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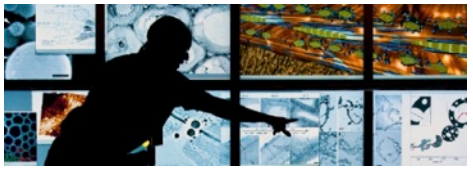
Revealing the Differences Between Free and Complexed Enzyme Mechanisms and Factors Contributing to Cell Wall Recalcitrance



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U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

Michael G. Resch

10th European Symposium on Biochemical Engineering Sciences and 6th International Forum on Industrial Bioprocesses

Biocatalysis Session

Lille, France

September 8, 2014

NREL/PR-5100-62724

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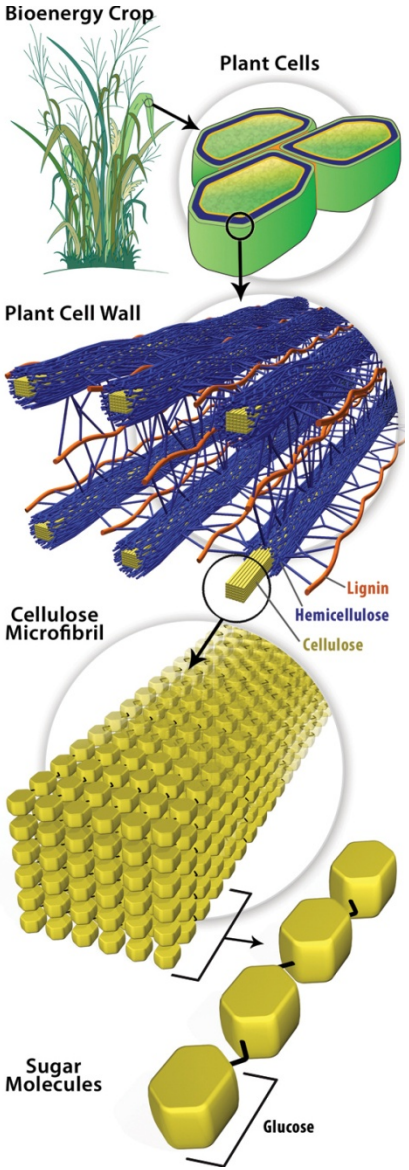
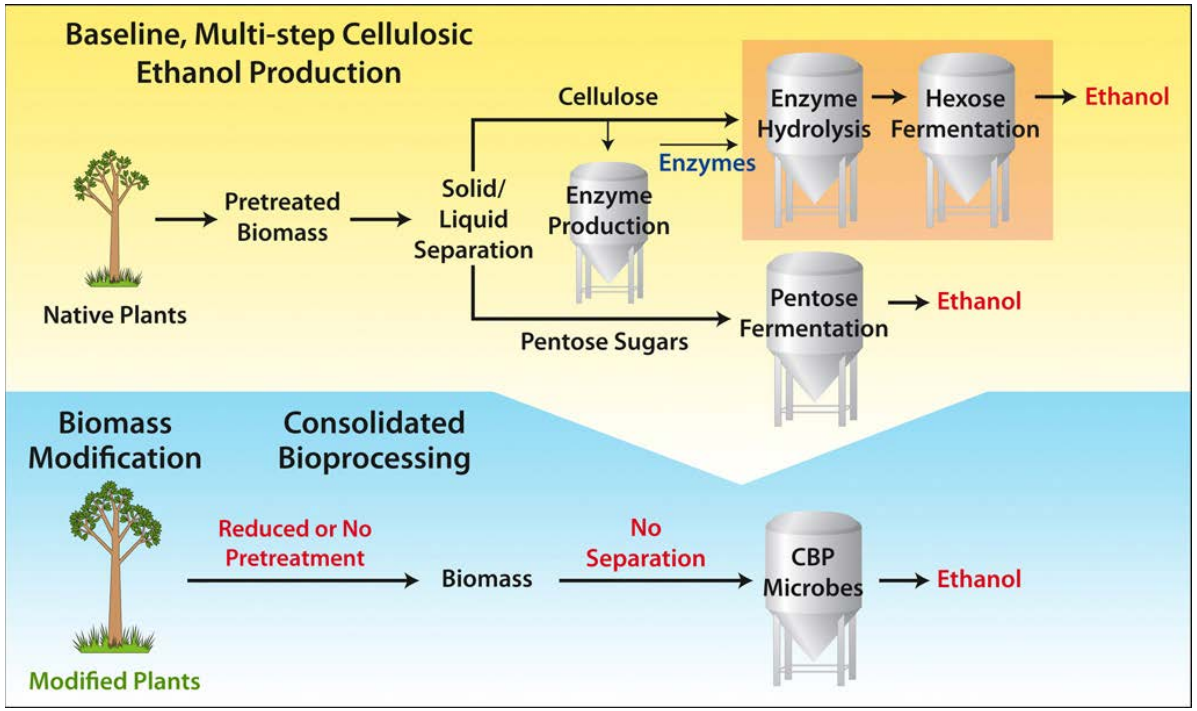
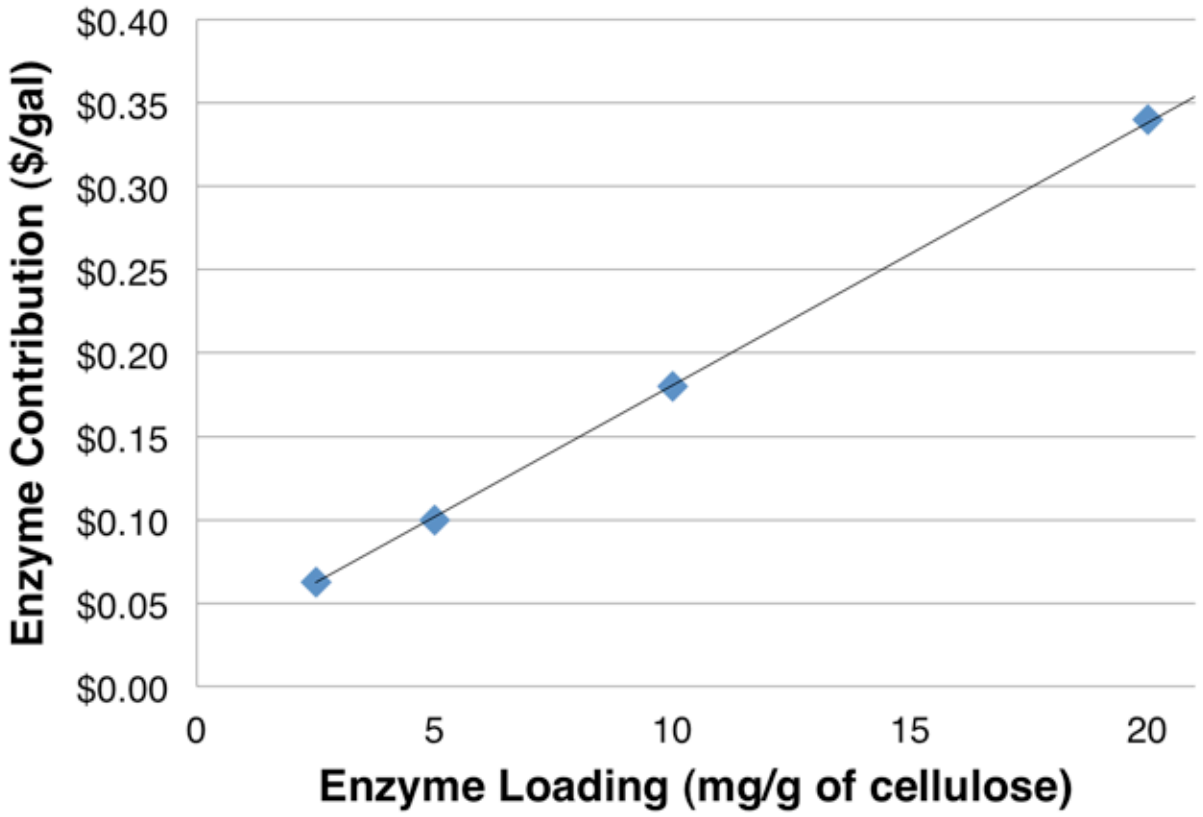
Biocatalytic Conversion of Biomass to Fuels





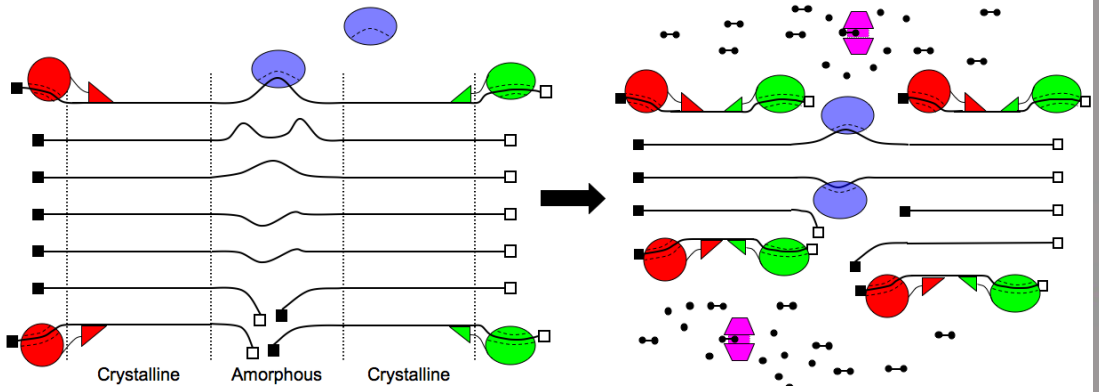
Economic Impact of Reducing Enzyme Loading



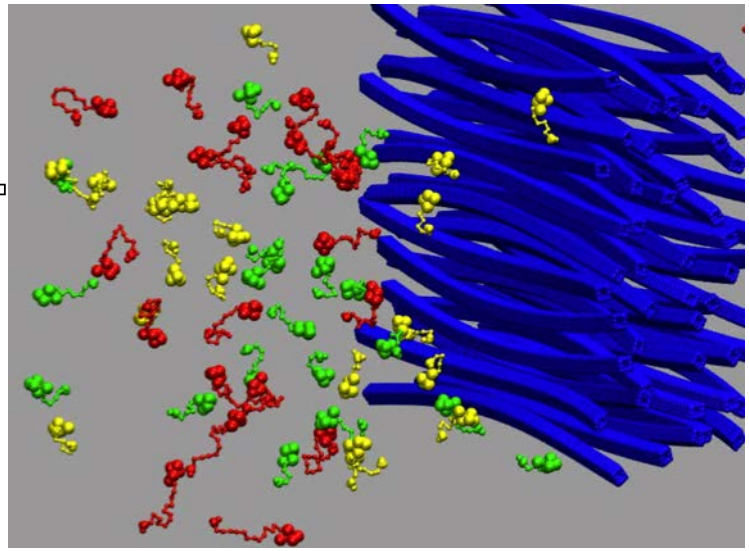
Humbird *et al*. 2011

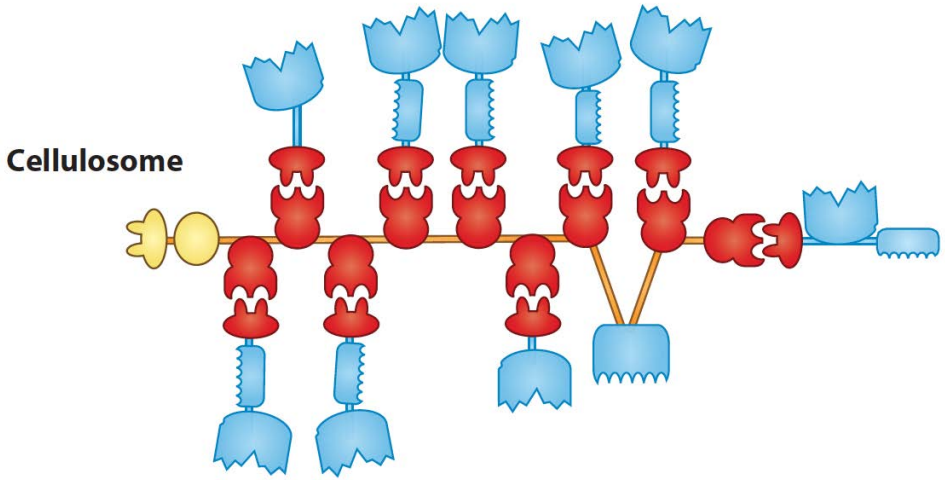
“Free” vs. Complexed Enzymes

“free” cellulases

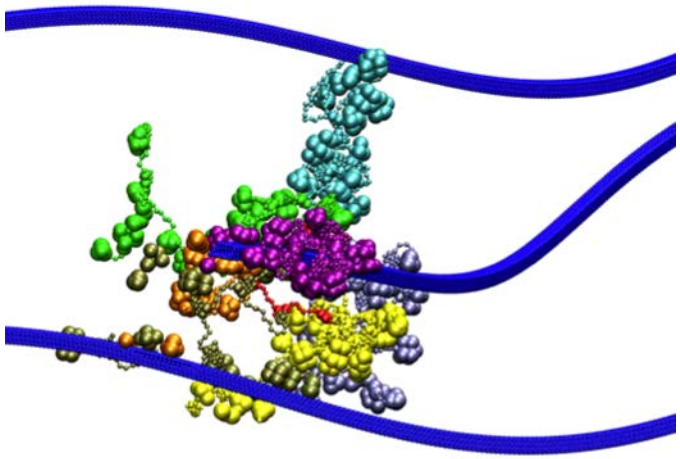


Lynd *et al. MMR* 2002

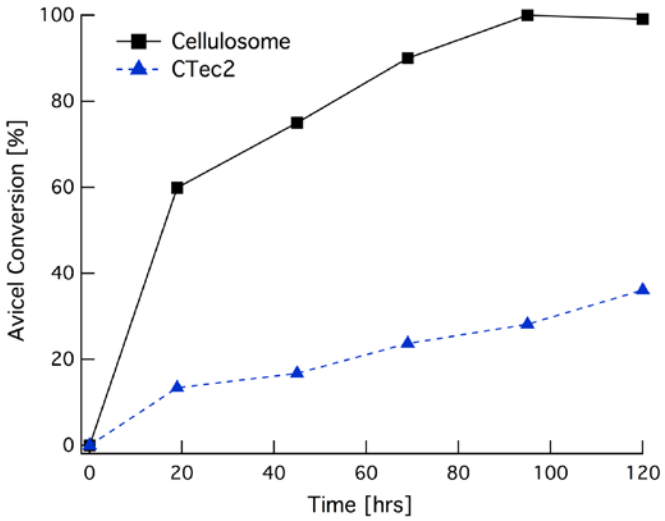




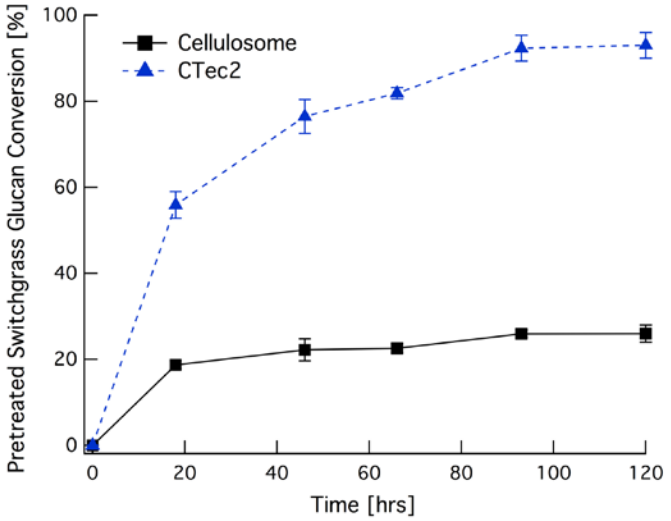
Gilbert and Fontes, *Ann. Rev. Biochem*. 2010



Avicel conversion at 5 mg/g



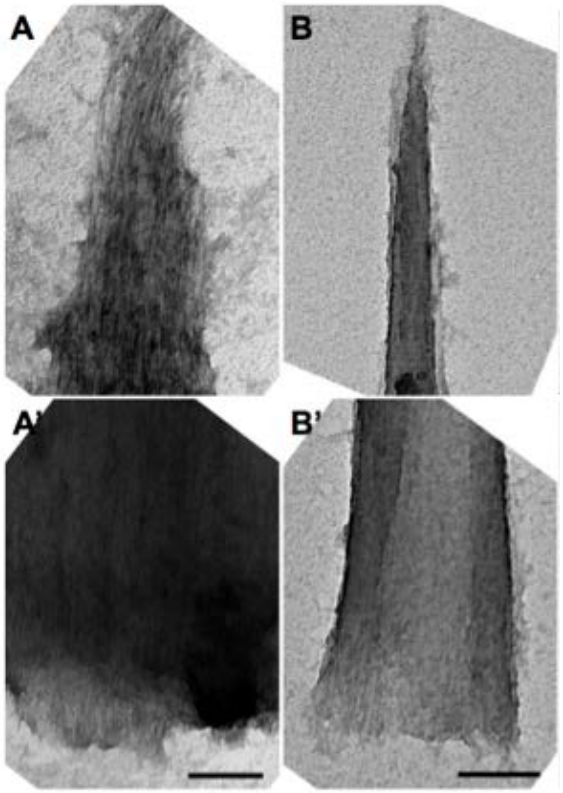
DA-SG conversion at 20 mg/g

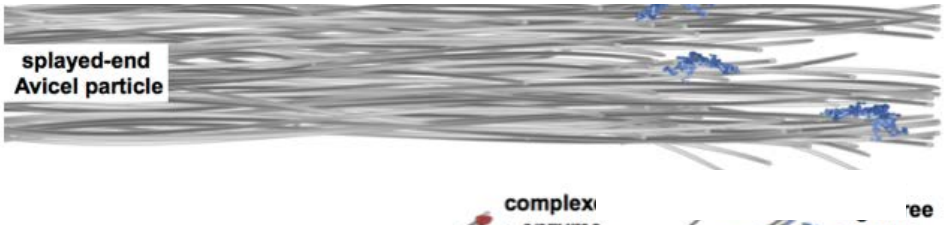


Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

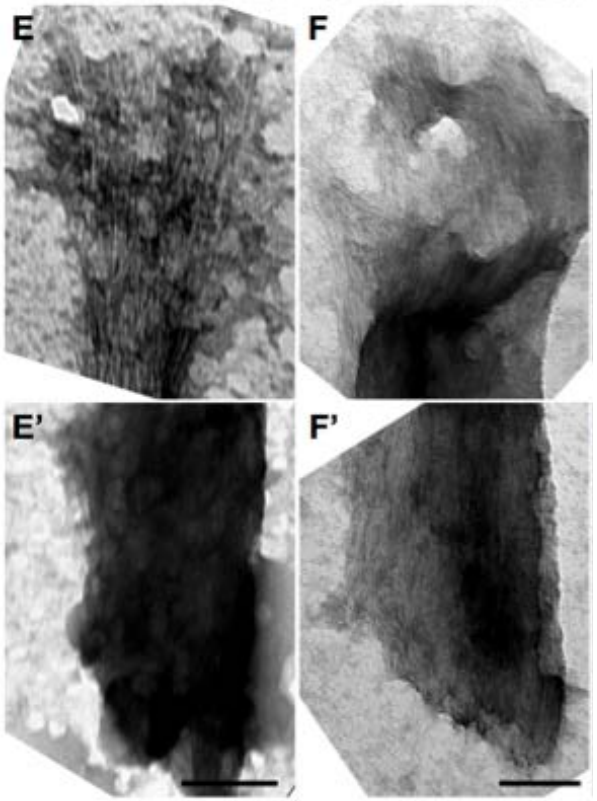
Different physical mechanisms of denconstruction

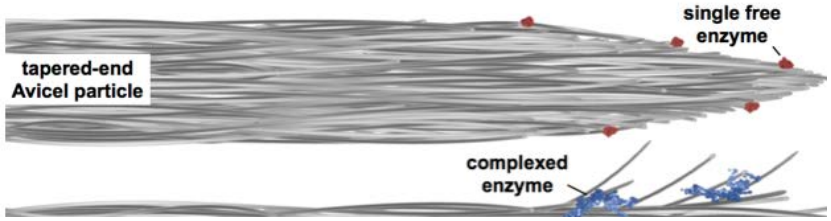
"Free" Cellulases





Cellulosomes

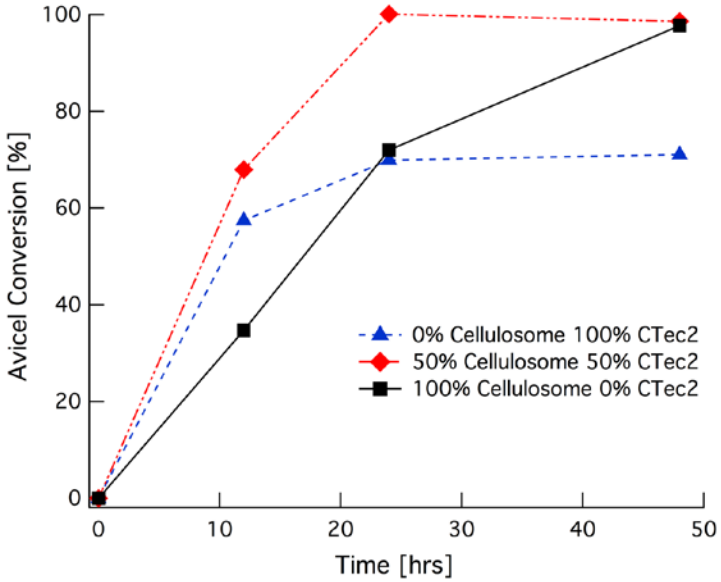


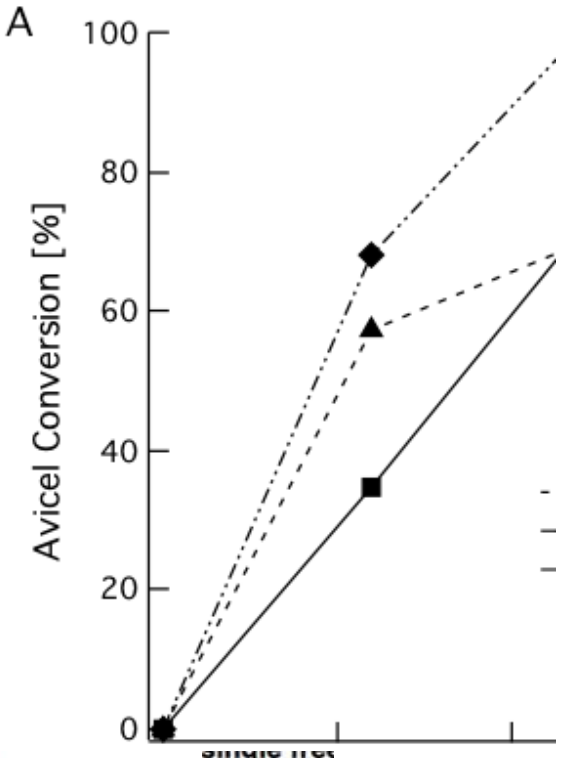


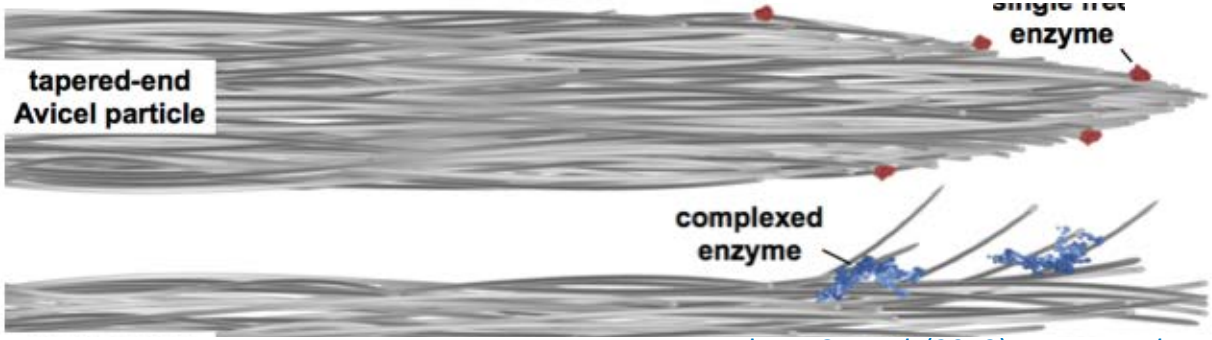
Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

Do Free and complexed enzymes degrade cellulose synergistically?

Avicel conversion at 10 mg/g



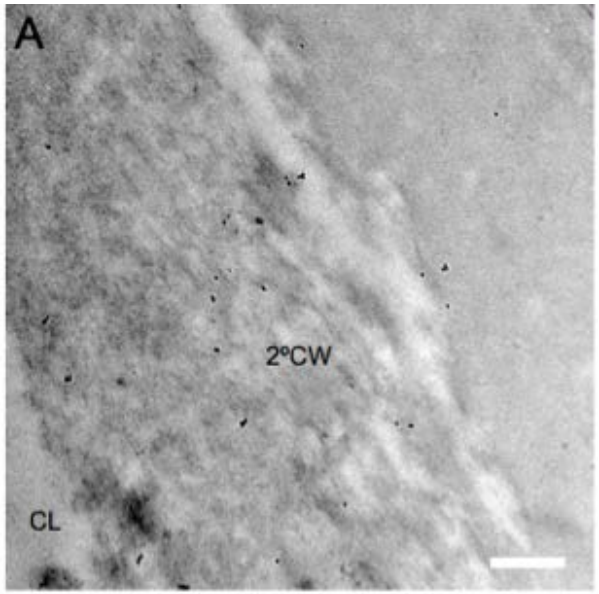




Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

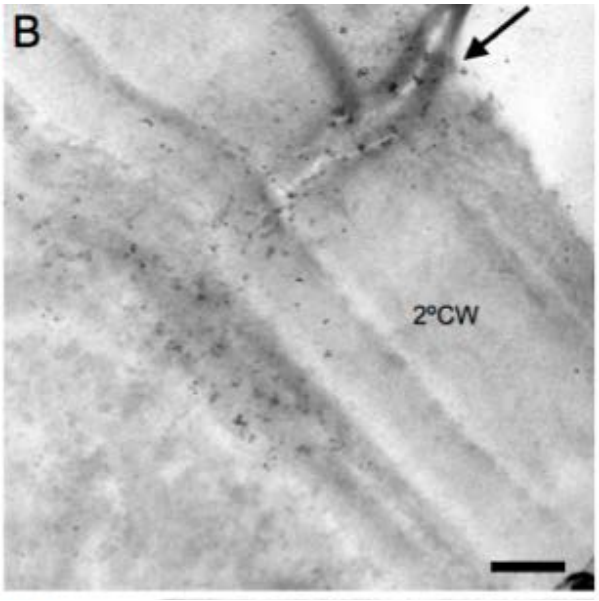
Enzyme Accessibility is Different

"Free" Enzymes



- Cellulases are evenly distributed
- Cellulosomes are near the cell walls

Cellulosomes



Possible factors limiting cellulosome activity on DA pretreated biomass

- Accessibility
- Low k_{off} compared to free cellulases
- Binding to lignin

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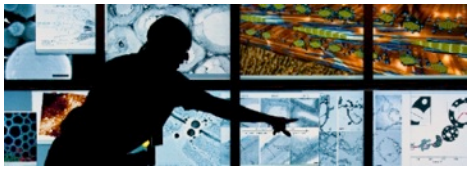
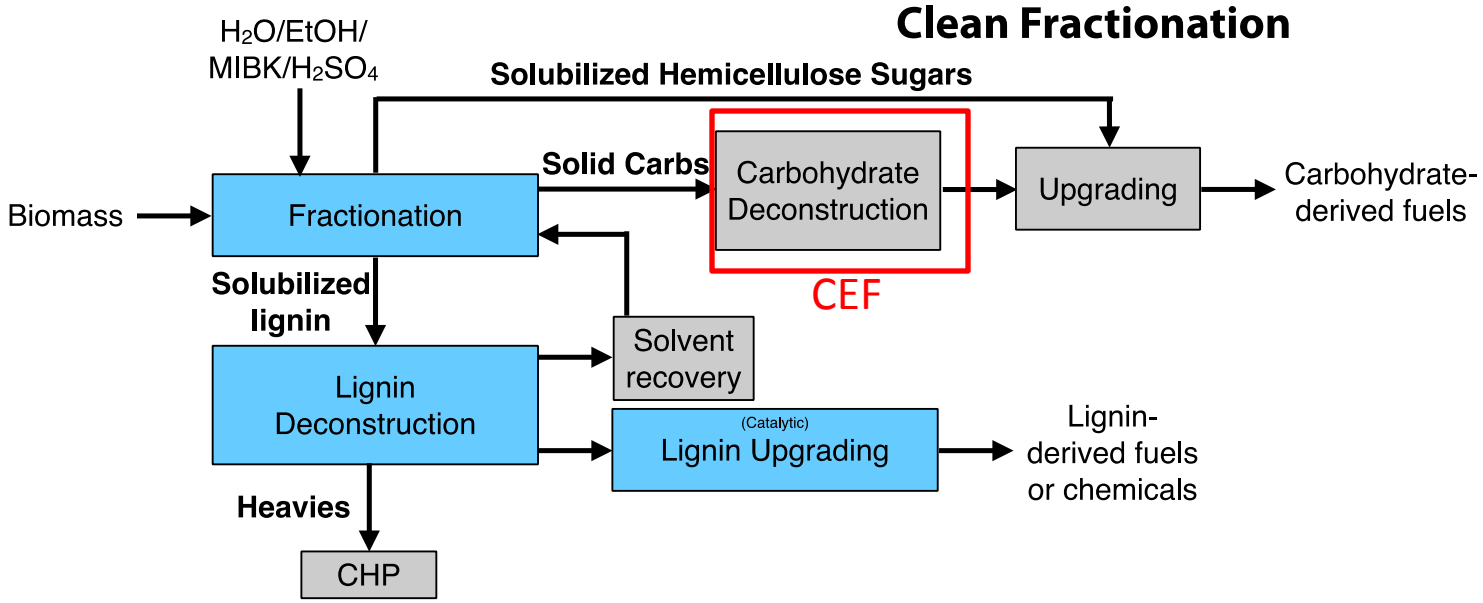


Clean Fractionation Pretreatment (Organosolve)

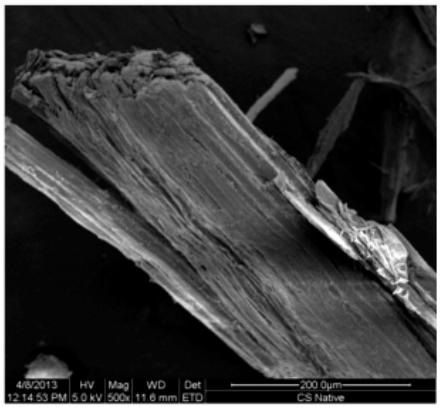


CEF Chemical Composition

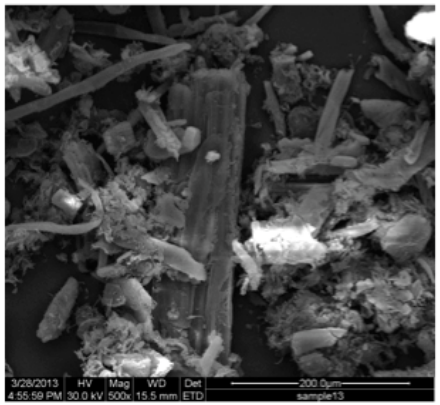
Solvent	Temp (°C)	Acid (mol/L)	Lignin (%)	Glucan (%)	Xylan (%)
MIBK/**Acetone**/H2O	140	0.025	10.63	63.31	17.47
MIBK/**Acetone**/H2O	140	0.05	3.67	82.28	6.56
MIBK/**Acetone**/H2O	140	0.1	3.33	87.19	3.08

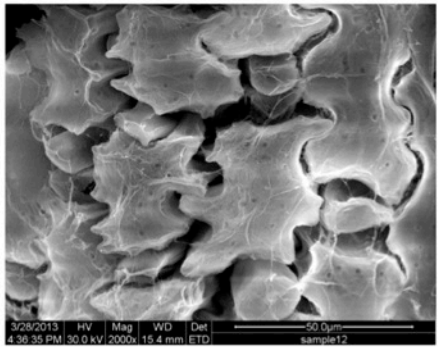
How does Clean Fractionation affect biomass morphology?

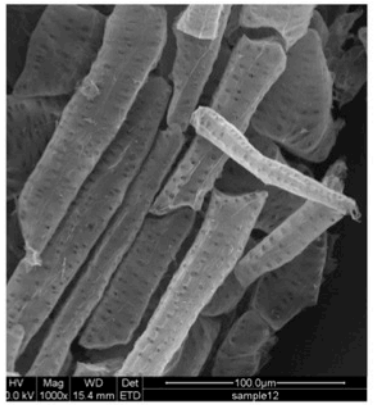
Native Vascular bundle



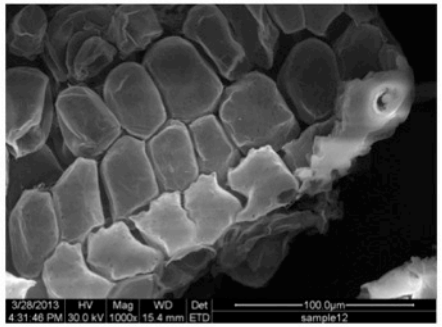
Dilute acid-treated





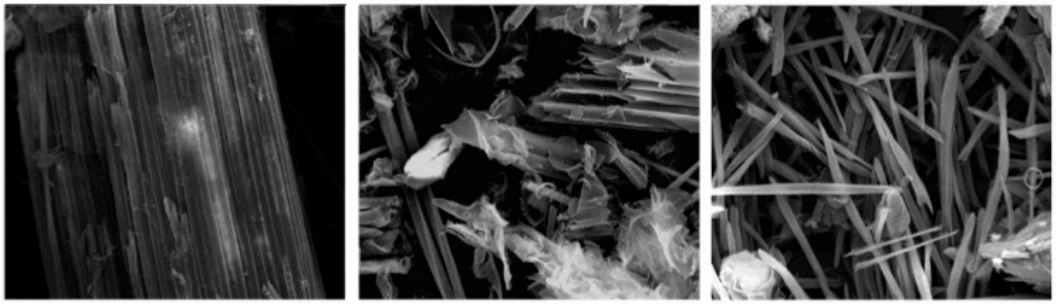


140° C 0.1M

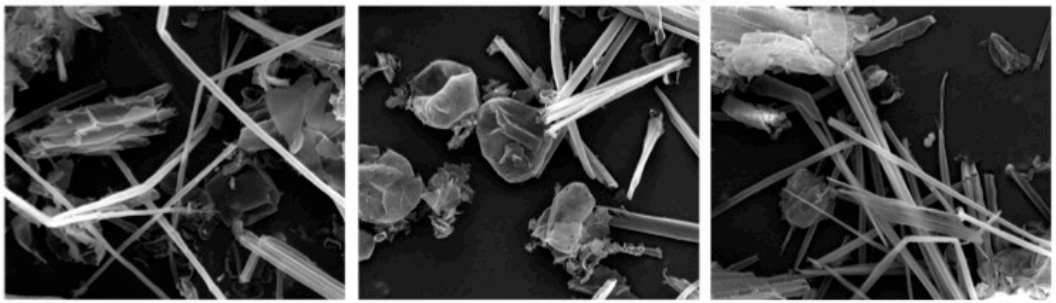


25 mM acid | 50 mM acid | 100 mM acid

Acetone 120° C

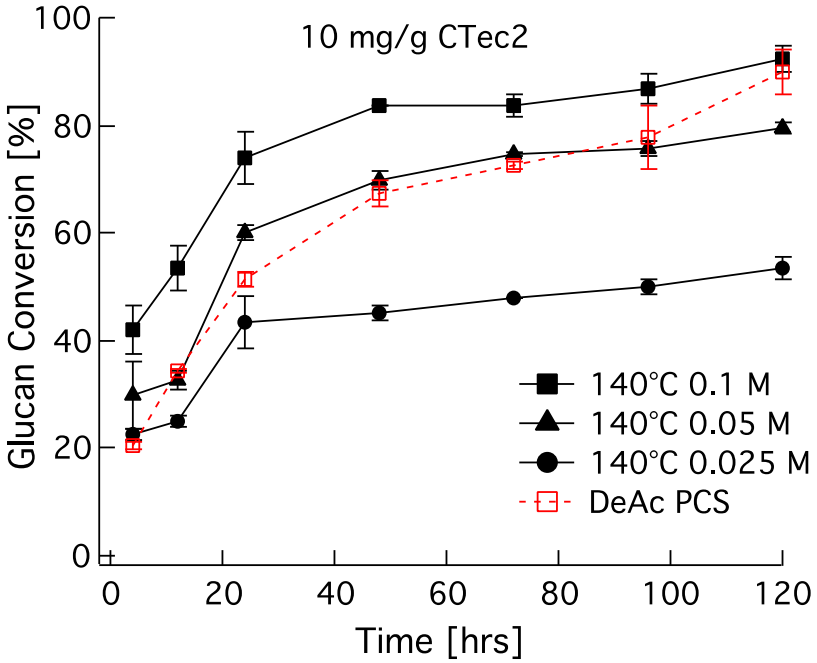


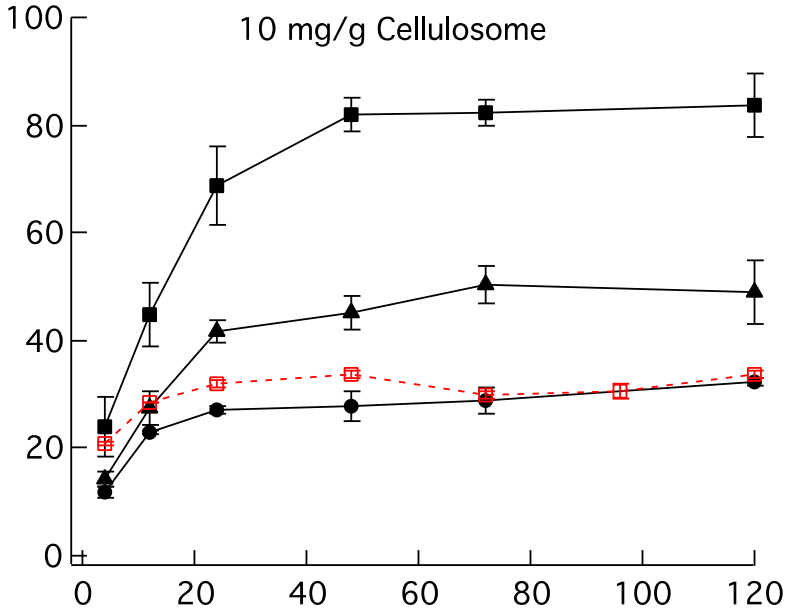
Acetone 140° C



Katahira, R. et *al* (2014) *ACS*

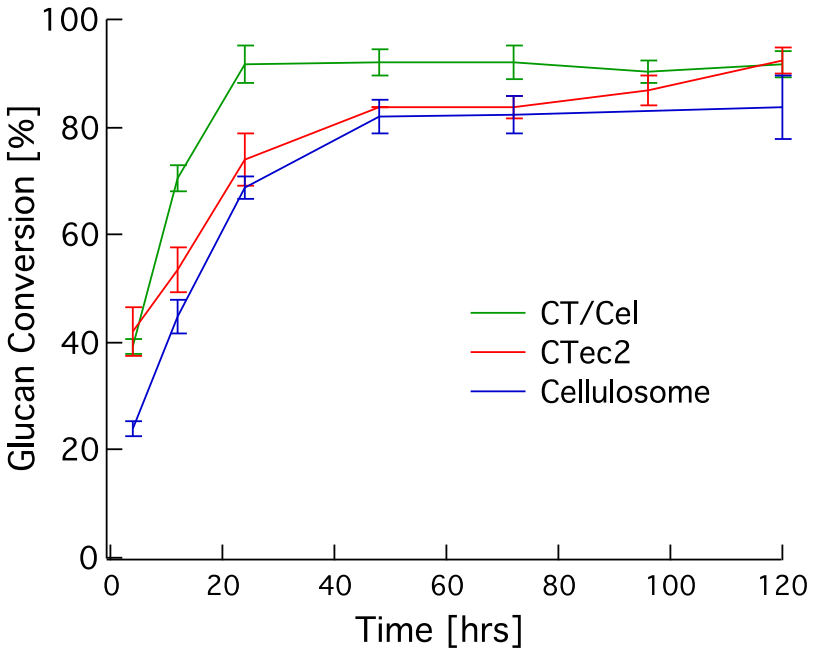
How does Clean Fractionation affect enzyme saccharification?

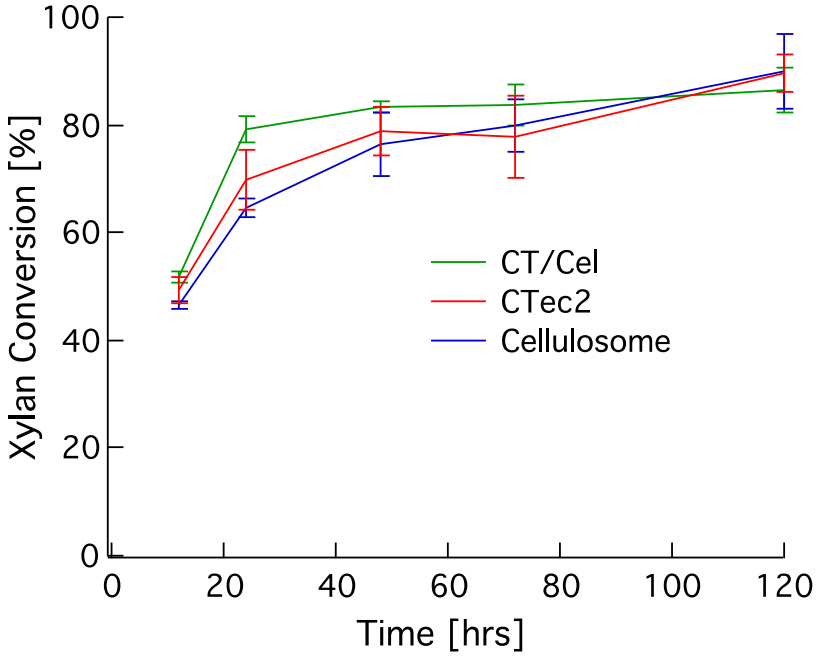




Resch, M. *et al* (2014) *ACS*

Synergistic Combination of CTec2 and Cellulosomes



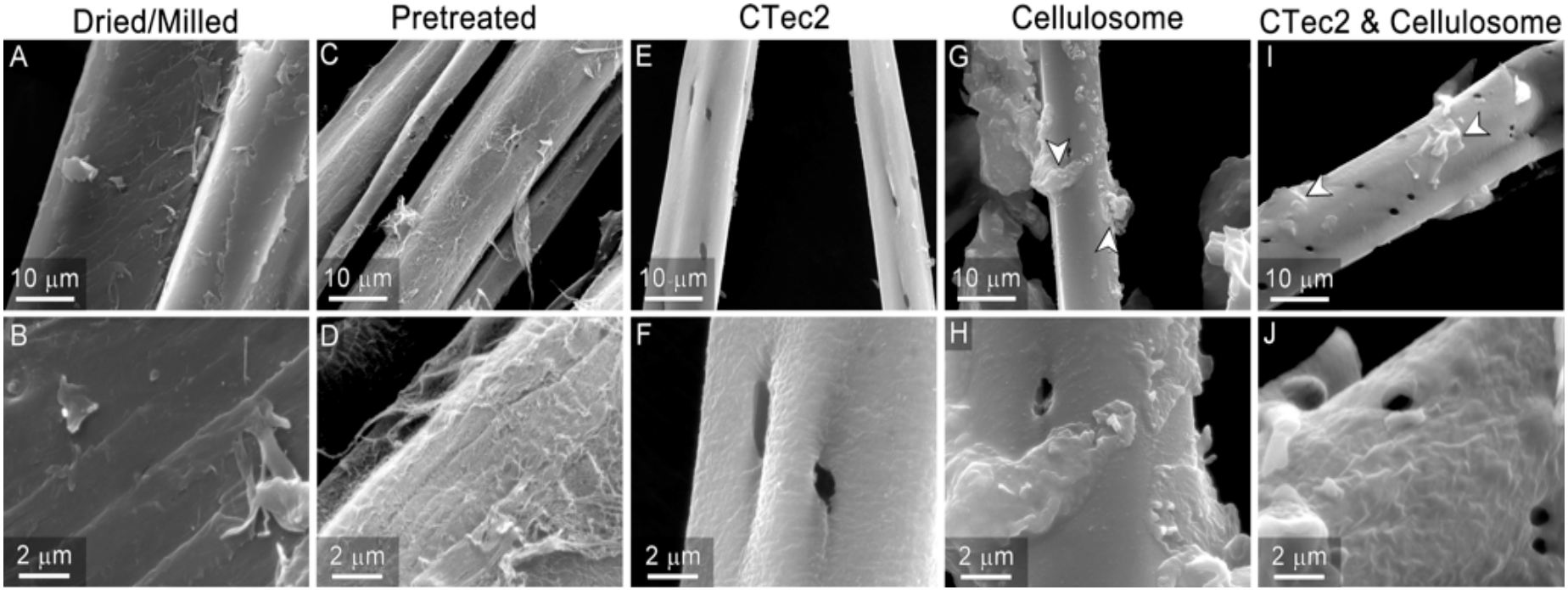


10 mg/g enzyme loading

CT/Cel = 5 mg/g CTec2 + 5 mg/g Cellulosomes

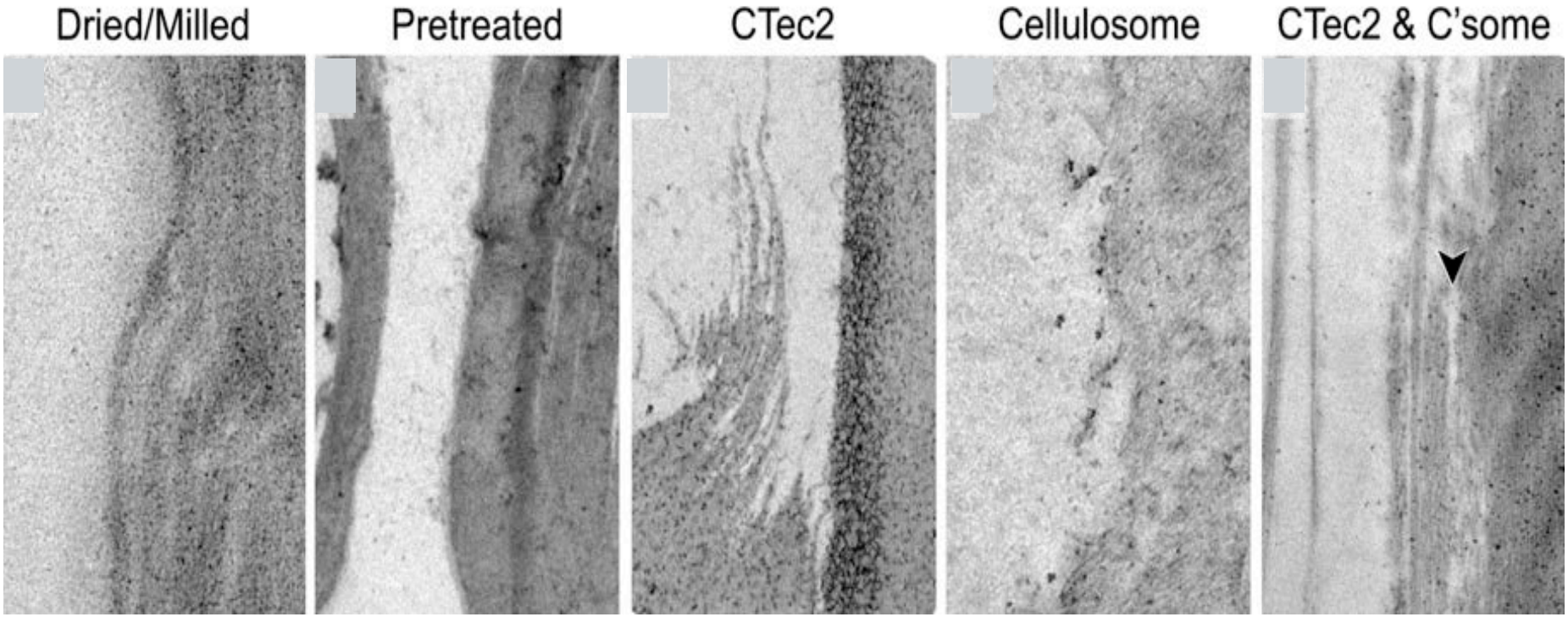
Resch, M. *et al* (2014) *ACS*

SEM of Digested Cellulose Enriched Fraction



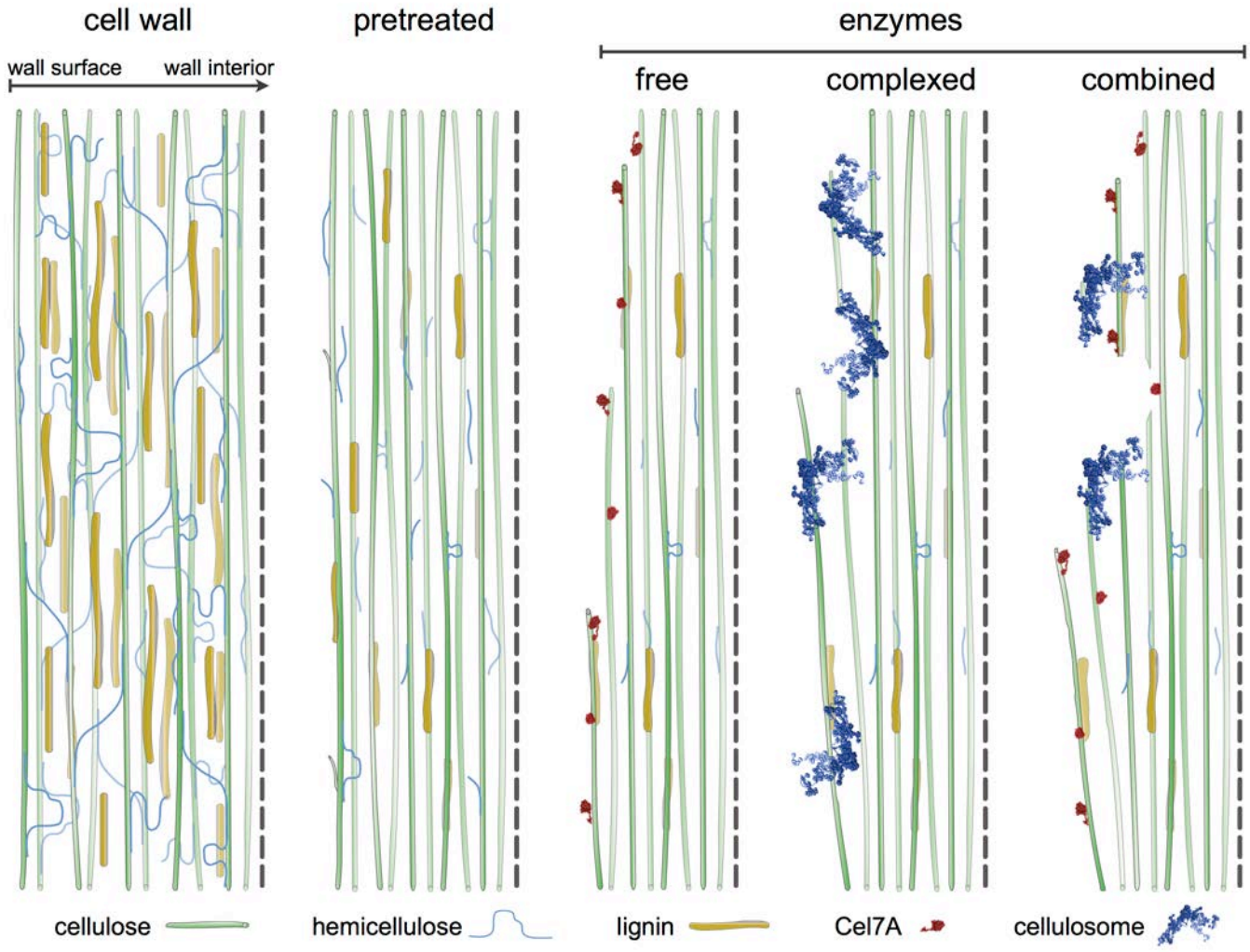
Resch, M. *et al* (2014) *ACS*

TEM of Digested Cellulose Enriched Fraction



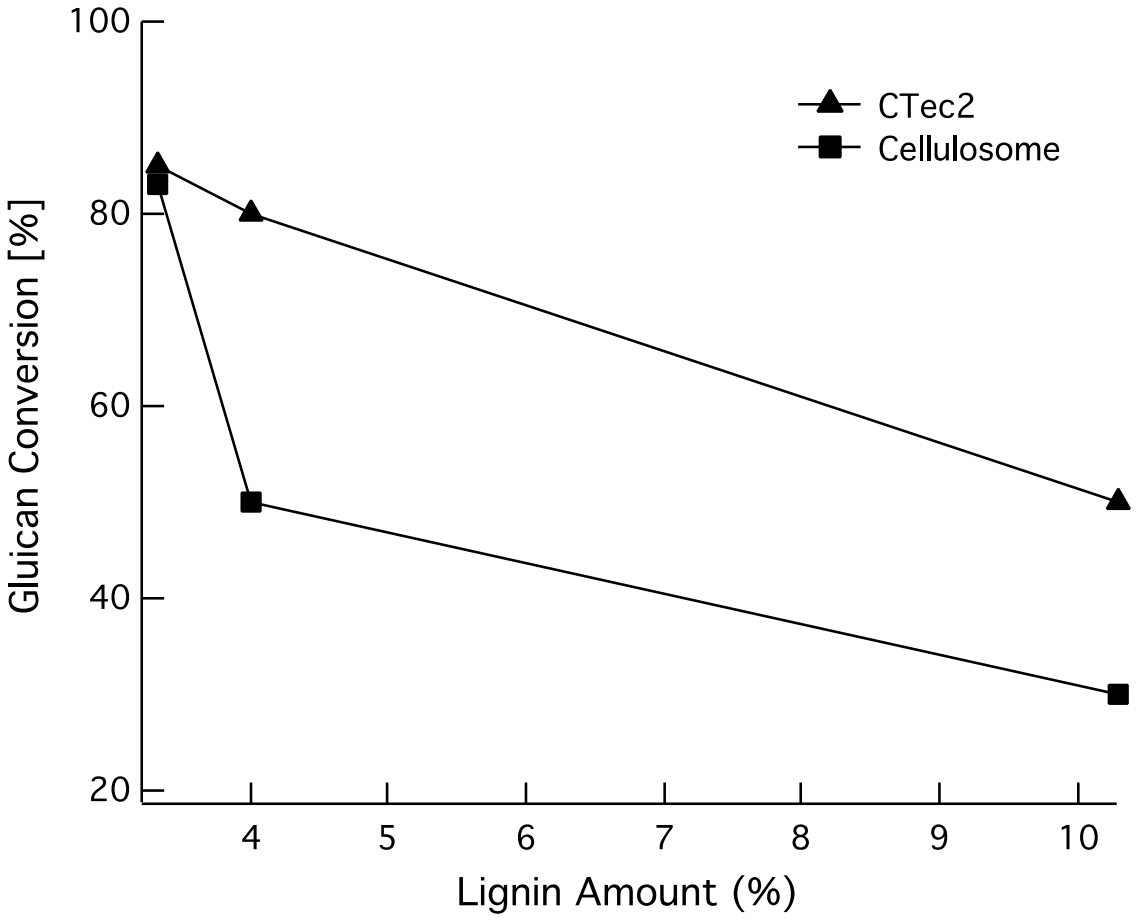
Resch, M. *et al* (2014) *ACS*

Model of Enzymatic Deconstruction Mechanisms



Resch, M. *et al* (2014) *ACS*

Affect of PT Severity on Enzymatic Saccharification



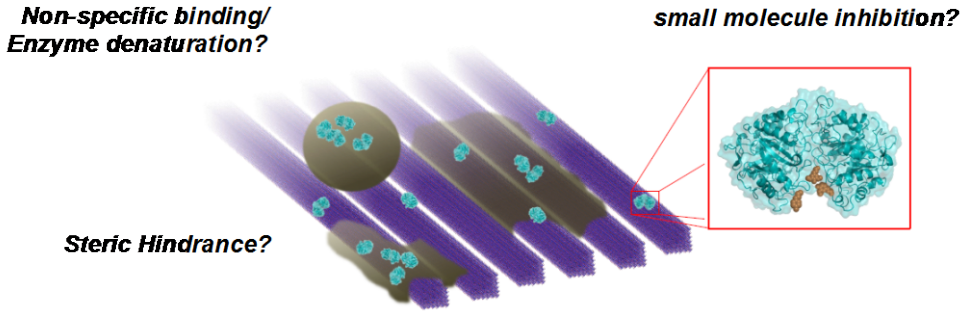
10 mg/g enzyme loading,
72 hours

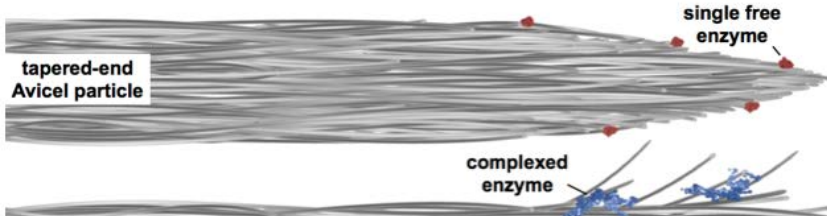
Resch, M. *et al* (in review)

Conclusions

- "Free" enzymes and cellulosomes have distinct mechanisms in cellulose and biomass deconstruction
- "Free" enzymes and cellulosomes are synergistic
- Biomass composition and enzyme accessibility to cellulose ends affect cellulosomes more than "free" enzymes
- Combining properties of "free" and multi-domain enzymes in commercial enzyme cocktails could lead to enzyme cost reductions

Is Lignin More Inhibitory to Cellulosomes than Free enzymes?





Acknowledgements

Funding

Office of Science

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Biomass Technologies Office (BETO)

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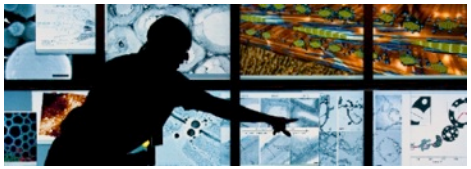

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