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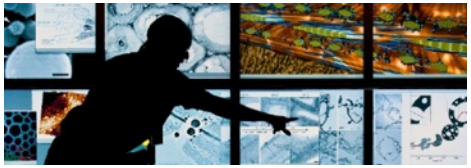
Revealing the Differences Between Free and Complexed Enzyme Mechanisms and Factors Contributing to Cell Wall Recalcitrance



BESC
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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

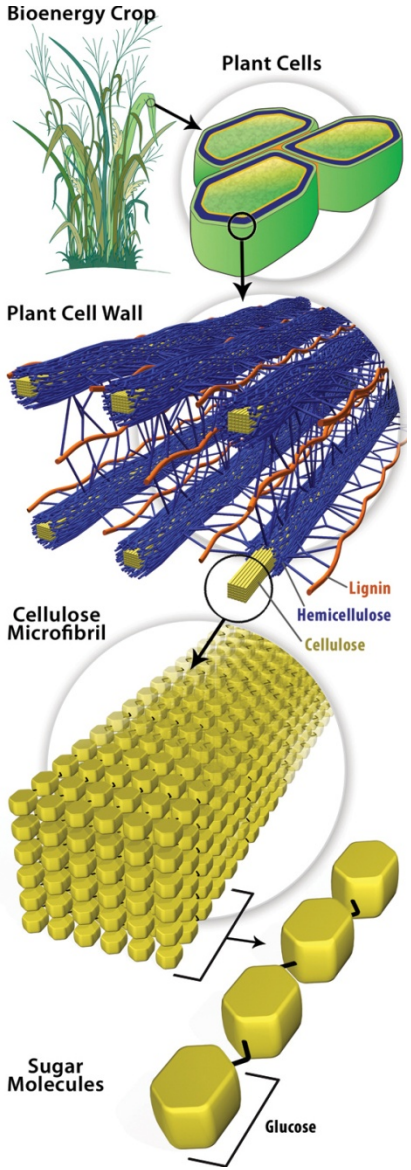
Lille, France

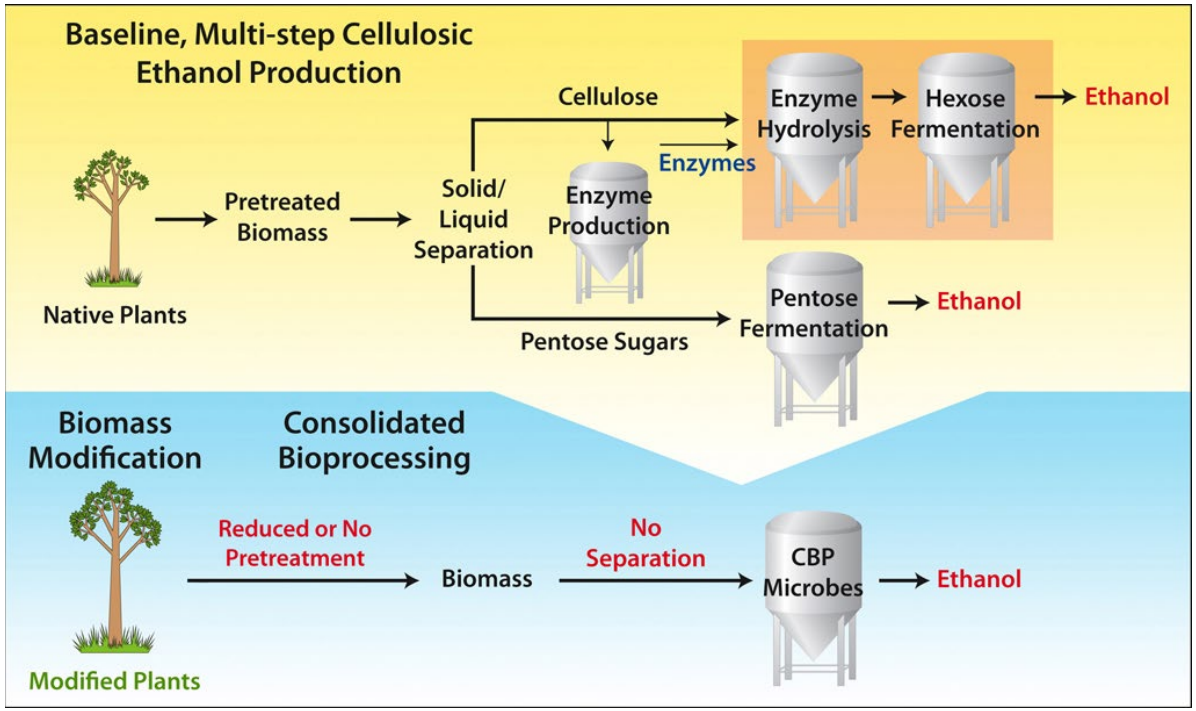
September 8, 2014

NREL/PR-2700-61394

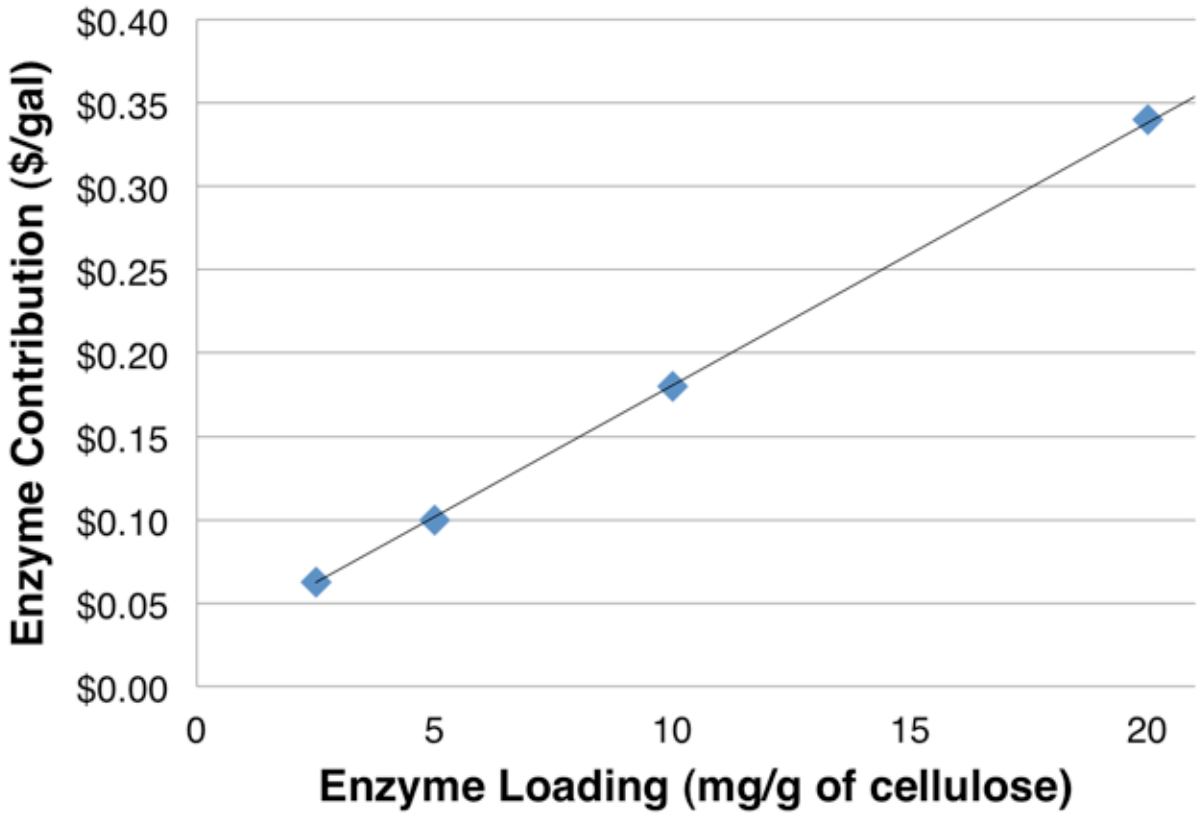
NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Biocatalytic Conversion of Biomass to Fuels





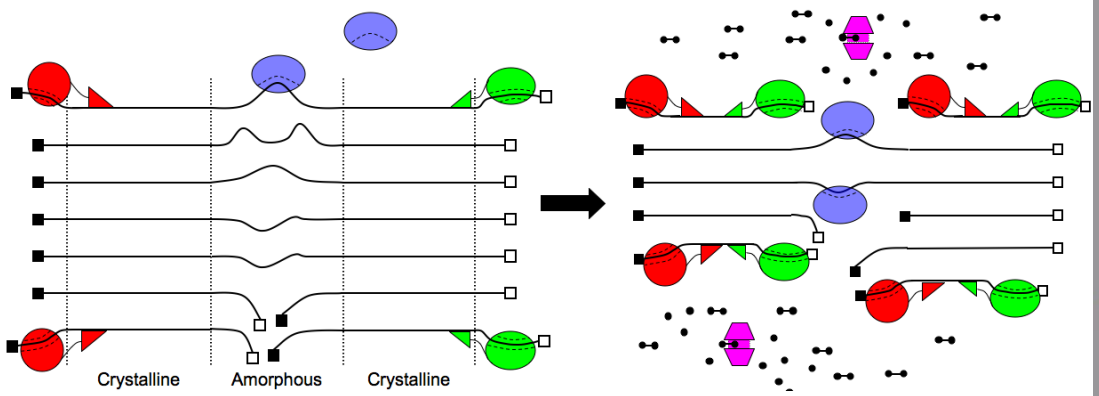
Economic Impact of Reducing Enzyme Loading



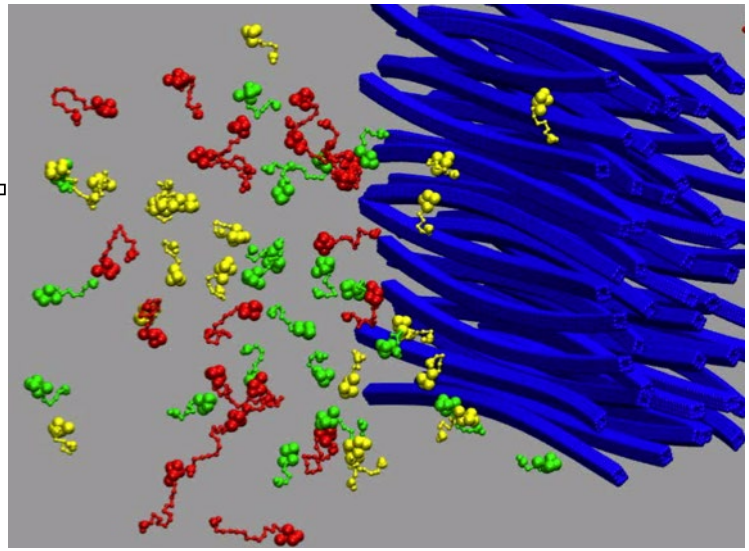
Humbird *et al*. 2011

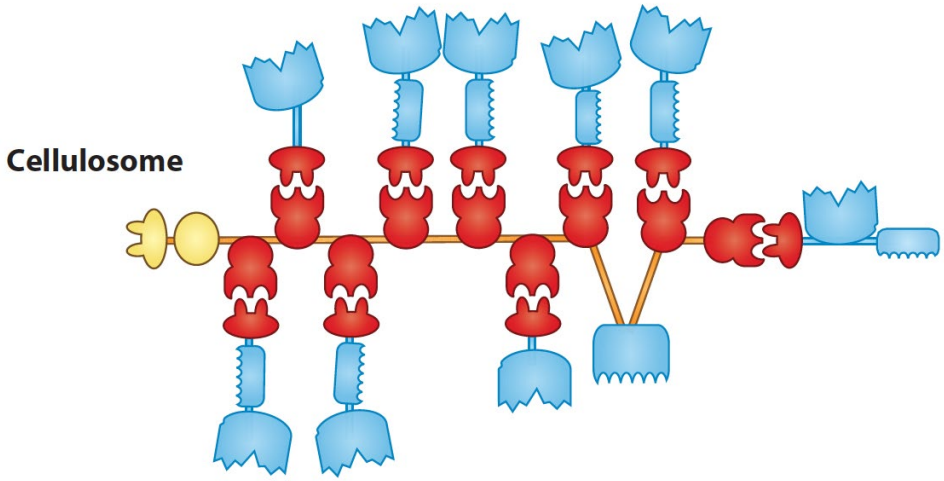
“Free” vs. Complexed Enzymes

“free” cellulases

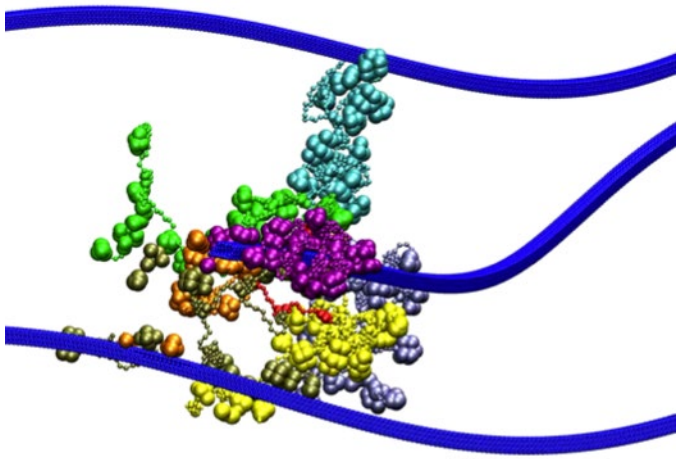


Lynd *et al. MMR* 2002

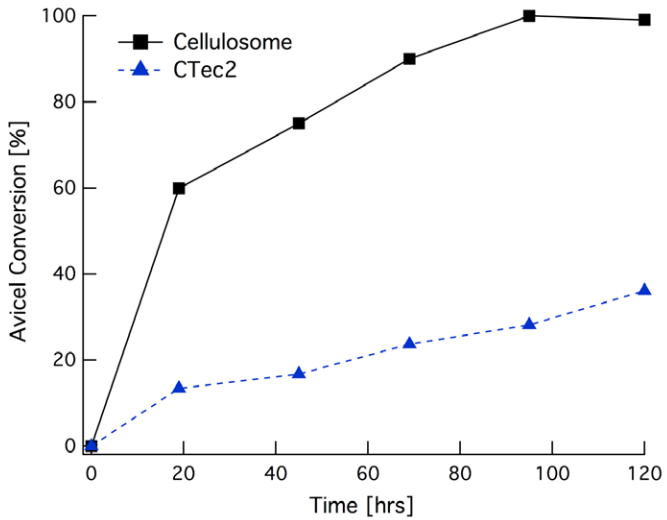




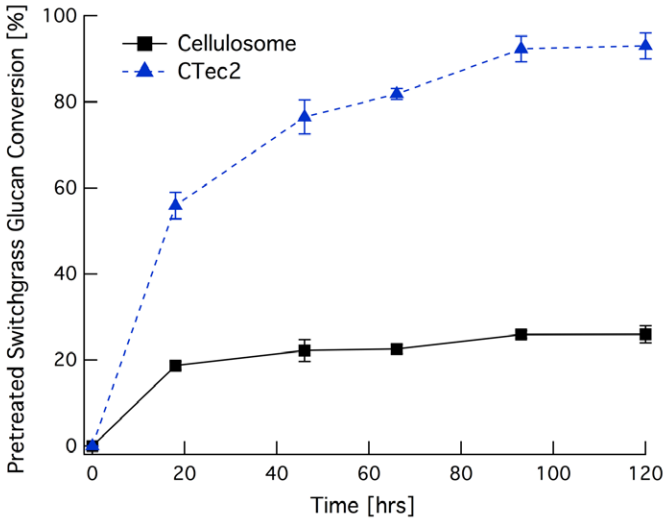
Gilbert and Fontes, *Ann. Rev. Biochem*. 2010



Avicel conversion at 5 mg/g



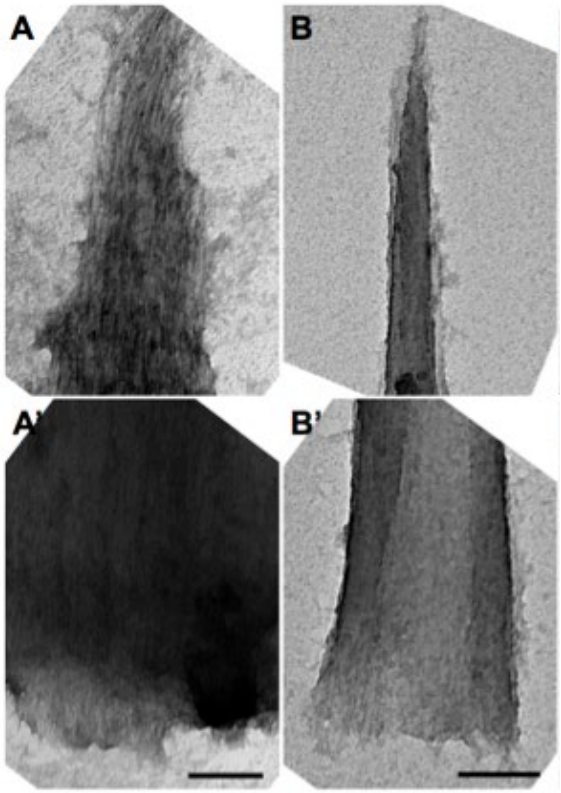
DA-SG conversion at 20 mg/g



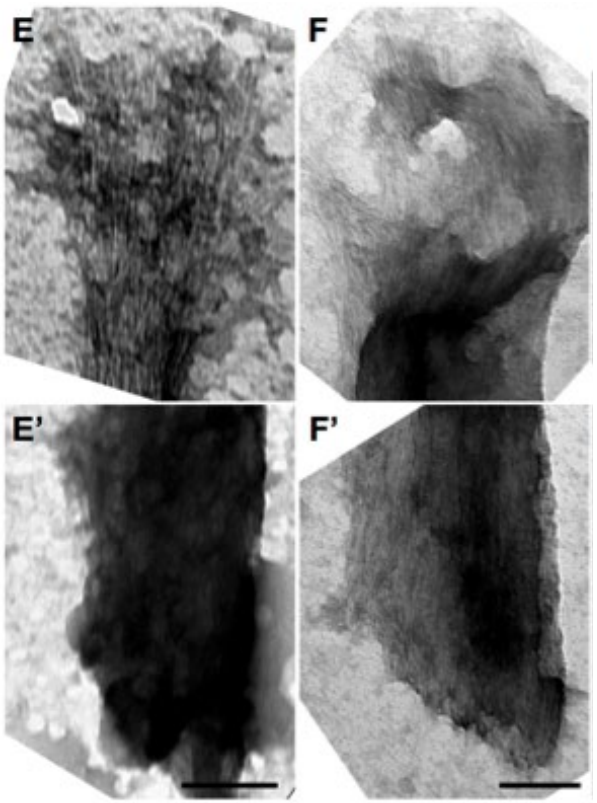
Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

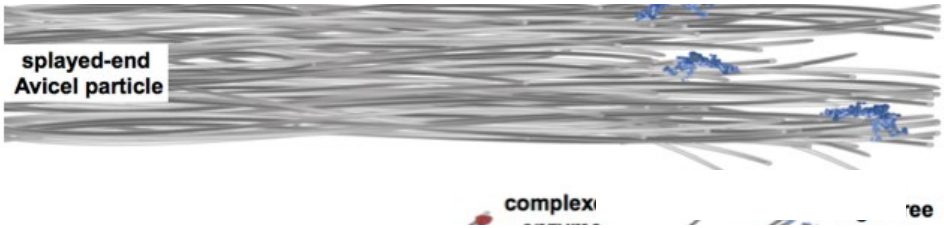
Different physical mechanisms of denconstruction

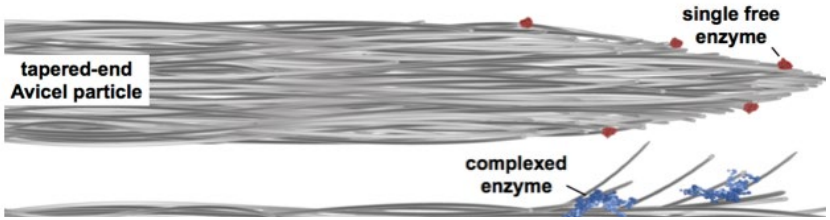
"Free" Cellulases



Cellulosomes



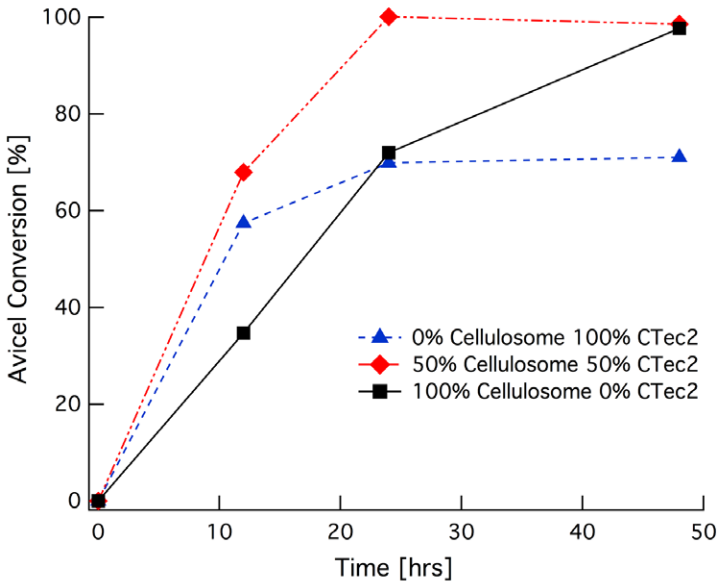


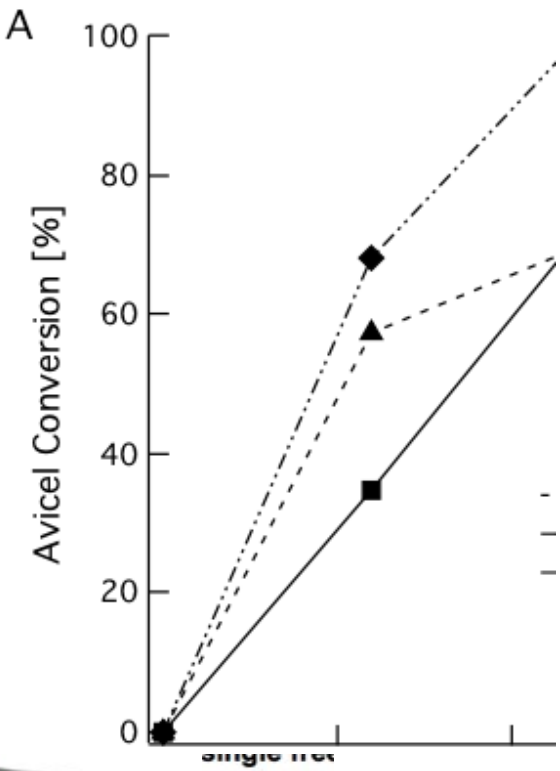


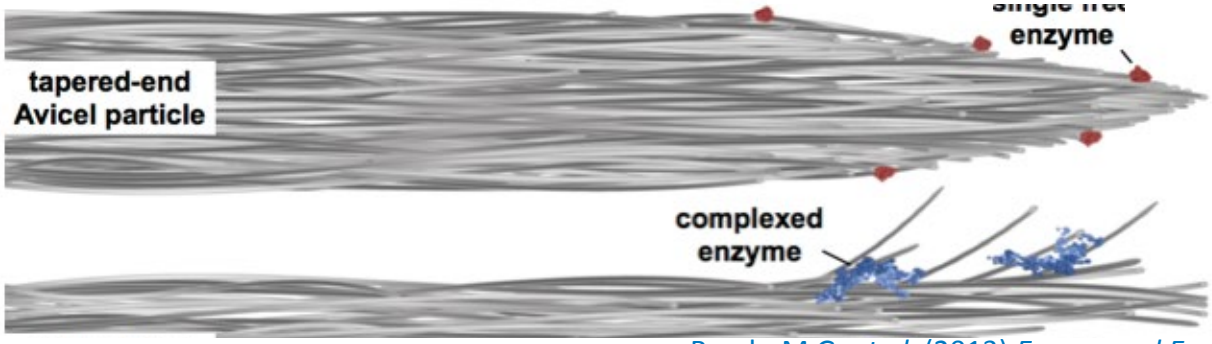
Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

Do Free and complexed enzymes degrade cellulose synergistically?

Avicel conversion at 10 mg/g



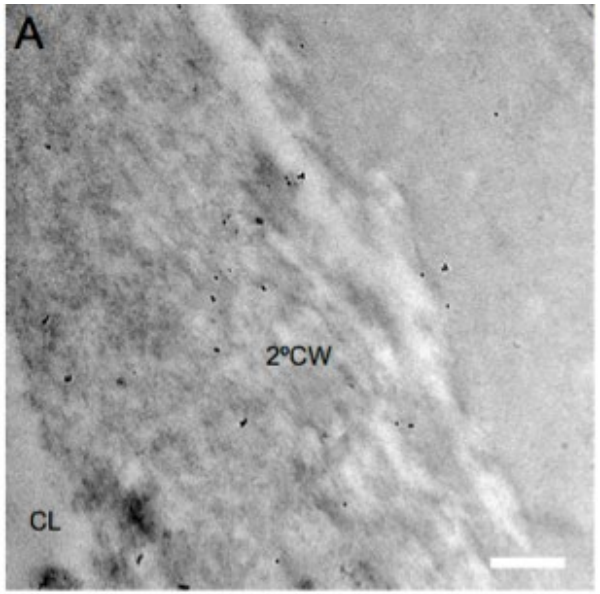




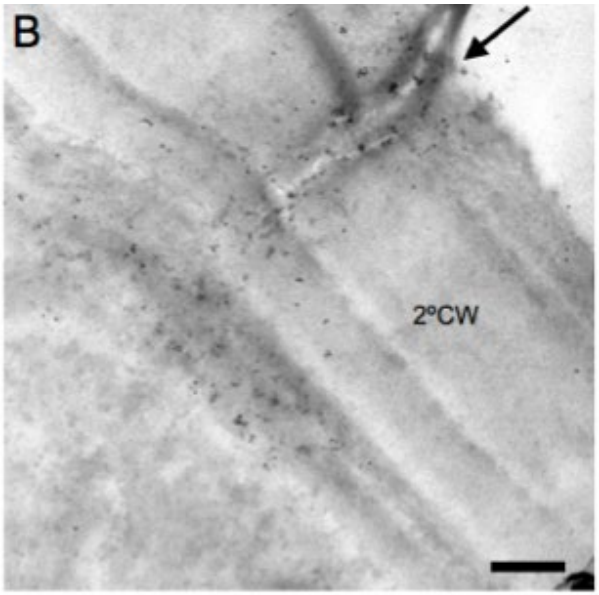
Resch, M.G. *et al.* (2013) *Energy and Environmental Science*

Enzyme Accessibility is Different

"Free" Enzymes



Cellulosomes



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

Possible factors limiting cellulosome activity on DA pretreated biomass

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

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

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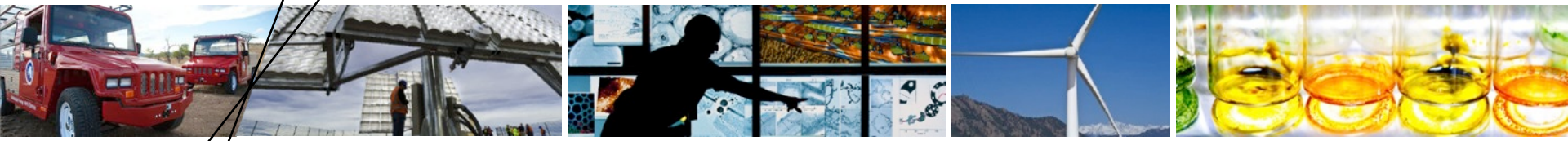
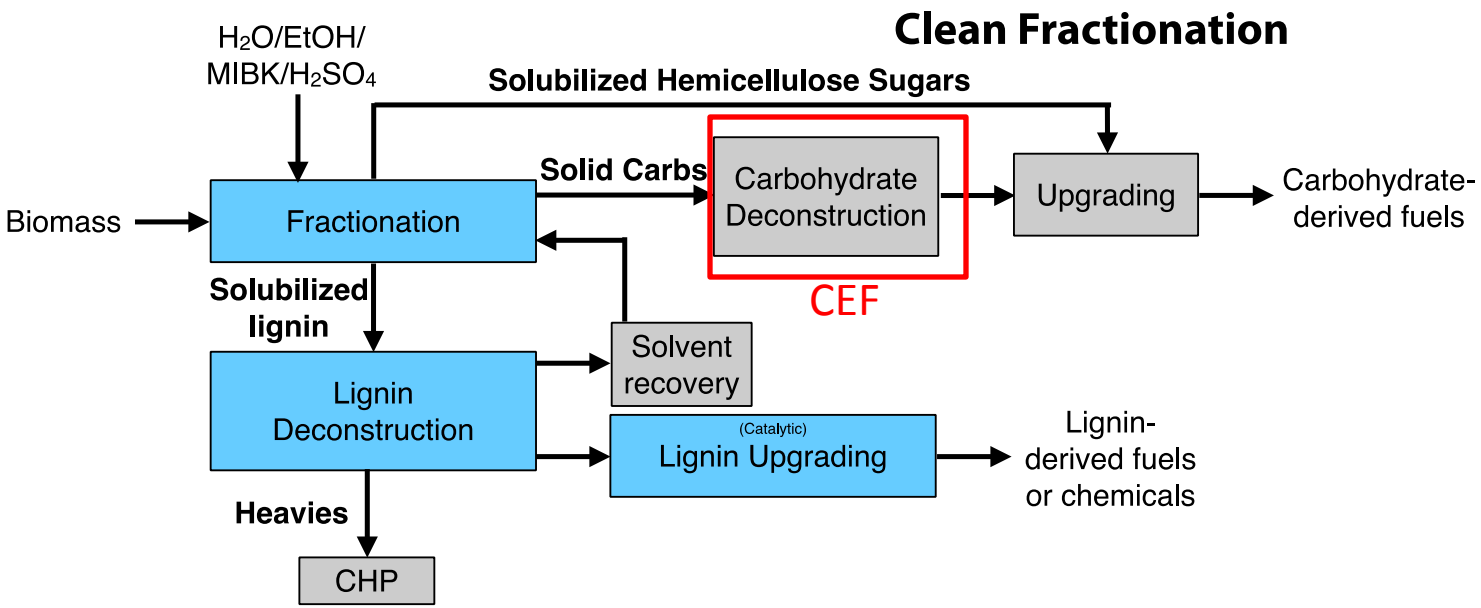


Clean Fractionation Pretreatment (Organosolve)

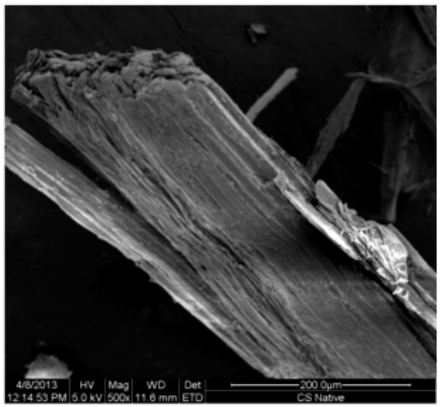


CEF Chemical Composition

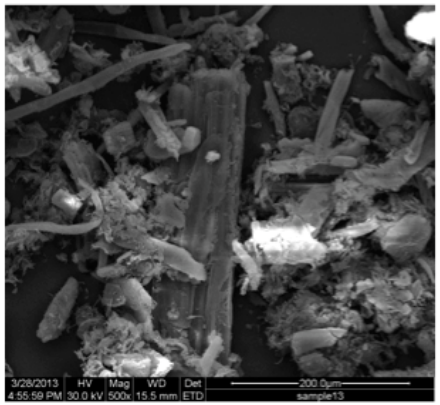
Solvent	Temp (°C)	Acid (mol/L)	Lignin (%)	Glucan (%)	Xylan (%)
MIBK/**Acetone**/H2O	140	0.025	10.63	63.31	17.47
MIBK/**Acetone**/H2O	140	0.05	3.67	82.28	6.56
MIBK/**Acetone**/H2O	140	0.1	3.33	87.19	3.08

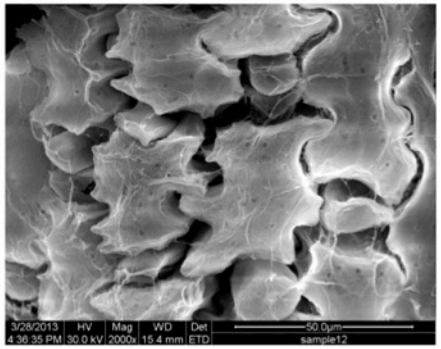
How does Clean Fractionation affect biomass morphology?

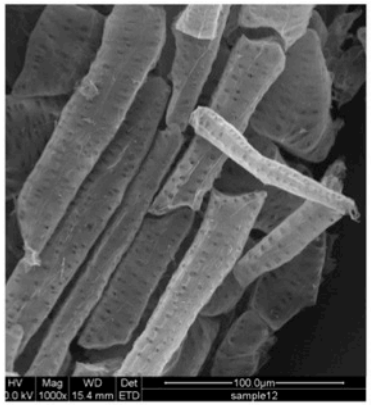
Native Vascular bundle



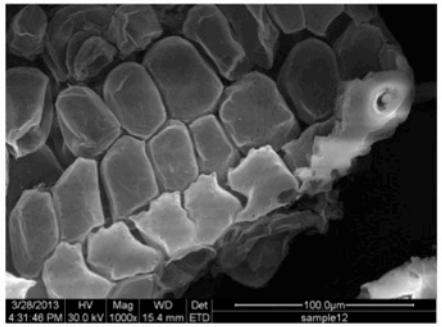
Dilute acid-treated

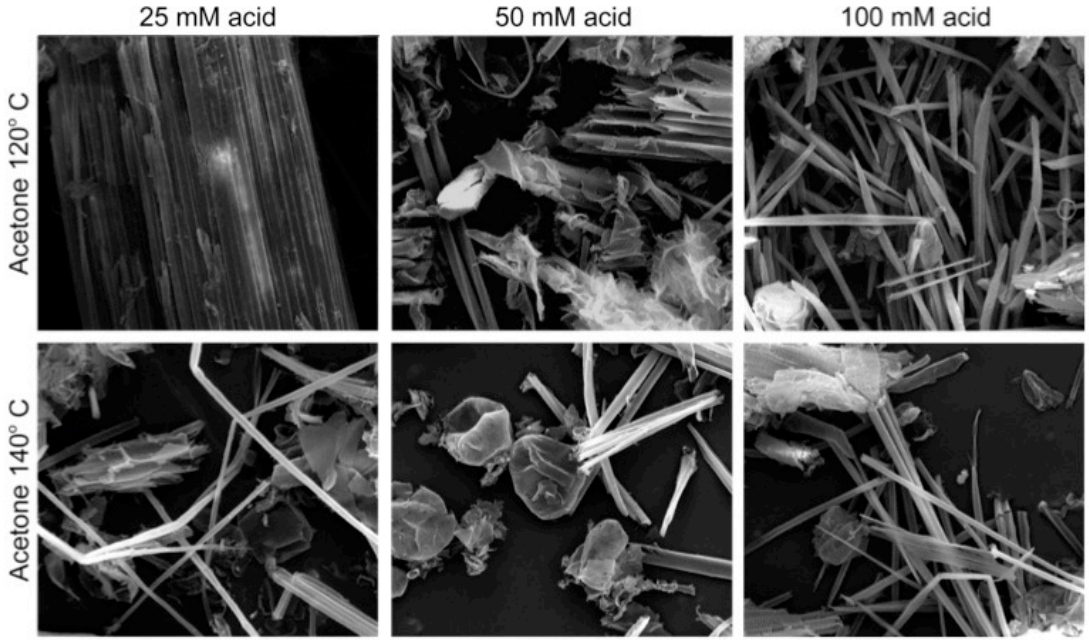






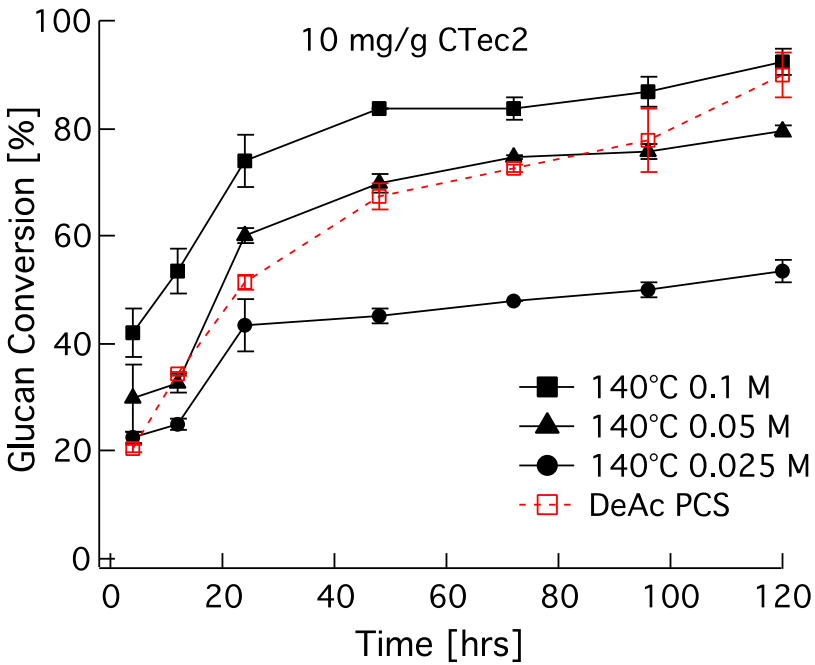
140° C 0.1M

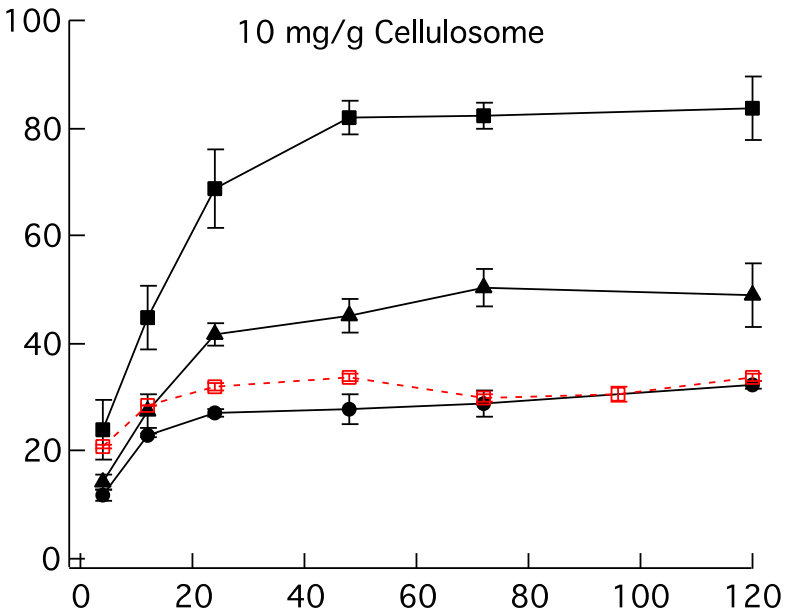




Katahira, R. et *al* (2014) *ACS*

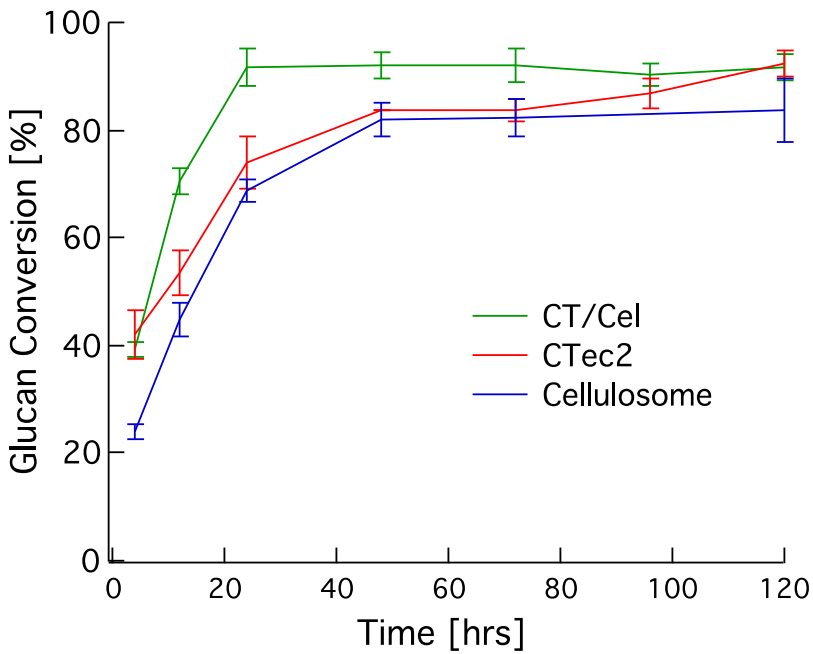
How does Clean Fractionation affect enzyme saccharification?

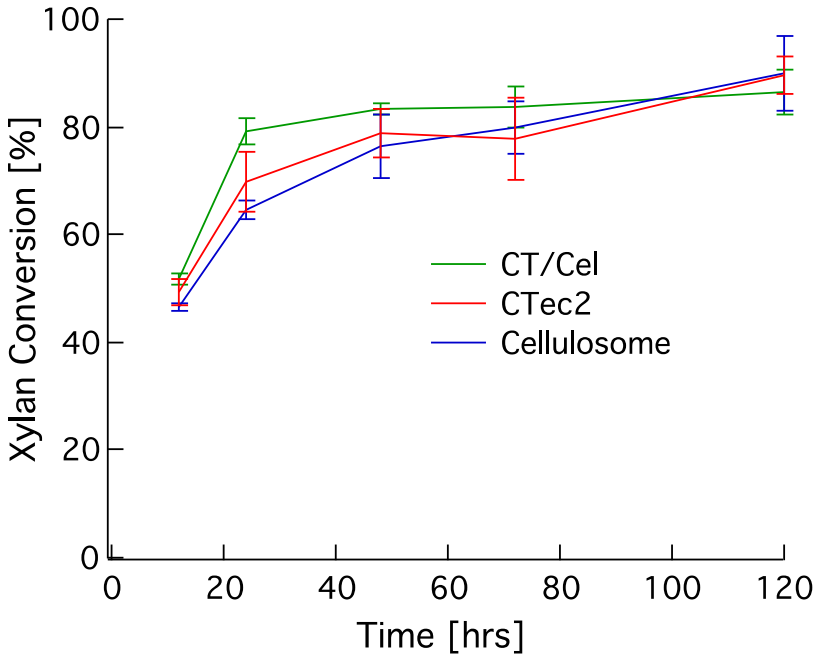




Resch, M. *et al* (2014) *ACS*

Synergistic Combination of CTec2 and Cellulosomes

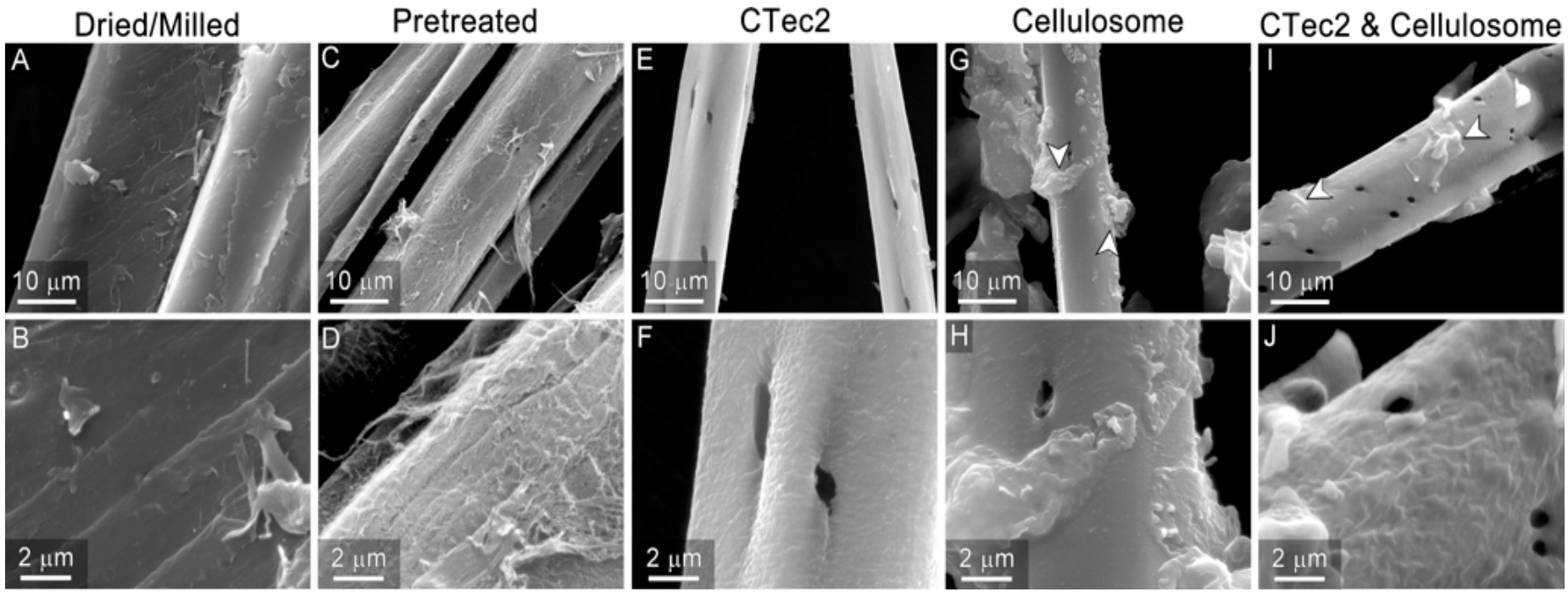




10 mg/g enzyme loading
CT/Cel = 5 mg/g CTec2 + 5 mg/g Cellulosomes

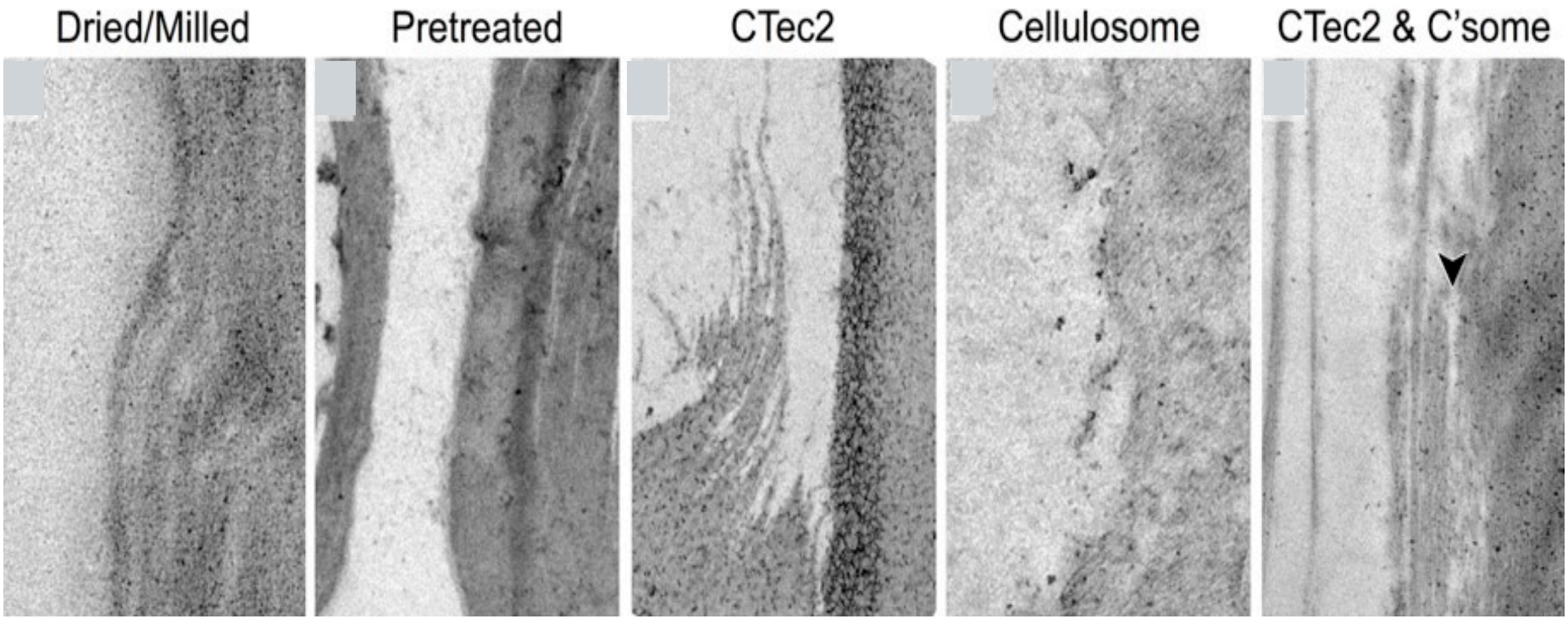
Resch, M. *et al* (2014) *ACS*

SEM of Digested Cellulose Enriched Fraction



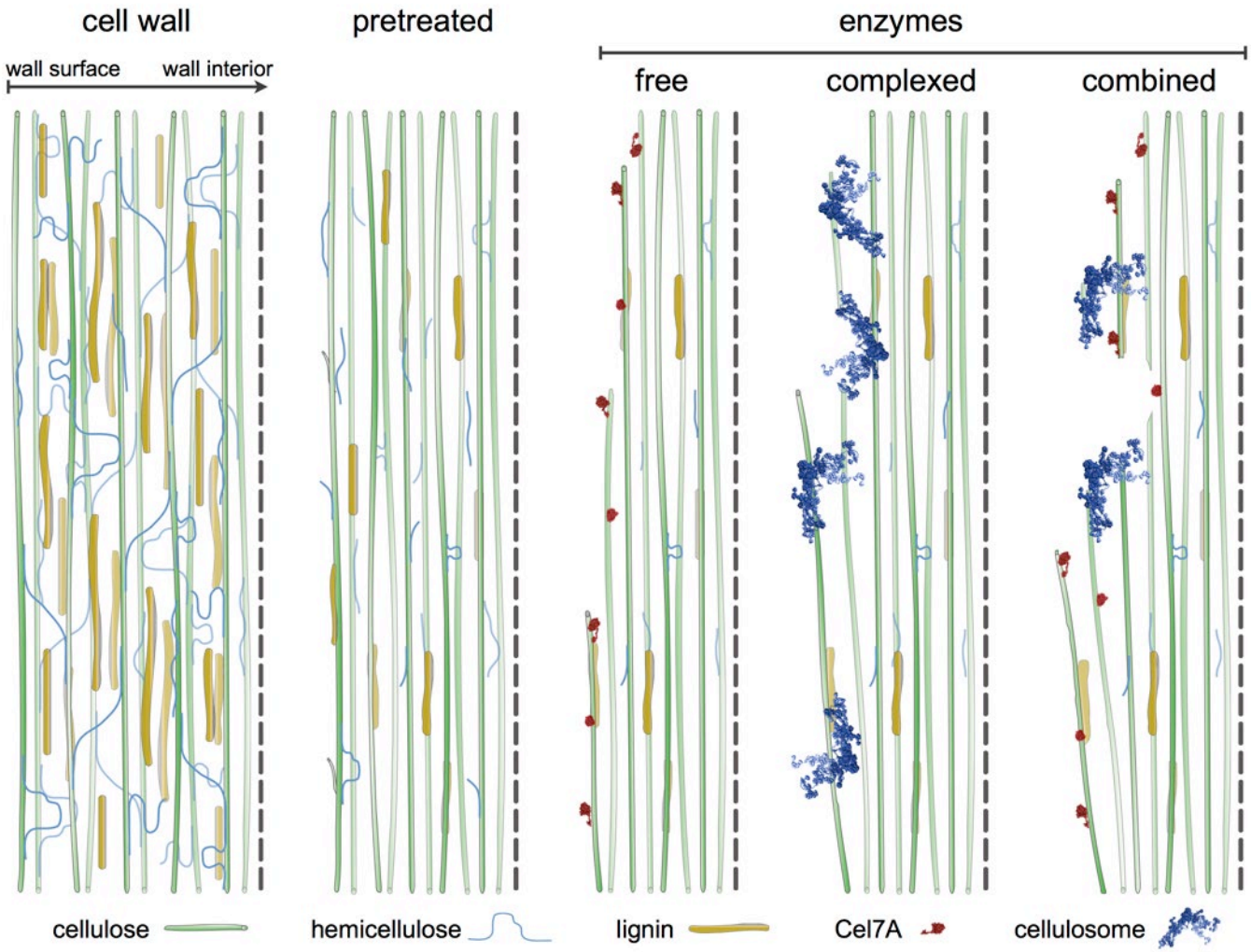
Resch, M. *et al* (2014) *ACS*

TEM of Digested Cellulose Enriched Fraction



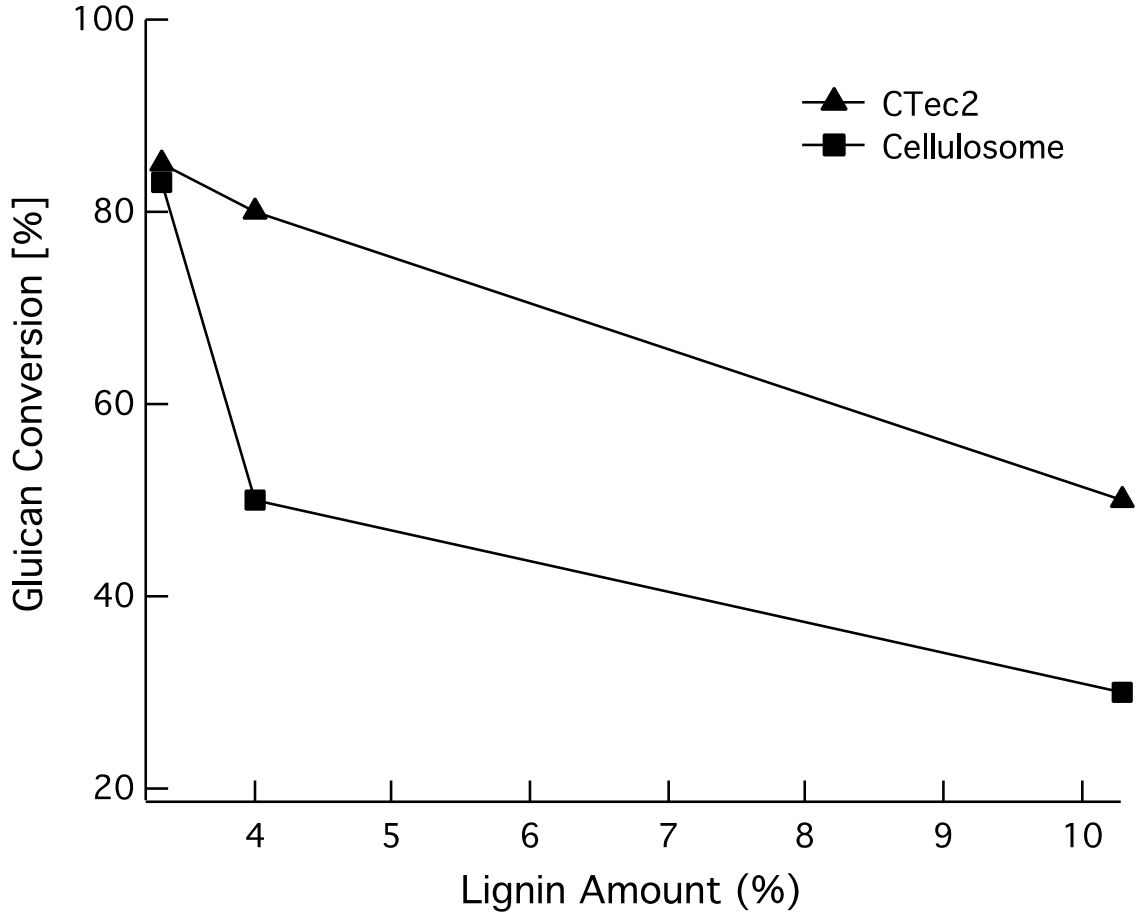
Resch, M. *et al* (2014) *ACS*

Model of Enzymatic Deconstruction Mechanisms



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

Affect of PT Severity on Enzymatic Saccharification



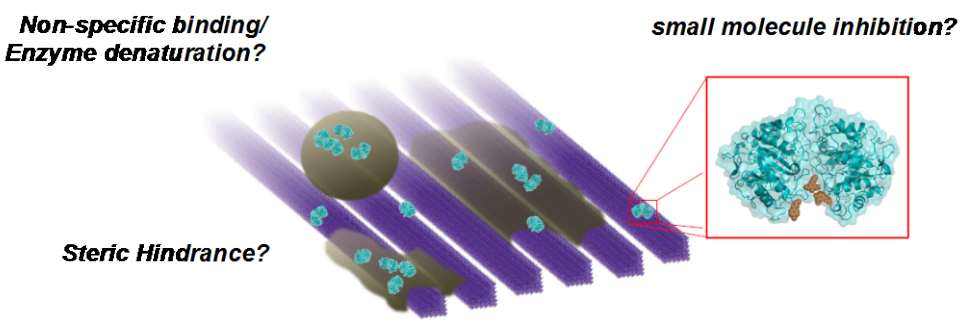
10 mg/g enzyme loading,
72 hours

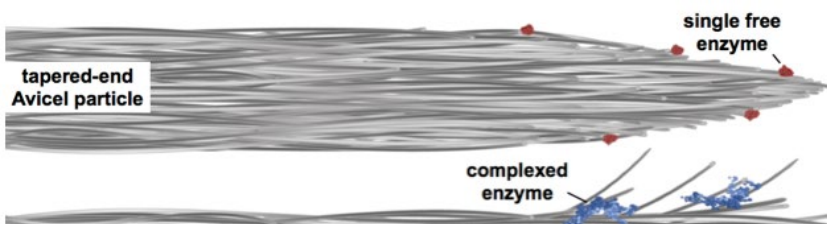
Resch, M. *et al* (in review)

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

BESC BioEnergy Science Center

Biomass Technologies Office (BETO)

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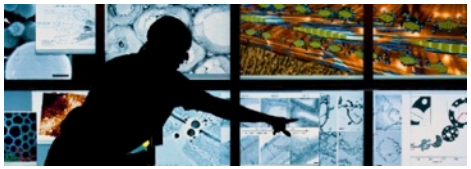

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