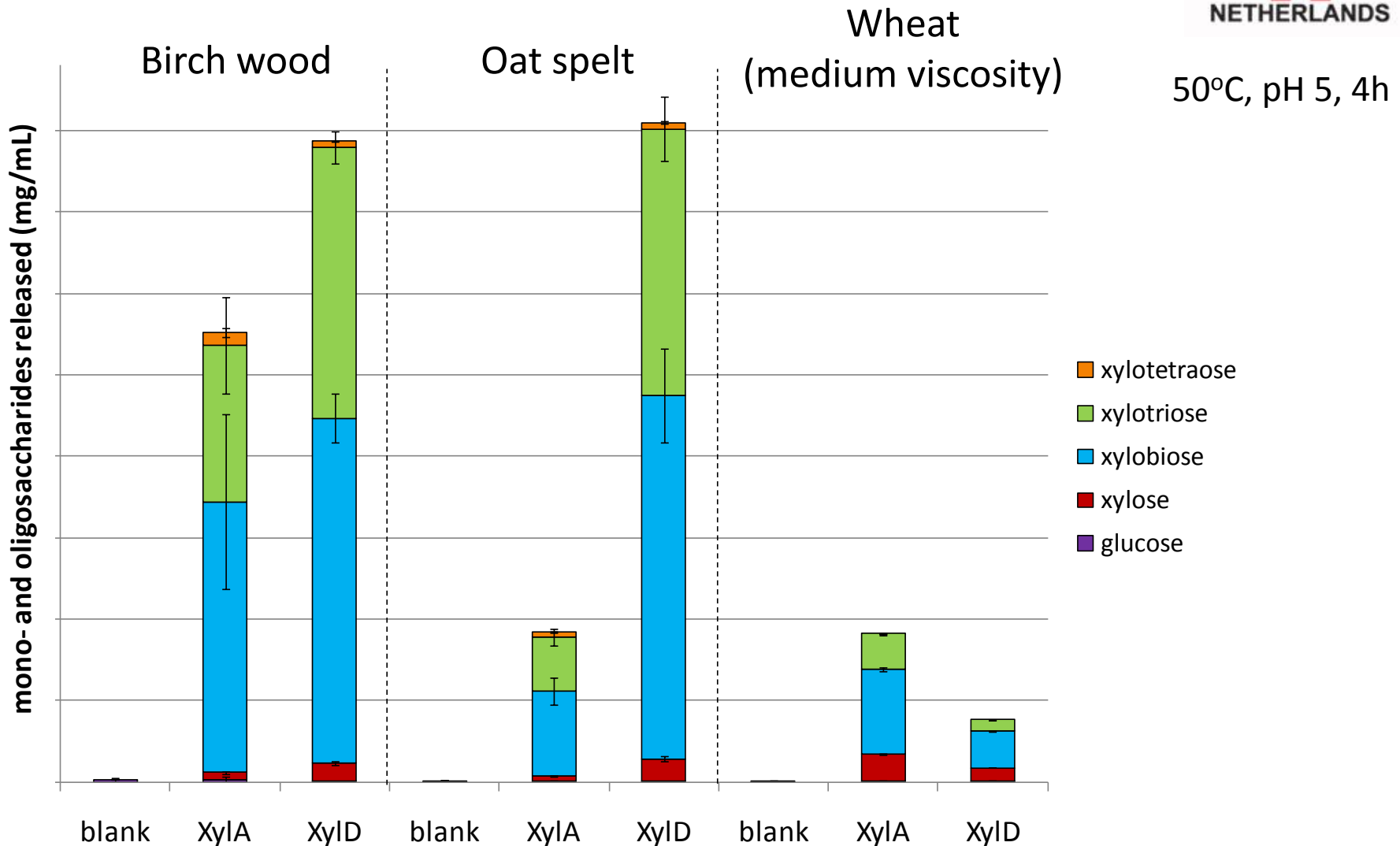


Activity of xylanases towards wheat straw

Conditions: 24h, pH5.0, 50°C



Activity towards xylans from different sources

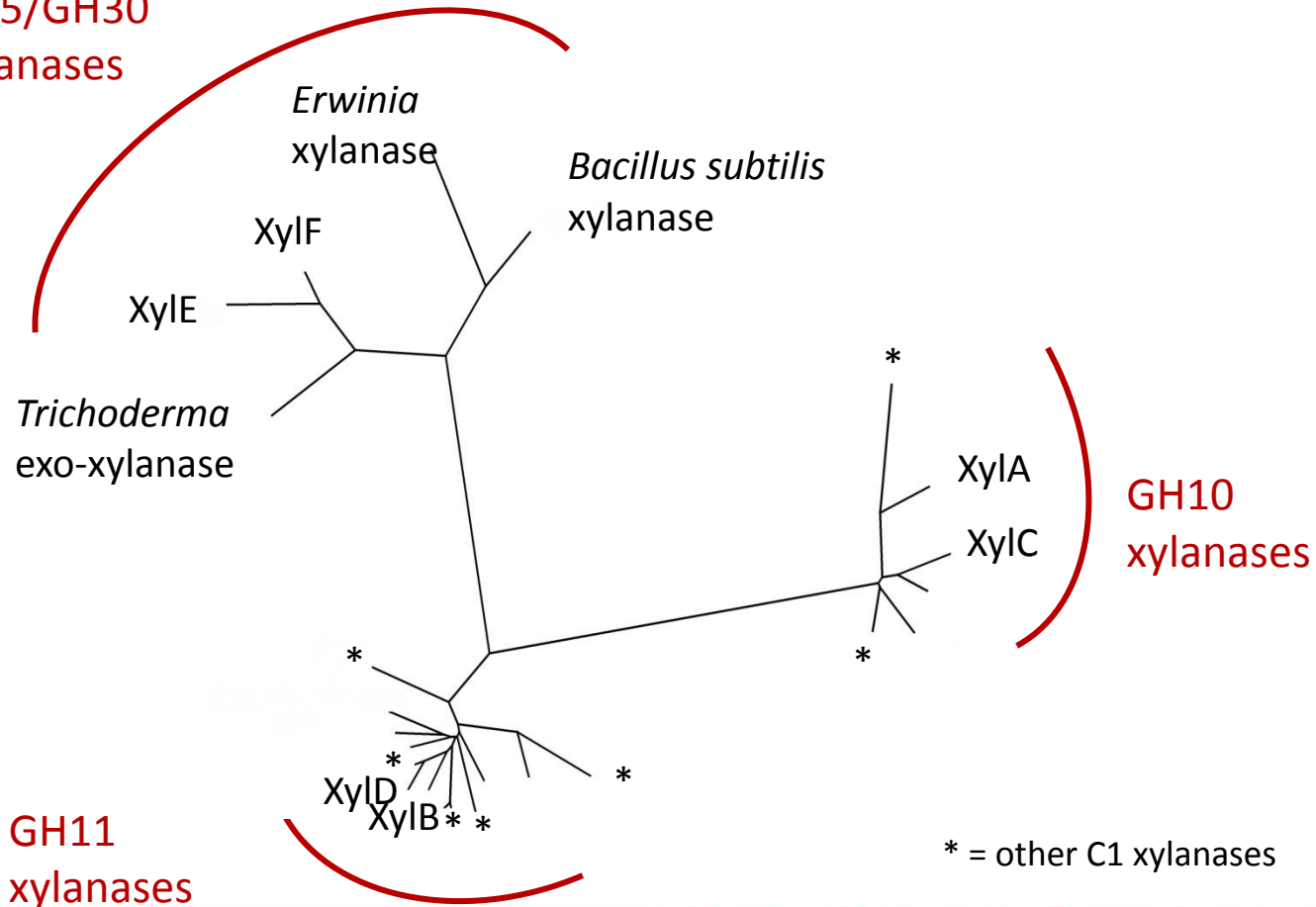


Two novel C1 xylanases

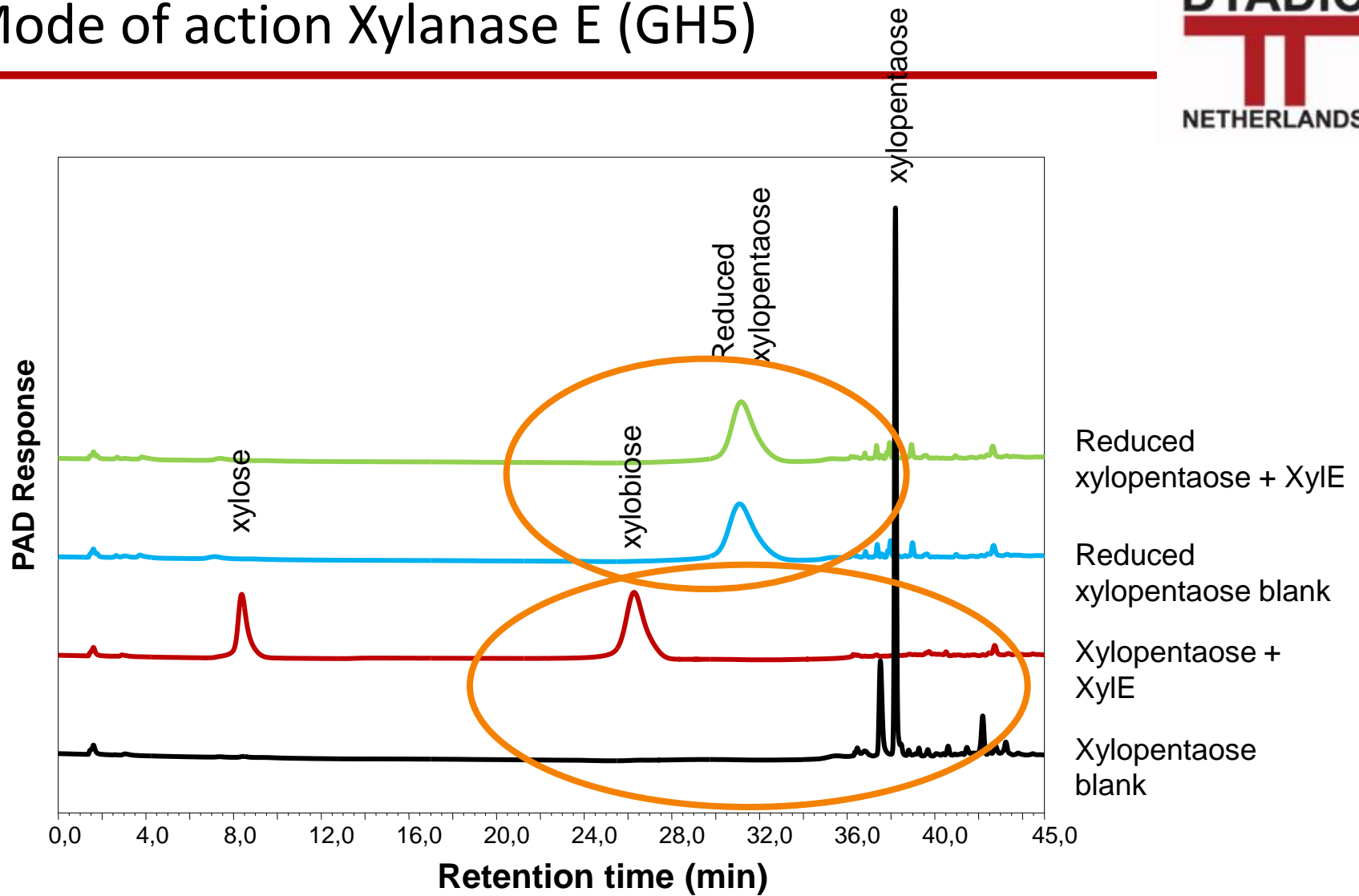
Two novel C1 xylanases have been identified and produced:

- XylE → GH5
- XylF → GH30?

GH5/GH30
xylanases

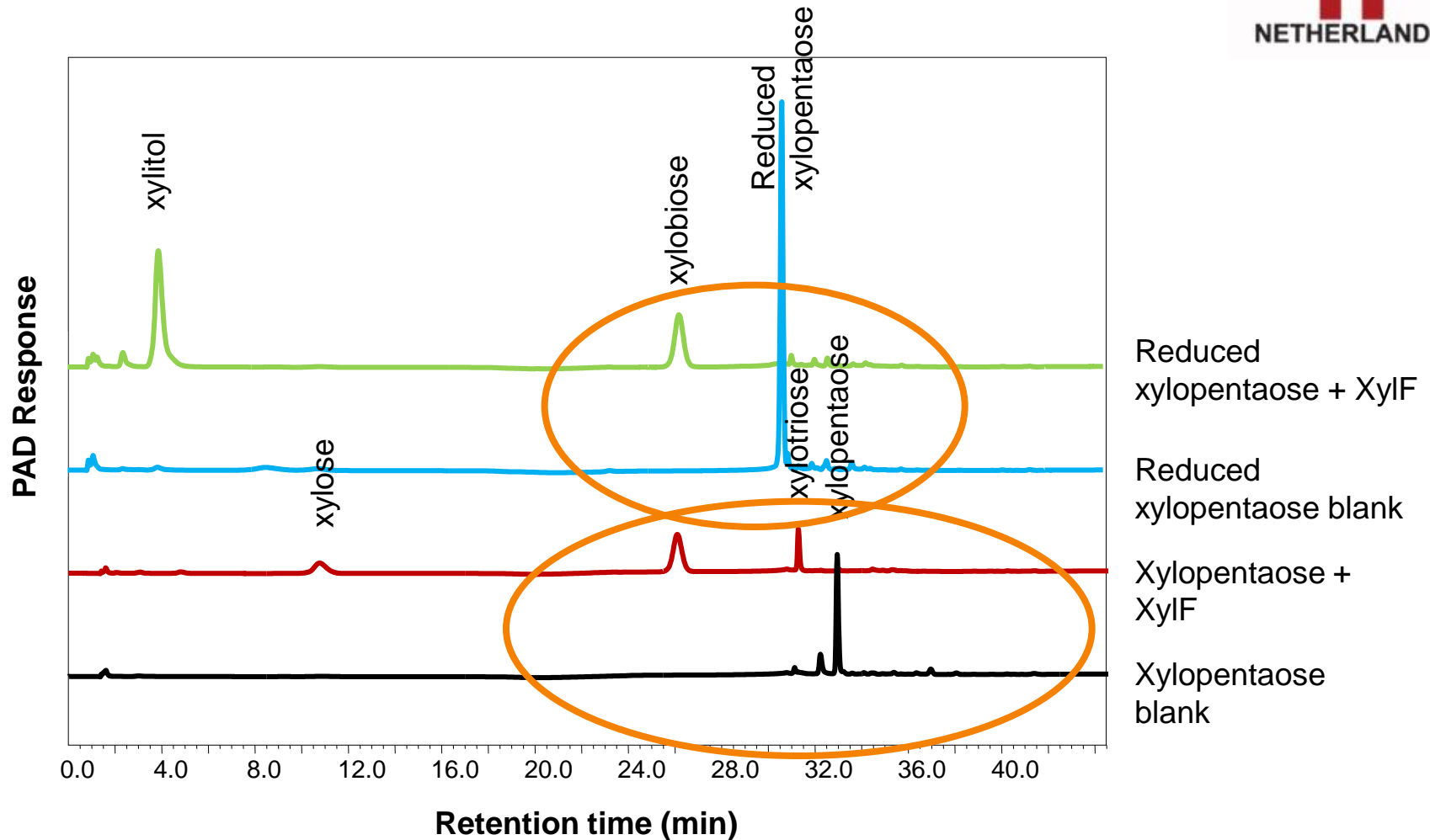


Mode of action Xylanase E (GH5)



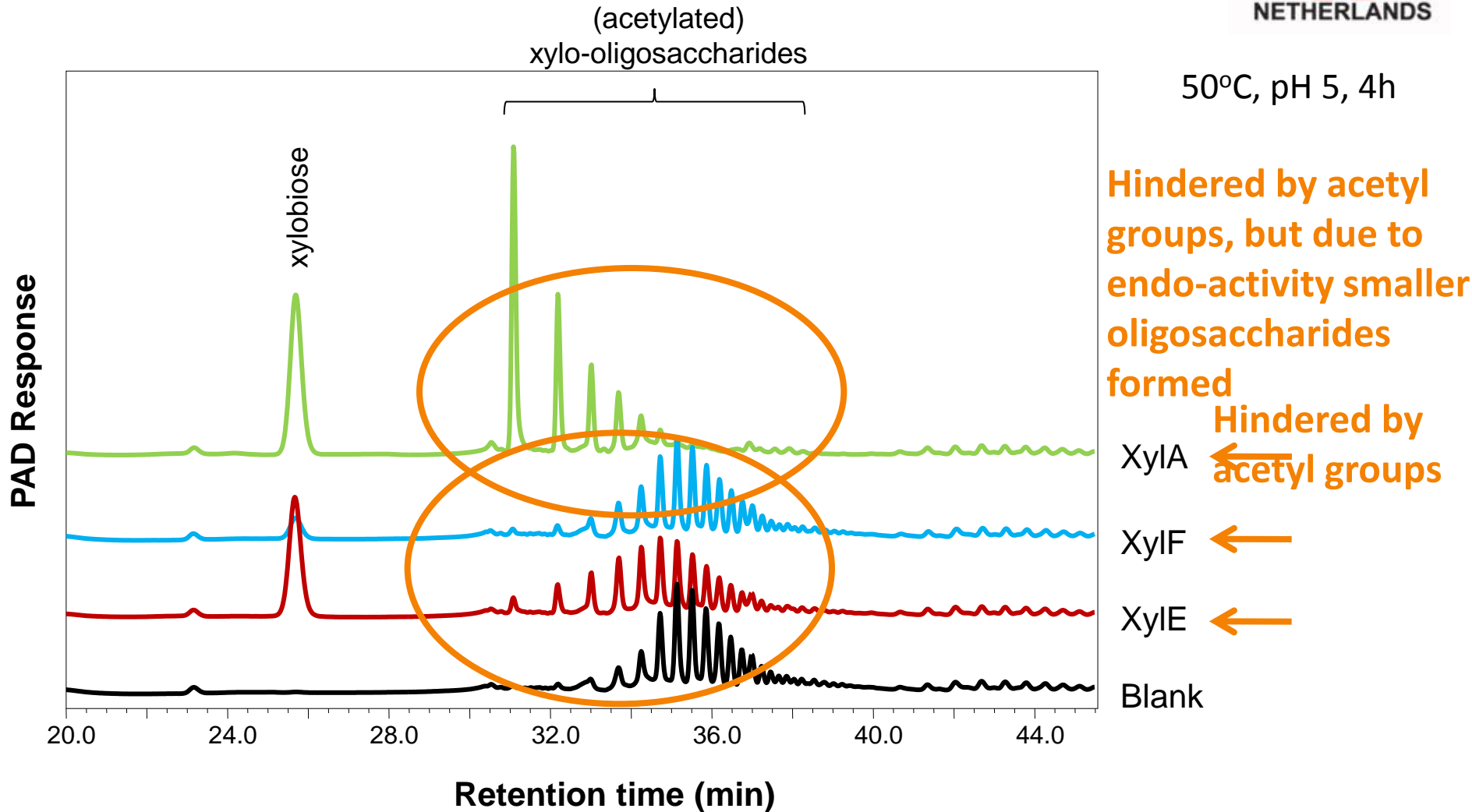
→ Release of xylobiose from xylopentaose
Acts from reducing end

Mode of action Xylanase F (GH30)



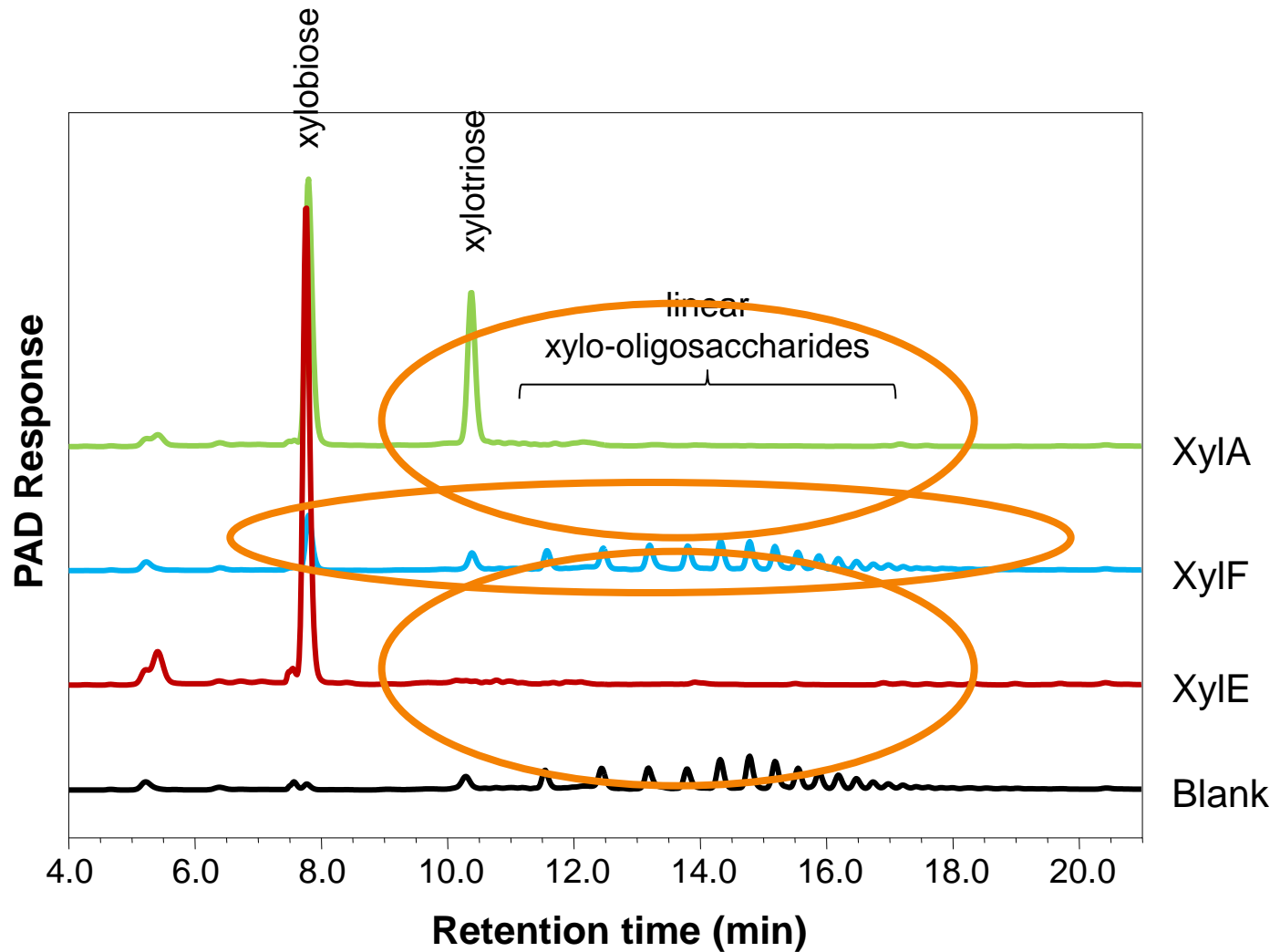
→ Acts as a source of xylobiose and xylopentaose

Activity towards acetylated xylo-oligosaccharides



Activity towards saponified xylo-oligosaccharides

50°C, pH 5, 4h

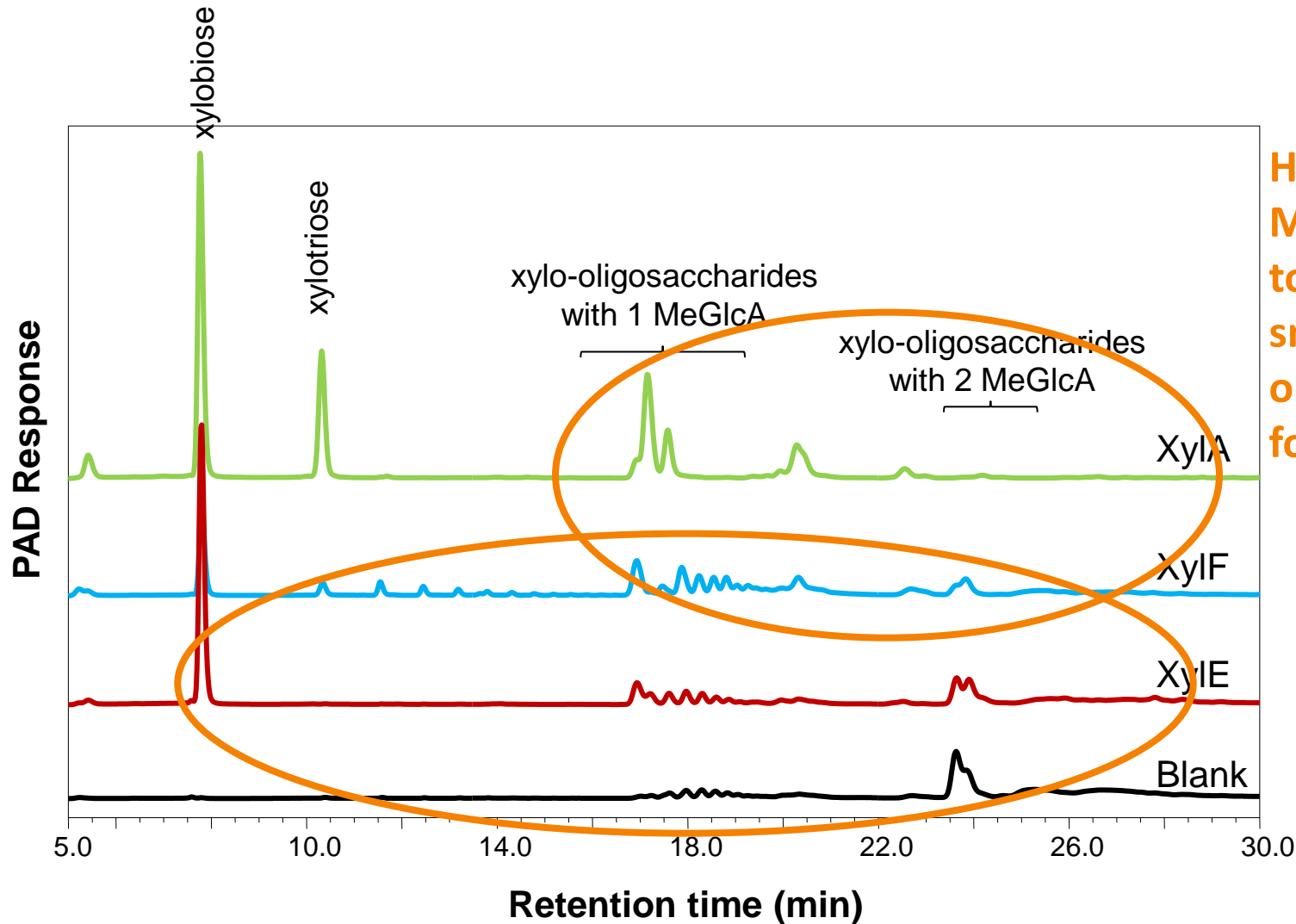


← Enzyme is slower than XylE

← Complete degradation

Activity towards acid xylo-oligosaccharides

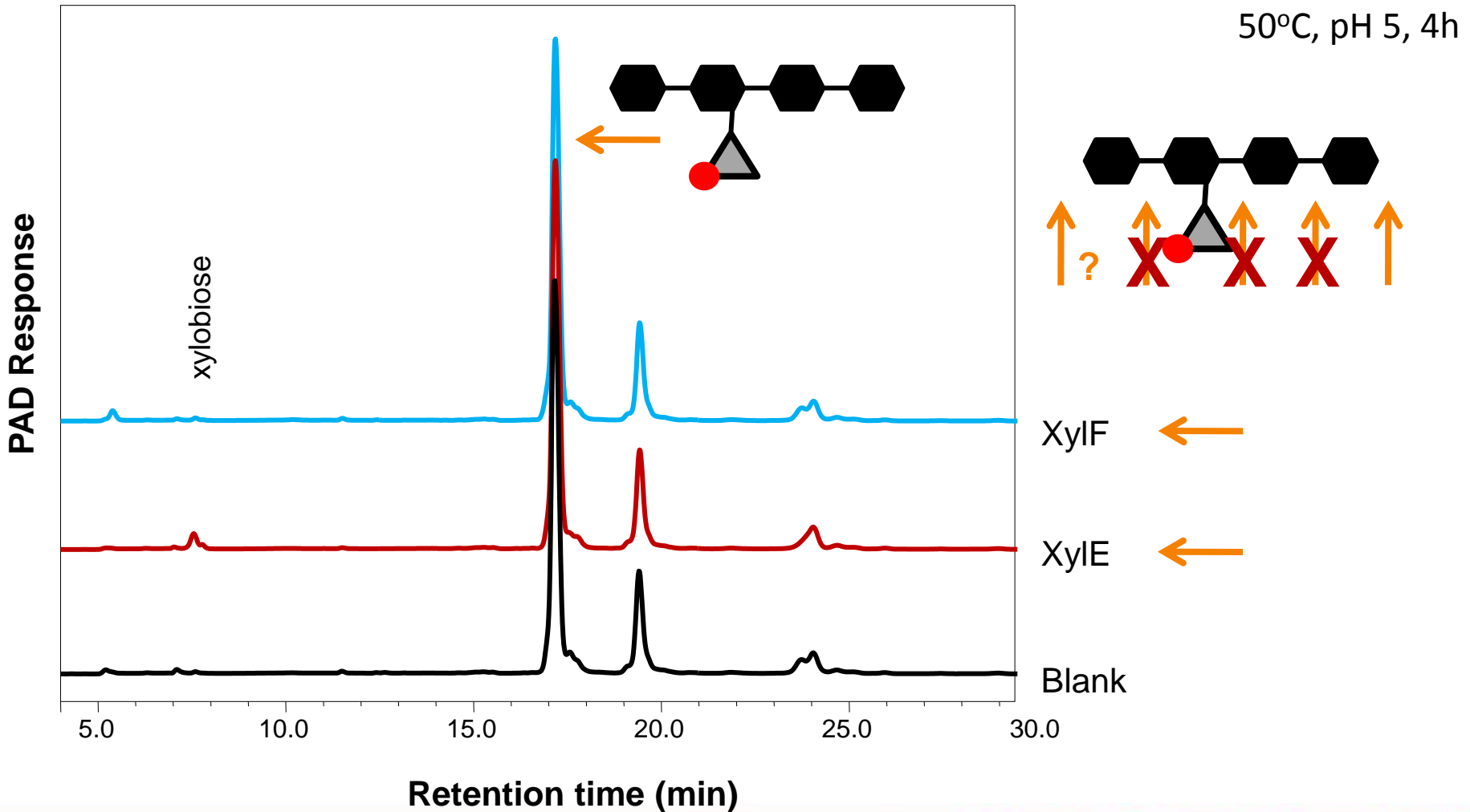
50°C, pH 5, 4h



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

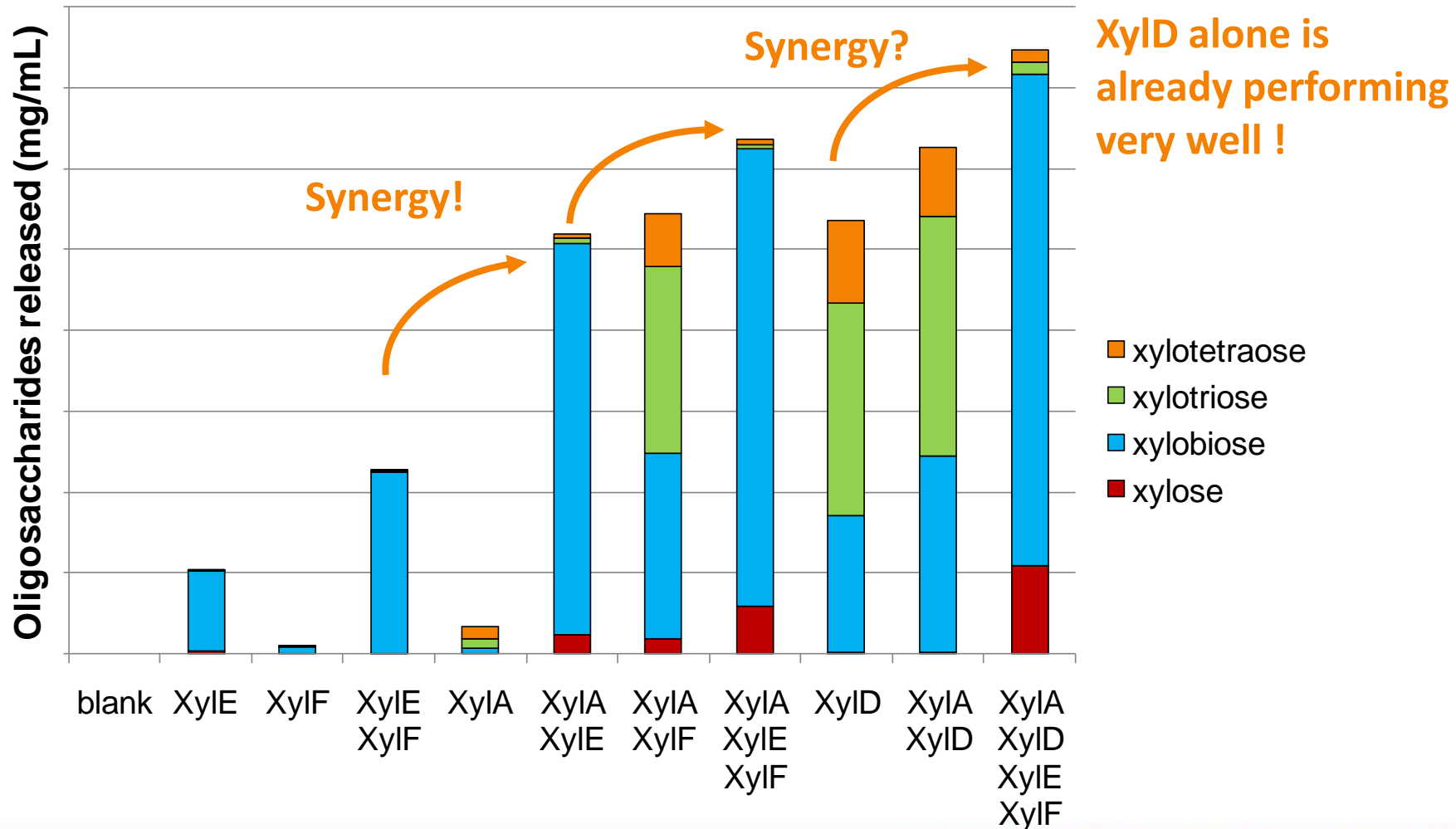


Activity towards tetra-aldouronic acid



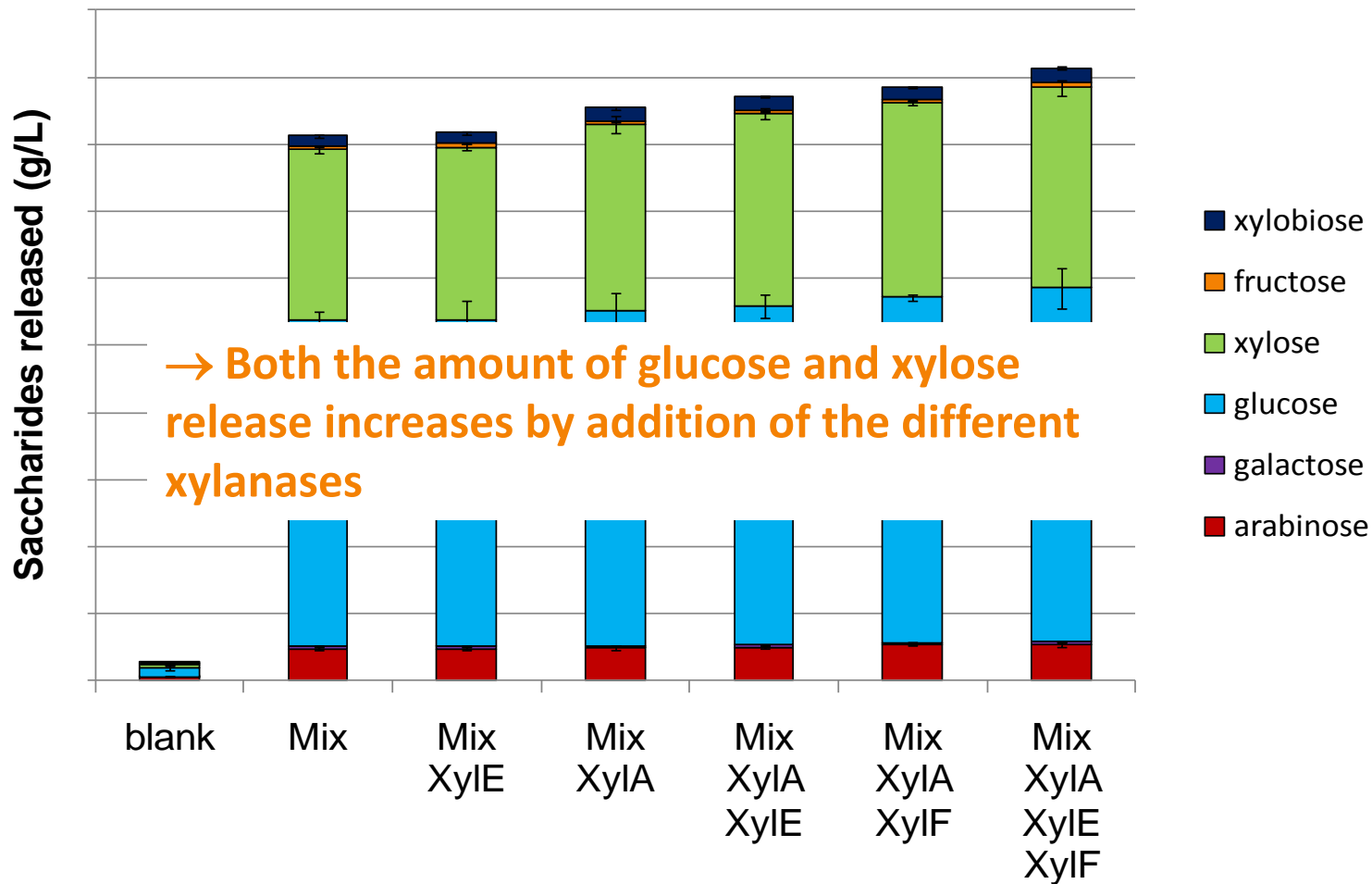
Synergistic action of the xylanases

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



Synergistic action on complex substrates

Pretreated wheat straw (10% DM), 50°C, pH 5, 24h, HPAEC analysis



Conclusions

- C1 has a broad spectrum of xylanases
- XylE:
 - Exo-xylanase releasing xylobiose
 - Acts from the reducing end of the xylan
 - Hindered by acetyl groups and MeGlcA
- XylF:
 - Exo-xylanase releasing mainly 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

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