New developments in enzyme research

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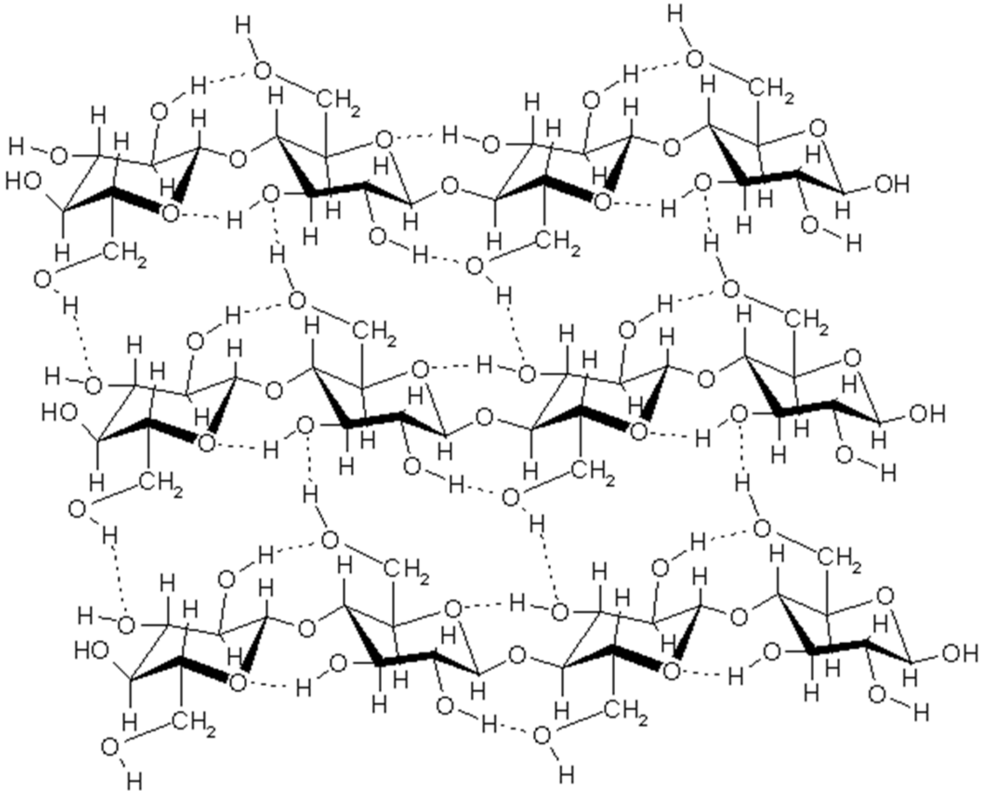
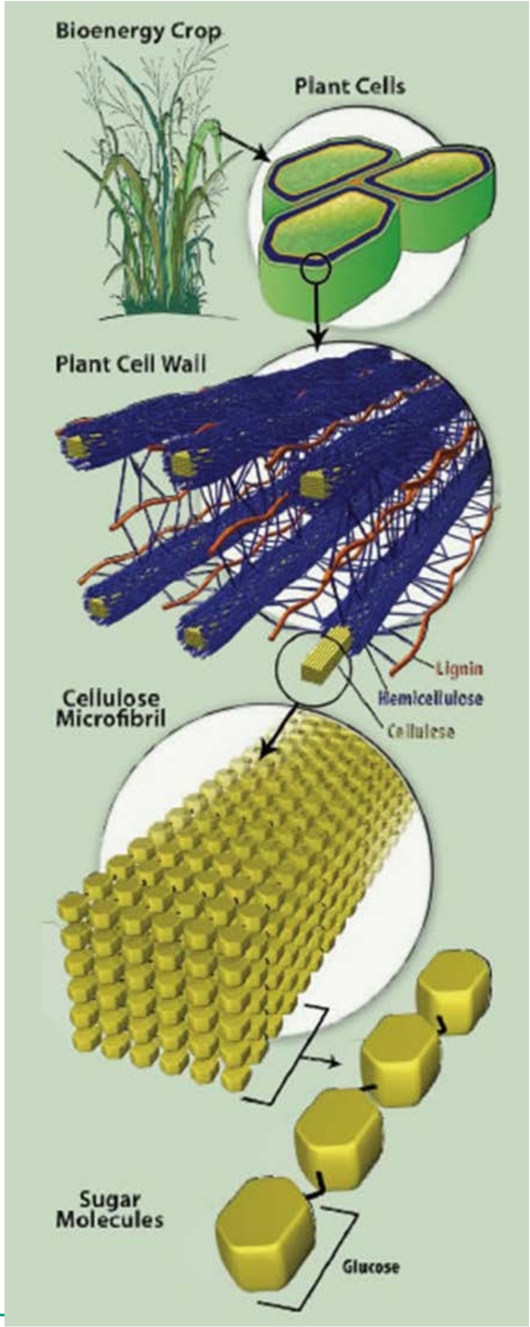
Bio4Fuels Days 2017

Efficient depolymerization of plant poly-saccharides is key to succesful biorefining

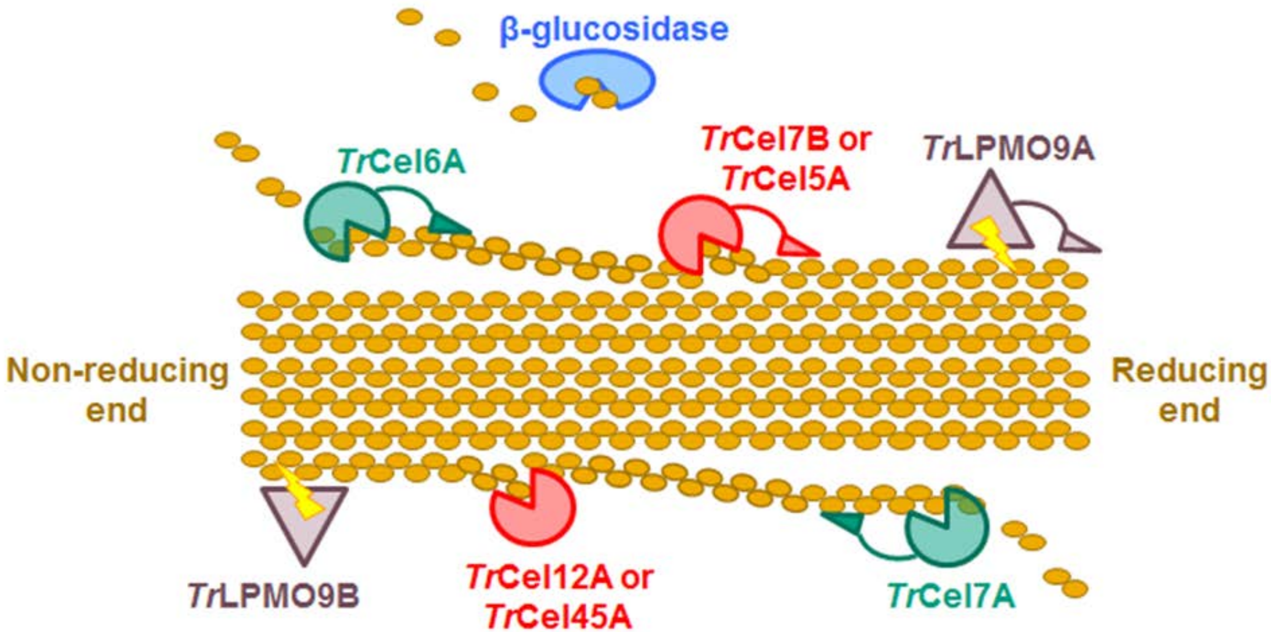


Cellulose

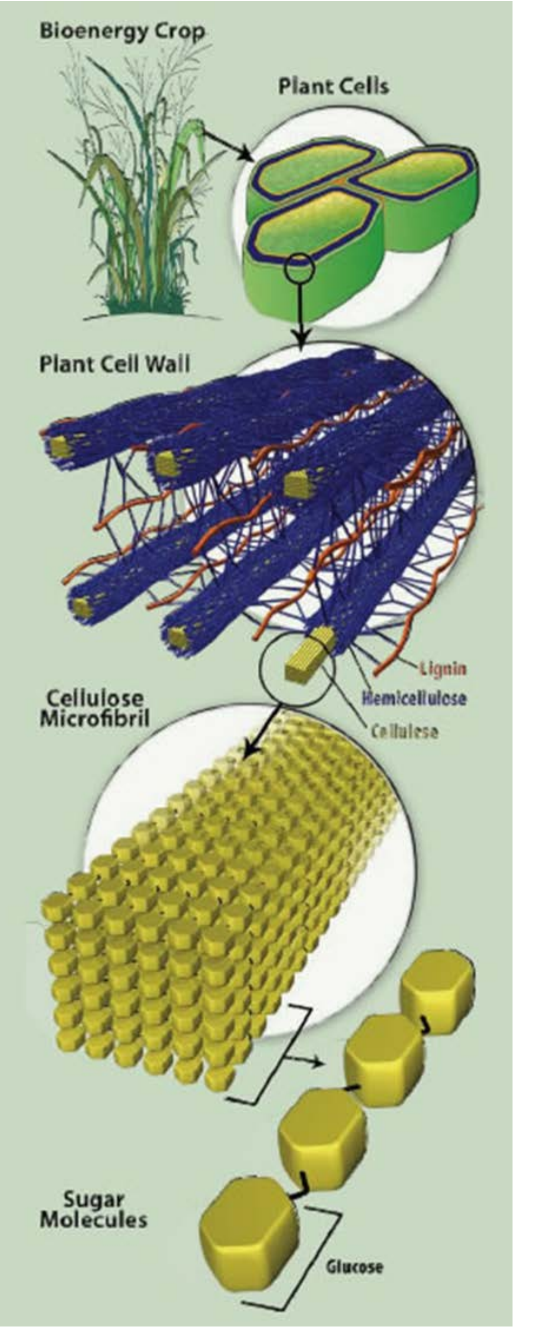


Ritter, 2008. *Plant Biochem.* 86(49):15.

We need enzyme *systems*

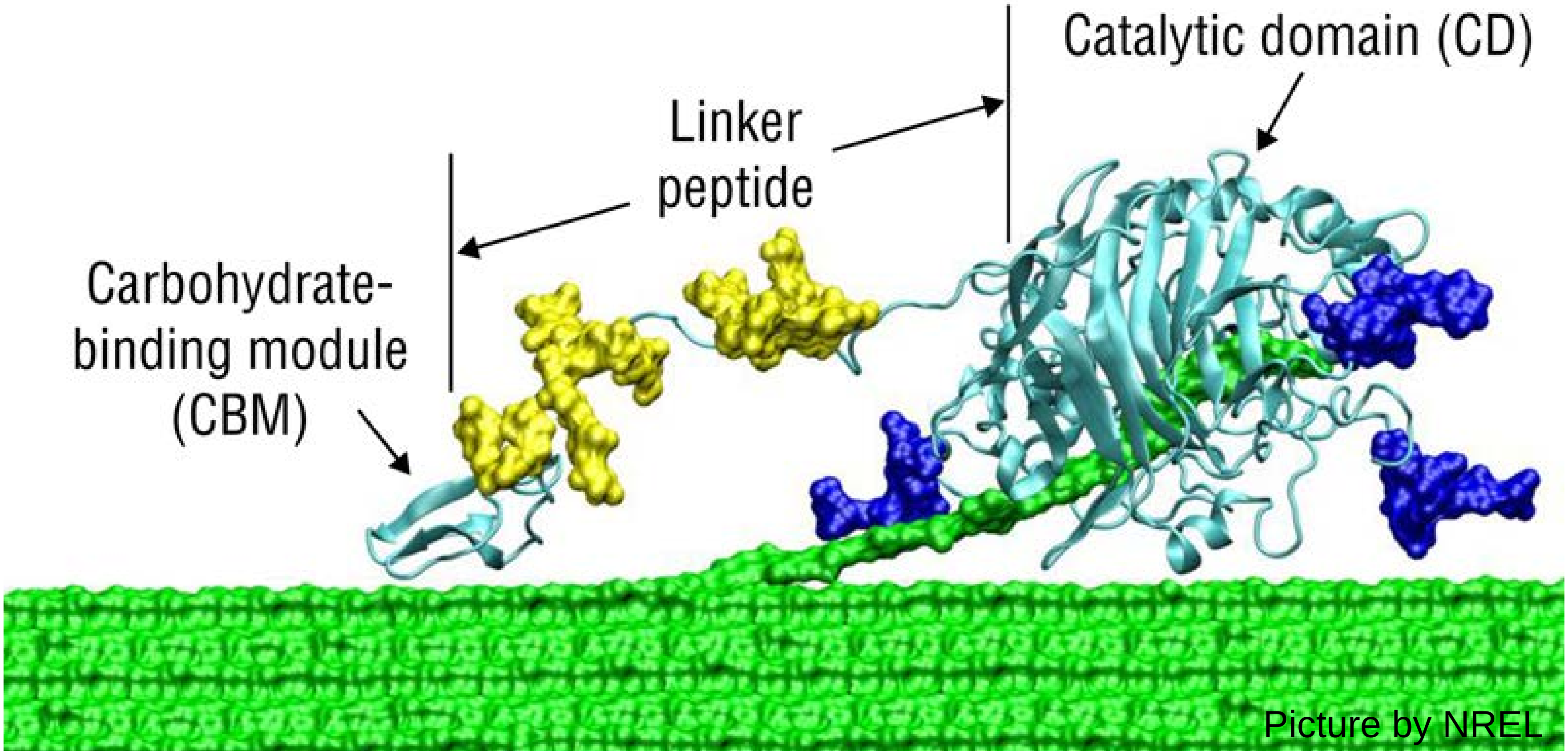


A cellulolytic enzyme system (picture by A. Varnai)

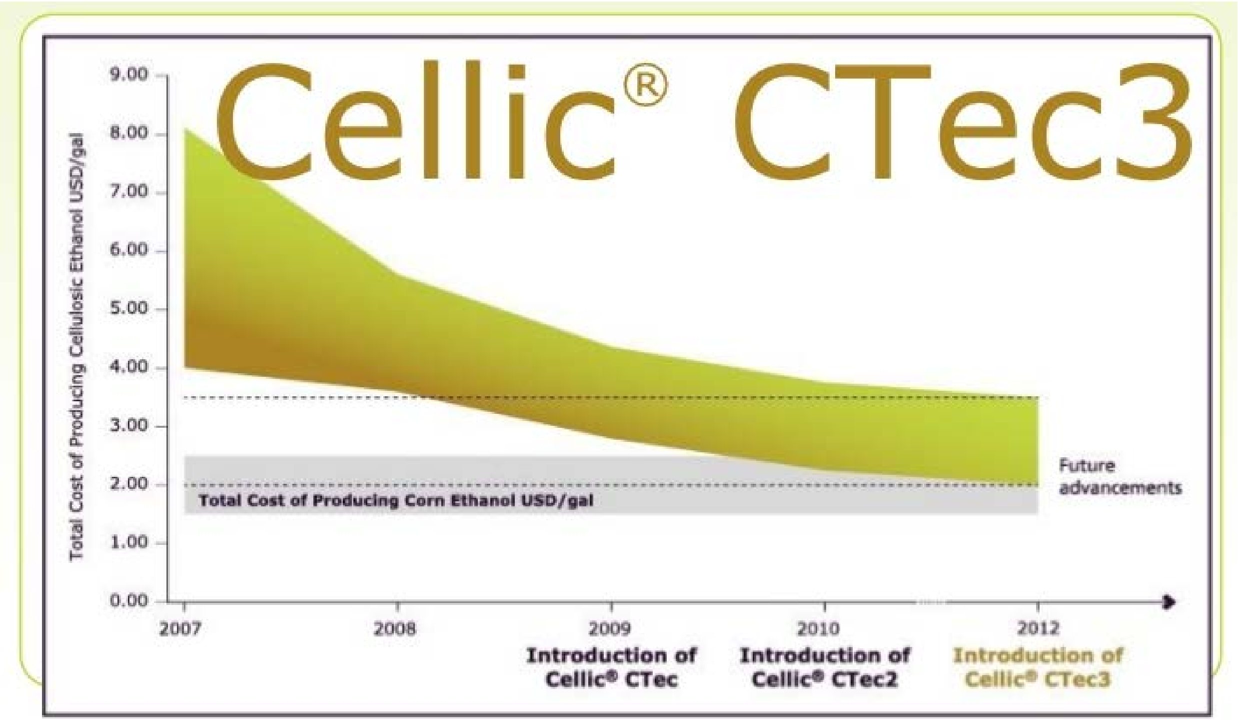


Ritter, 2008. *Plant Biochem.* 86(49):15.

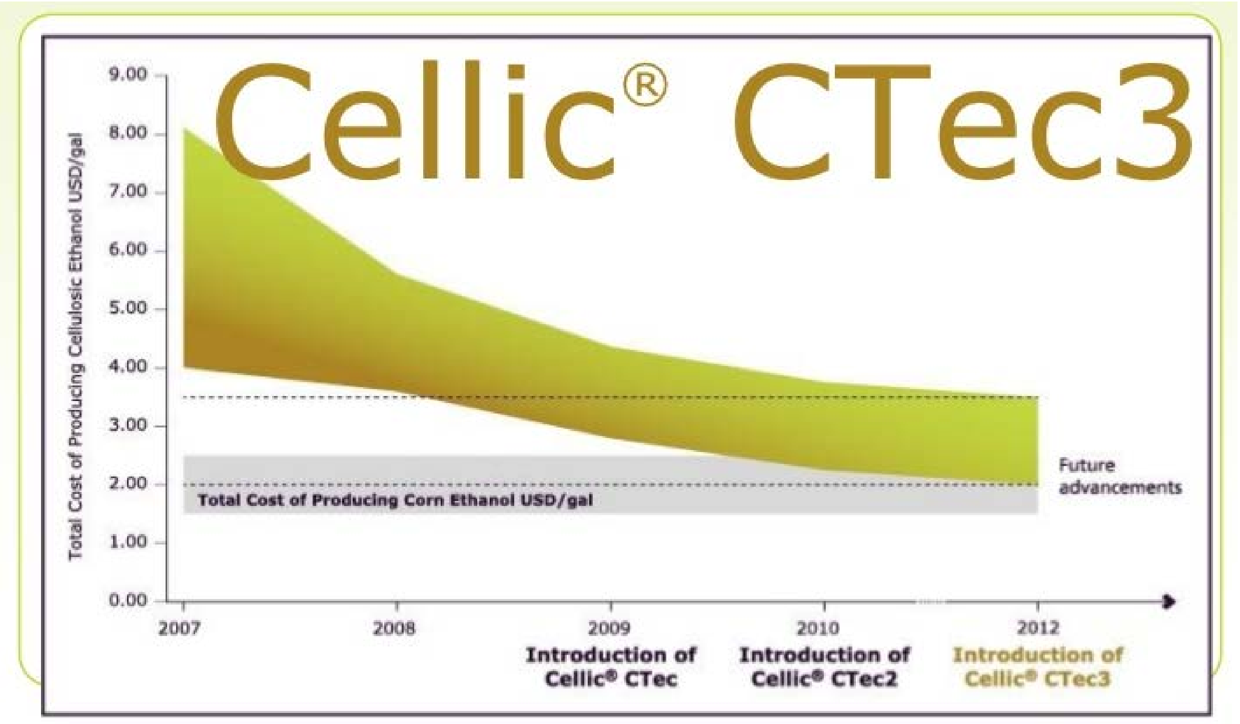
These enzymes are complex and have a daunting task



There are efficient enzyme cocktails,

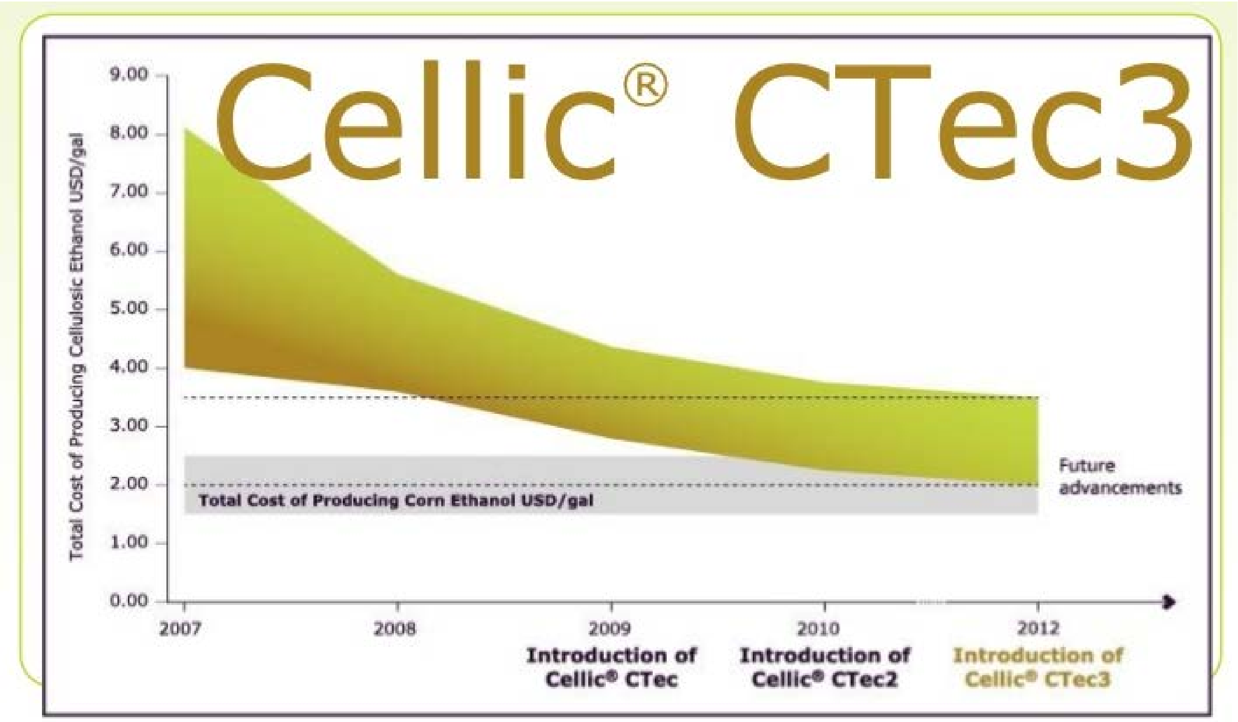


There are efficient enzyme cocktails, which are……



- Not optimized for Nordic biomass

There are efficient enzyme cocktails, which are……

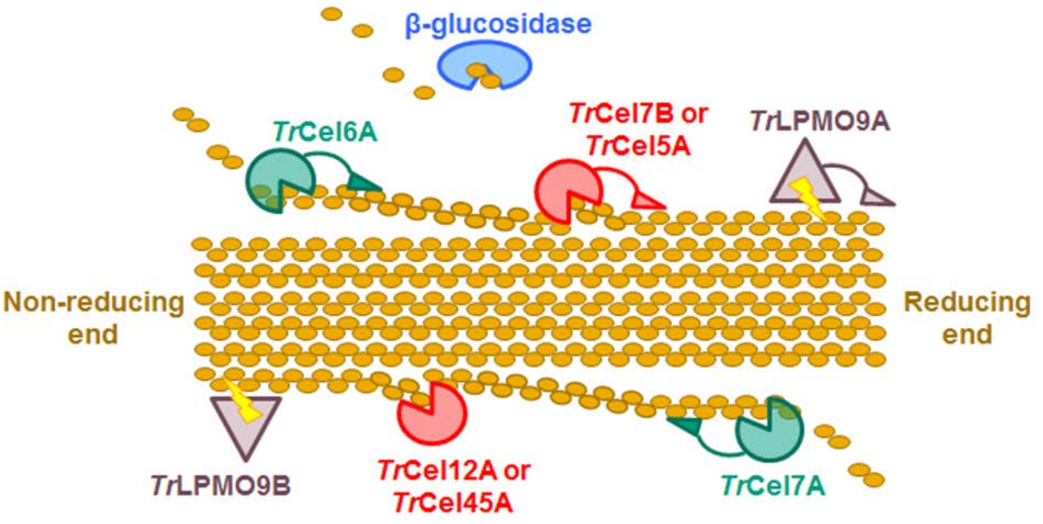


- Not optimized for Nordic biomass
- Still an important cost driver

Bio4Fuels topics - pretreatment

The relationship between pretreatment and substrate degradability:

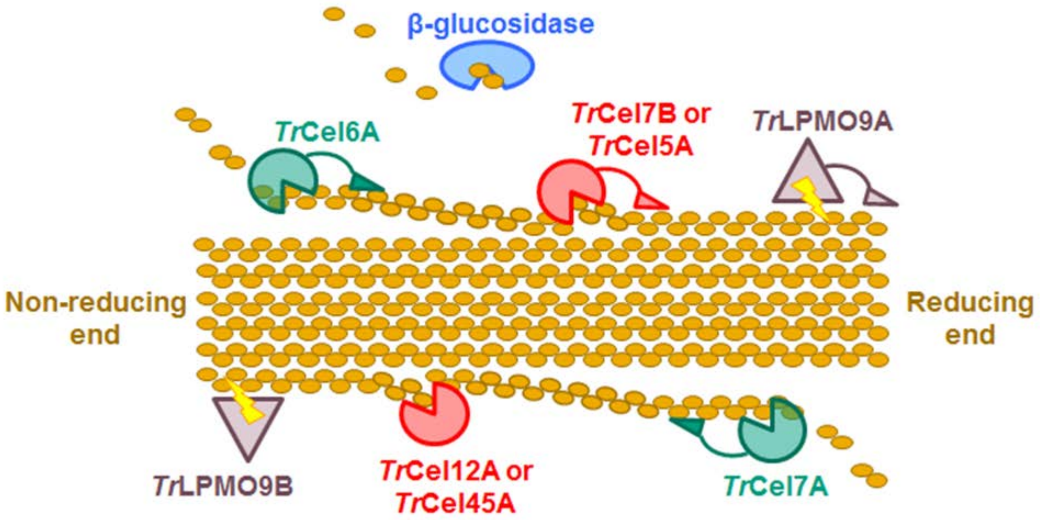
- Borregaard process
- St1 process
- NMBU steam explosion
- PFI processes



Bio4Fuels topics – enzymes (*with new ENERGIX project*)

Enzyme topics:

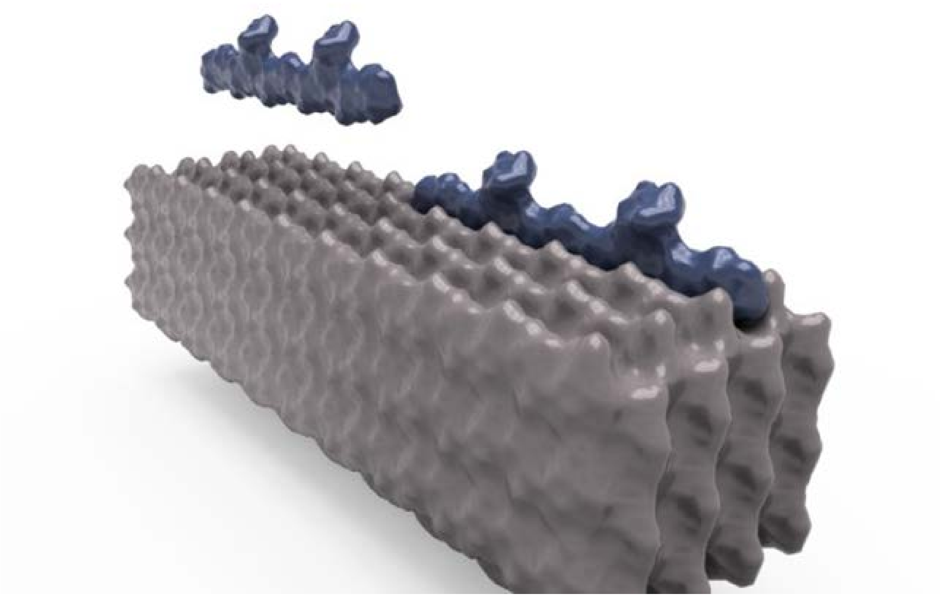
- Why are enzymes lost during bioprocessing? Where do they go? Who do they get stuck? Is processivity favourable (see Horn et al., 2006, PNAS)
- What is the role of CBMs? (see Várnai et al., 2013, Biotechnol Biofuels 6:30)

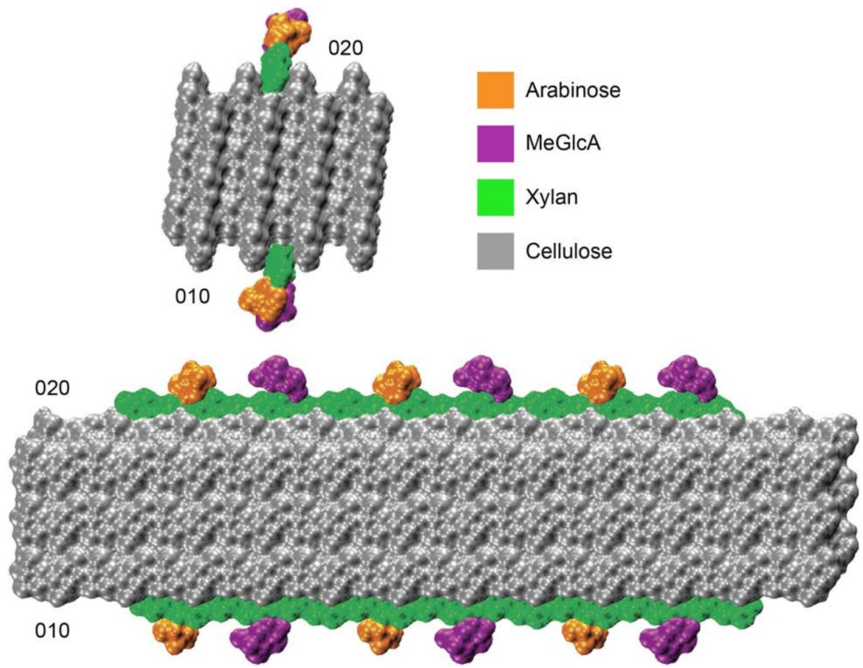


Bio4Fuels topics - enzymes

Enzyme topics:

- What is the effect of remaining hemicelluloses and can negative effects be mitigated by use of hemicellulases?



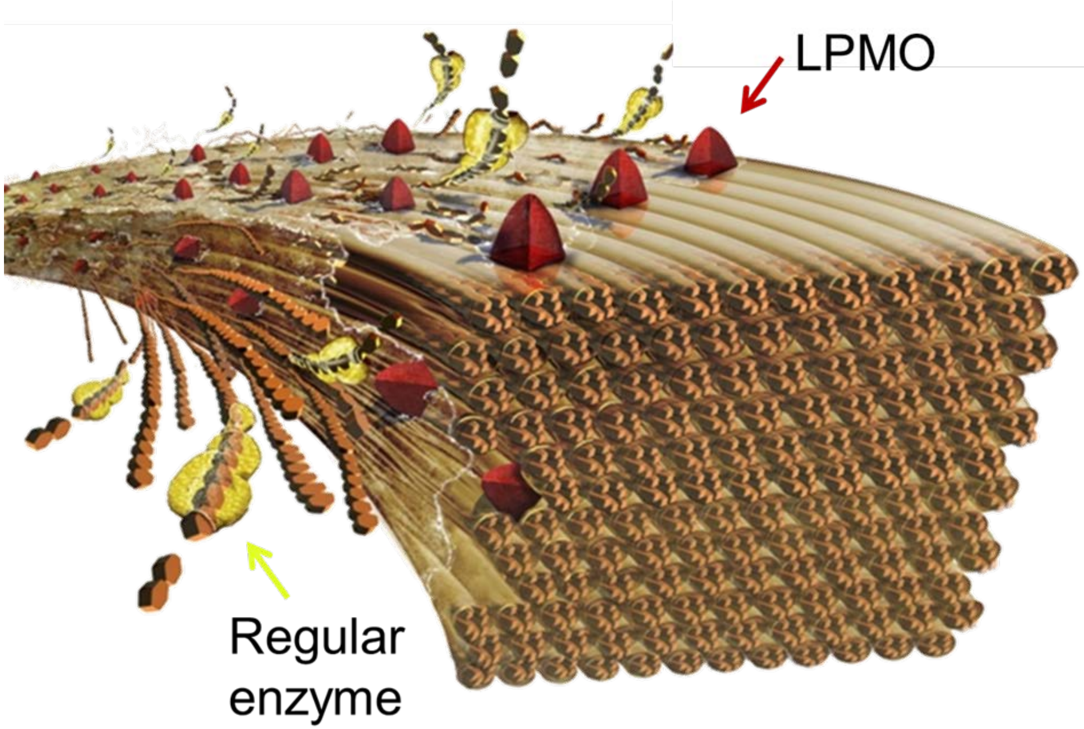


Pictures by Paul Dupree and colleagues

Bio4Fuels topics - enzymes

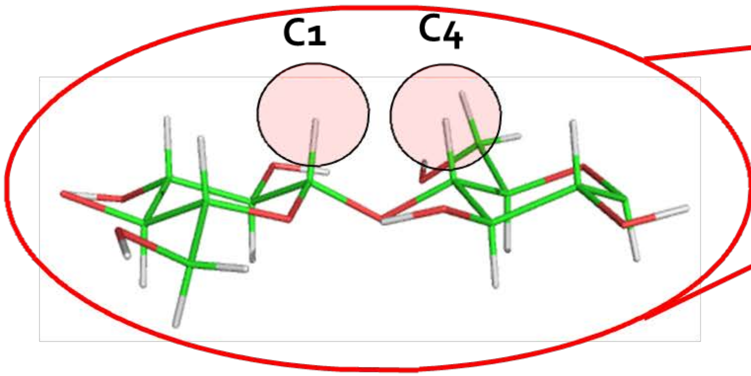
Enzyme topics:

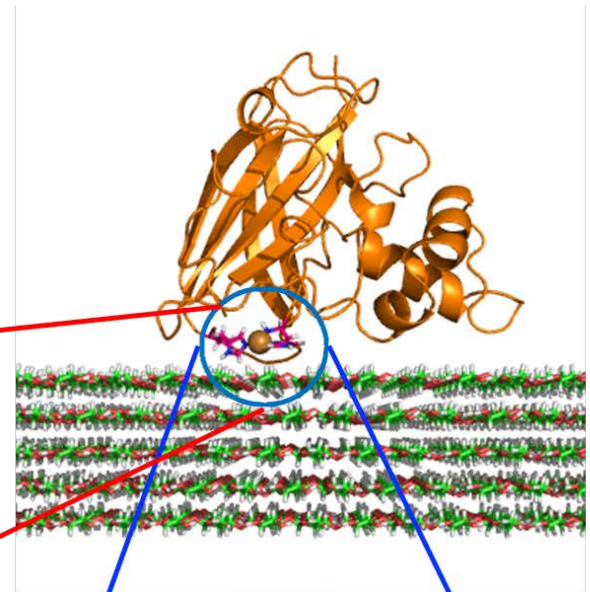
- Harnessing the potential of LPMOs

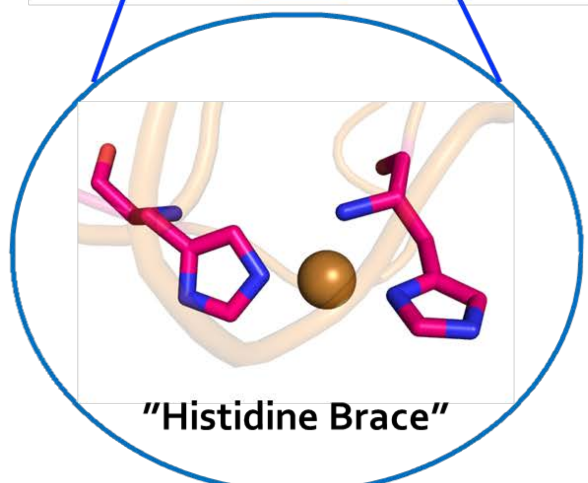


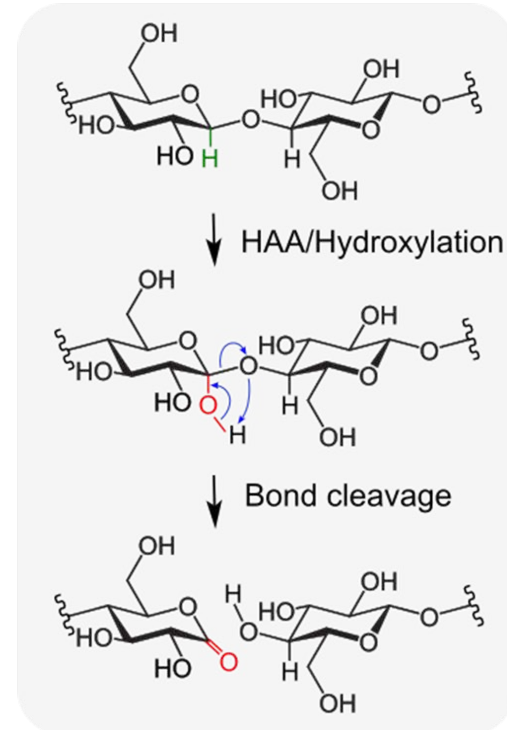
Vaaje-Kolstad et al., 2010, *Science*; Horn et al., 2012 *Biotechnology for Biofuels*; Aachmann et al., 2012, *PNAS*; Agger et al., 2014, *PNAS*; Forsberg et al., 2014, *PNAS*; Courtade et al., 2016, *PNAS*; Kracher et al., 2016, *Science*; Bissaro et al., 2017, *Nature Chem Biol*

LPMO catalysis





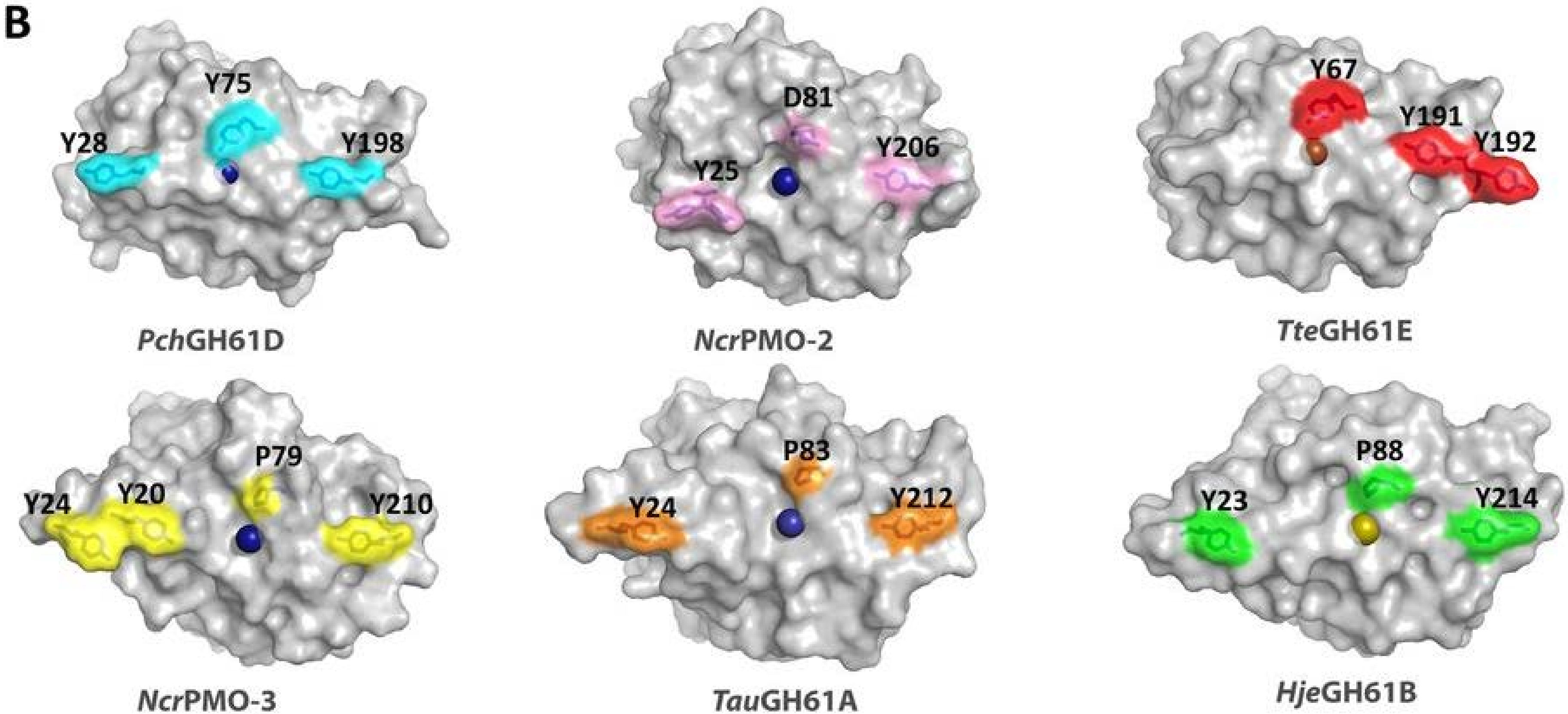




Hydrogen Atom Abstraction
≈ 95 kcal/mol

Quinlan et al, *PNAS*, 2011
Beeson et al., *JACS*, 2012
Beeson et al., *Ann. Rev. Biochem.*, 2015
Walton and Davies, *Curr. Opin. Chem.Biol.*, 2016

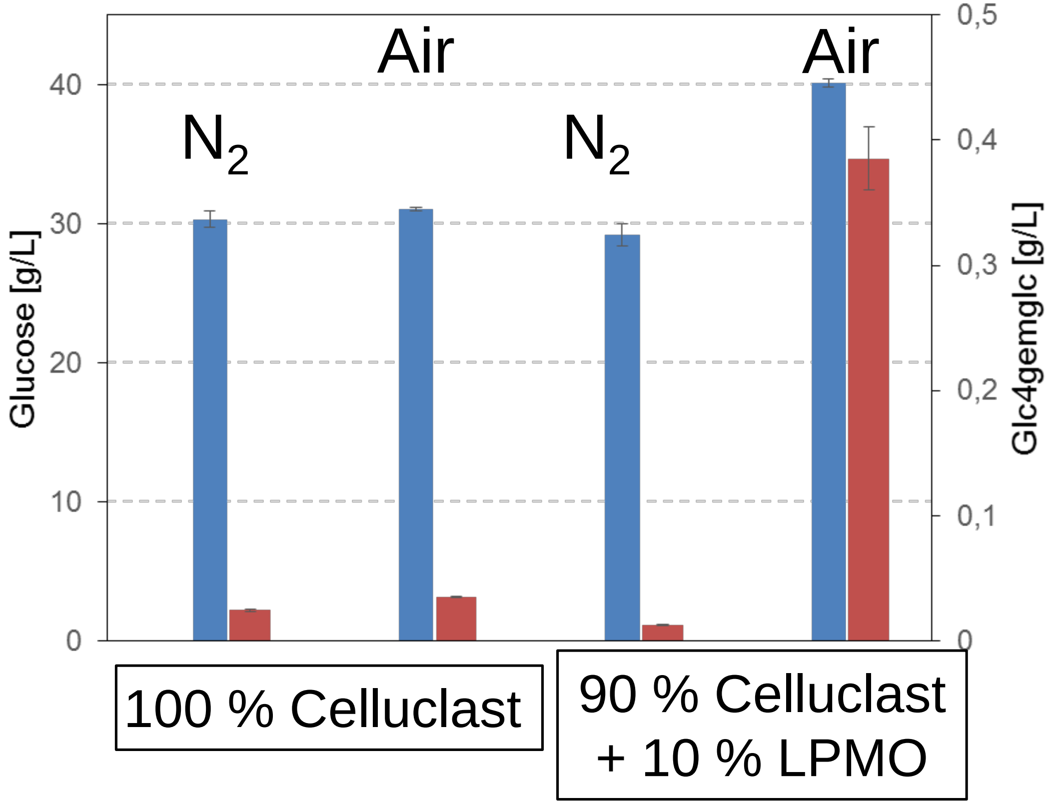
There are many LPMOs……



Wu et al., 2013, JBC 288,12828

See also: Aachmann et al., 2012, PNAS; Agger et al., 2014, PNAS; Borisova et al., 2015, JBC; Vaaje-Kolstad et al., 2017, Curr Opin Struct Biol 44,67

LPMOs really help, and they need oxygen and electrons



SE Birch
10 % DM

Blue: glucose

Red: LPMO products

Müller et al., 2015, Biotech Biofuels

2016 Discovery: LPMOs are (also) peroxygenases

nature chemical biology

ARTICLE

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Oxidative cleavage of polysaccharides by monocopper enzymes depends on H_2O_2

Bastien Bissaro[1,2*], Åsmund K Røhr[2], Gerdt Müller[2], Piotr Chylenski[2], Morten Skaugen[2], Zarah Forsberg[2], Svein J Horn[2], Gustav Vaaje-Kolstad[2] & Vincent G H Eijsink[2*]

Enzymes currently known as lytic polysaccharide monooxygenases (LPMOs) play an important role in the conversion of recalcitrant polysaccharides, but their mode of action has remained largely enigmatic. It is generally believed that catalysis by LPMOs requires molecular oxygen and a reductant that delivers two electrons per catalytic cycle. Using enzyme assays, mass spectrometry and experiments with labeled oxygen atoms, we show here that H_2O_2, rather than O_2, is the preferred co-substrate of LPMOs. By controlling H_2O_2 supply, stable reaction kinetics are achieved, the LPMOs work in the absence of O_2, and the reductant is consumed in priming rather than in stoichiometric amounts. The use of H_2O_2 by a monocopper enzyme that is otherwise cofactor-free offers new perspectives regarding the mode of action of copper enzymes. Furthermore, these findings have implications for the enzymatic conversion of biomass in Nature and in industrial biorefining.

+ several papers in the publishing pipeline

The LPMO reaction revised

2010 $$R\text{-}H + O_2 + 2e^- + 2H^+ \xrightarrow[\text{LPMO-Cu(II)}]{} R\text{-}OH + H_2O$$

2016 $$R\text{-}H + H_2O_2 \xrightarrow[\text{LPMO-Cu(I)}]{} R\text{-}OH + H_2O$$

The LPMO reaction revised

2010 $R\text{-}H + O_2 + 2e^- + 2H^+ \xrightarrow[\text{LPMO-Cu(II)}]{} R\text{-}OH + H_2O$

2016 $R\text{-}H + H_2O_2 \xrightarrow[\text{LPMO-Cu(I)}]{} R\text{-}OH + H_2O$

And: ***supplying a reduced non-substrate-bound LPMO with H_2O_2 leads to oxidative self-inactivation (at very low H_2O_2 concentrations)***

H_2O_2 as a co-substrate of LPMOs

Avicel 10% DM, treated with Cellic CTec2, anaerobically

Müller, Chylenski, Bissaro, Eijsink & Horn, unpublished data

H_2O_2 as a co-substrate of LPMOs

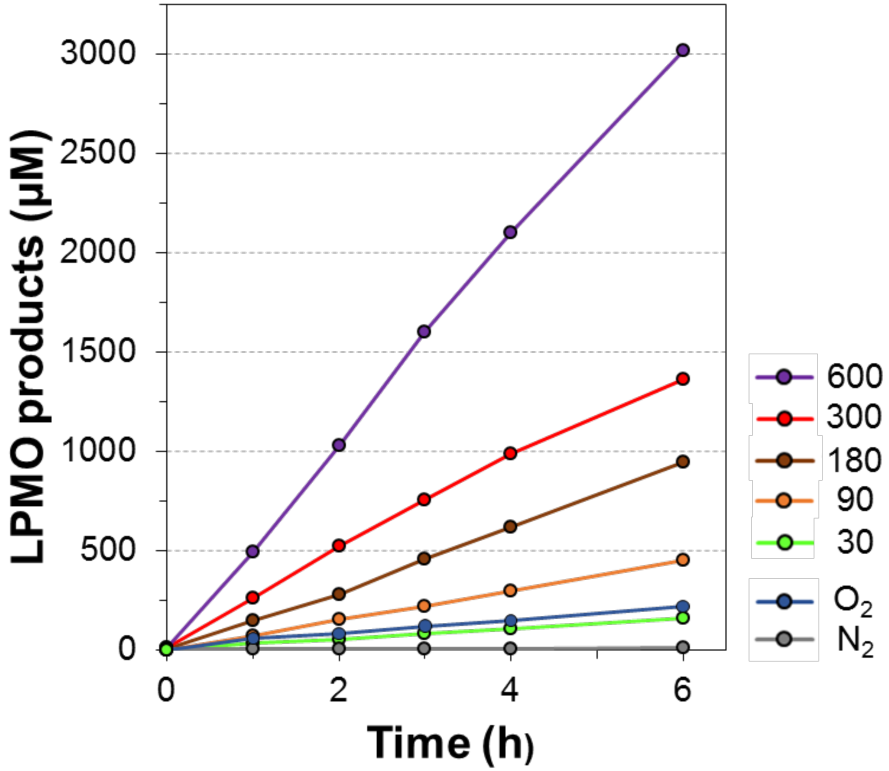
H_2O_2 feeding (Avicel, 48 hours)

Effects are substrate-dependent

Sulfite-pulped Norway spruce
- 10% w/w DM
- Cellic® CTec2 (4 mg/g DM)
- 1 mM ascorbic acid

Steam exploded birch
- 10% w/w DM
- Cellic® CTec2 (2 mg/g DM)

H_2O_2 feeding on the model substrate Avicel – note:



Appr. LPMO rates are still ***very*** low:

0.3 min^{-1} (O_2) – 3 min^{-1} (600 $\mu M.h^{-1}$)

Concluding remarks

- LPMO activity can be controlled and boosted by controlling H_2O_2.

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- We are far from harnessing the full potential of LPMOs, and LPMO containing cellulase cocktails.
- Optimization of enzyme cocktail and/or process conditions based on this new LPMO knowledge may be key to further improvements in biomass processing.

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- LPMO self-inactivation is a highly relevant issue and needs to be controlled.
- We are far from harnessing the full potential of LPMOs, and LPMO containing cellulase cocktails.
- Optimization of enzyme cocktail and/or process conditions based on this new LPMO knowledge may be key to further improvements in biomass processing.
- *Other central Bio4Fuels enzyme topics: Hemicellulases, CBMs, Enzyme fate*