NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Harnessing Industrial Biotechnology to Produce Liquid Biofuels from Cellulosics – Focus on Enzymatic Hydrolysis

BBEST Conference

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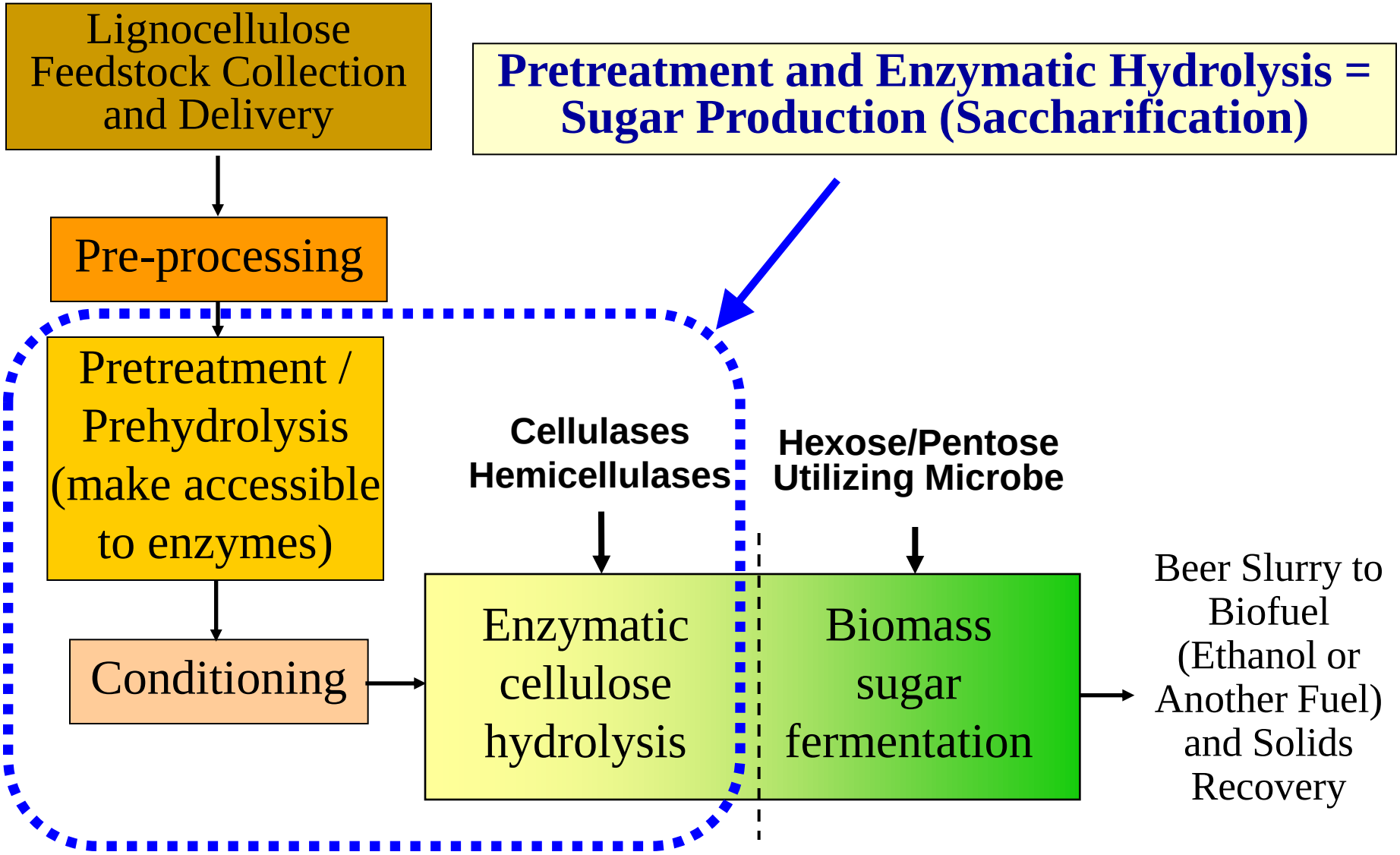
Campos do Jordão, São Paulo, Brazil

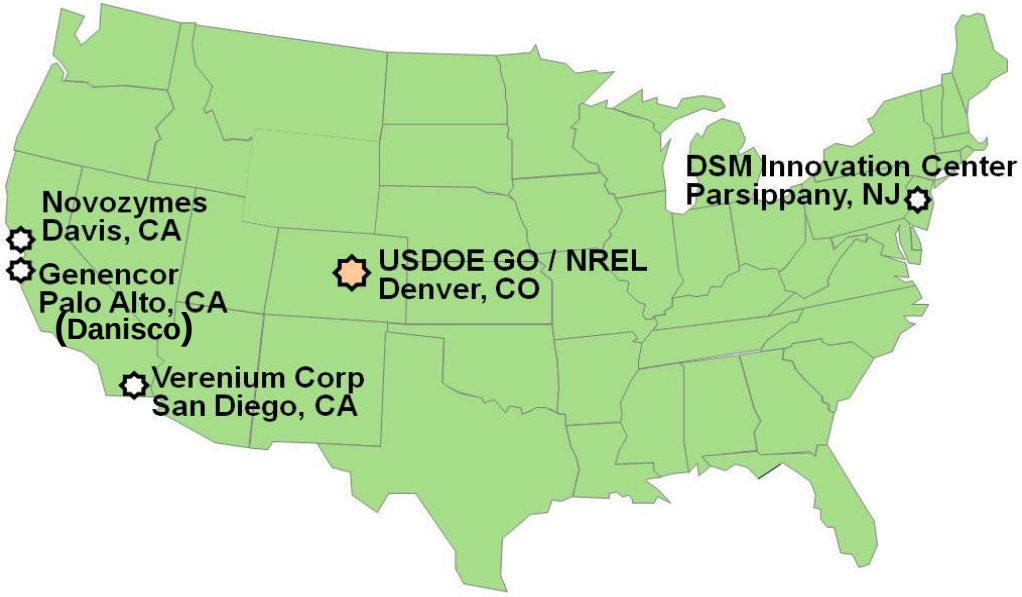
August 18, 2011

Economic Sugar Production Remains Biggest Challenge for BC Route



USDOE's Enzyme Improvement Projects

- Goal: Decrease enzyme cost.
- Approach: Cost-shared enzyme improvement projects awarded to DSM, Genencor (Danisco), Novozymes and Verenium (BP) through USDOE FOA DE-PS36-07GO97034 "Development of Saccharifying Enzymes for Commercial Use."
- Awards (on-going) require NREL to test and publish in a non-attributed manner the performance of all companies' benchmark enzymes on NREL prepared dilute acid pretreated corn stover (PCS).
- Testing completed, manuscript accepted for publication in *Biotechnology for Biofuels*.



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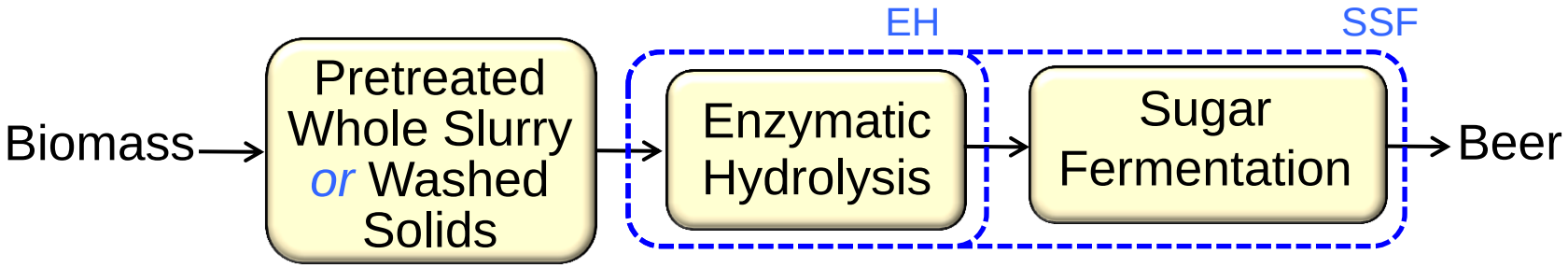
Quantifying Enzyme Performance

Table B: Enzyme Performance

			Benchmark	Intermediate Target	Final Target
		% Improvement in cost contribution from benchmark			
C_E	Enzyme Cost	$/gal ethanol	$C_E = \frac{E_P E_L}{B_N Y}$		
E_P	Enzyme Price	$/L-product			
E_L	Enzyme Loading (7 day residence time)	g protein/ (g cellulose + g xylan entering enzymatic hydrolysis)			
B_N	Enzyme Concentration	g protein/ L-product			
Y	Ethanol Process Yield	gal ethanol/ (g cellulose + g xylan in raw feedstock)	$Y = \frac{(\frac{F_C C_C C_G}{R_C} + \frac{F_H C_H C_X}{R_H})}{F_C + F_H}$		
	Ethanol Process Yield	gal ethanol/ ton biomass			
F_C	*Cellulose Fraction in Raw Feedstock*	wt fraction			
F_H	*Xylan Fraction in Raw Feedstock*	wt fraction			
C_C	*Cellulose Conversion to Glucose (including pretreatment conversion)*	g cellulose converted/ g cellulose total	0.90		
C_H	*Xylan Conversion to Xylose (including pretreatment conversion)*	g xylan converted/ g xylan total	0.90		
C_G	*Glucose Conversion to Ethanol*	g glucose converted/ g glucose total	0.95		
C_X	*Xylose Conversion to Ethanol*	g xylose converted/ g xylose total	0.85		
R_C	*Theoretical Cellulose per gallon ethanol*	g cellulose/ gallon ethanol	5250		
R_H	*Theoretical Xylan per gallon ethanol*	g xylan/gallon ethanol	5135		

Comparative Testing Methodology

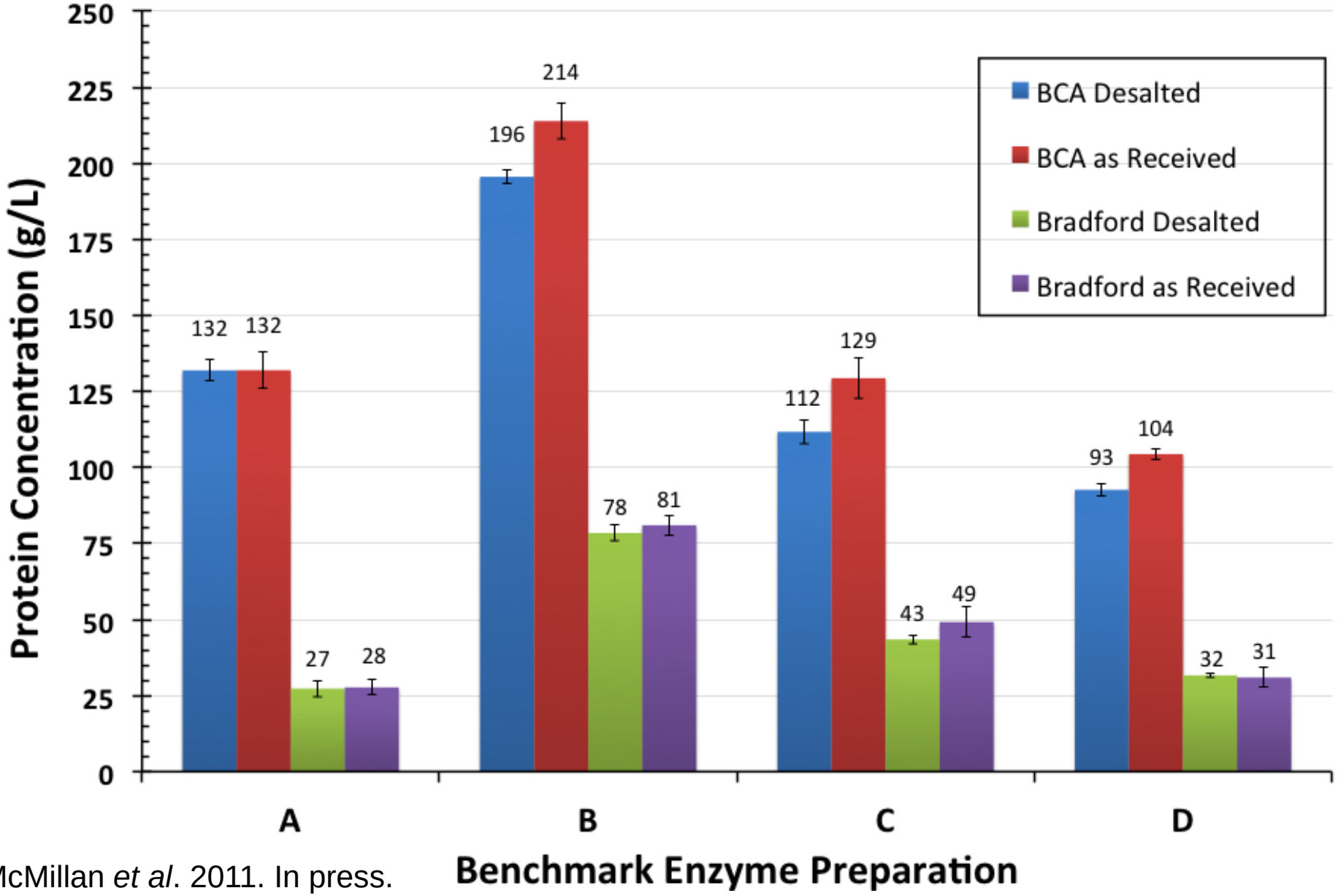
- All tests performed to mimic NREL's 2012 biochemical design case
  - 20% w/w PCS whole slurry ≡ 11% w/w insoluble solids ≡ 6.5% w/w cellulose
- Two conversion modes examined
  - EH: 7 days at pH 5 and 50° C using PCS whole slurry *or* washed PCS solids
  - SSF: 7 days at pH 5 and 37° C *using D5A yeast* using washed PCS solids



- Assessed glucose production dosage response at ≥ 3 enzyme loadings (in duplicate/triplicate), quantifying protein by BCA *and* Bradford assays
  - Shake flask method followed NREL Laboratory Analytical Procedure, "SSF Experimental Protocols − Lignocellulosic Biomass Hydrolysis and Fermentation" *http://www.nrel.gov/biomass/analytical_procedures.html#lap-009*
  - Calculated cellulose conversion yield from (net) glucose or ethanol produced

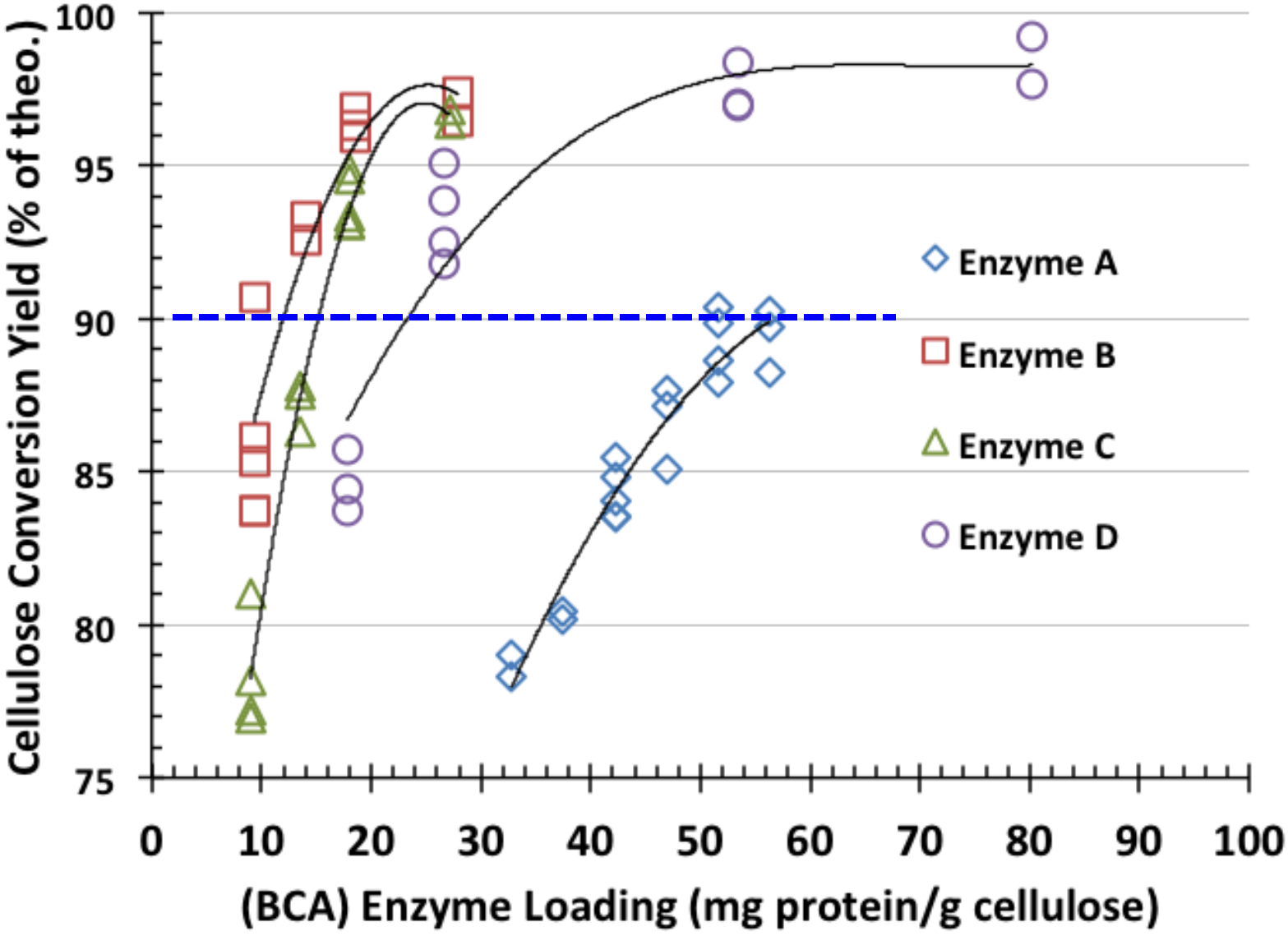
EH = Enzymatic Hydrolysis; SSF = Simultaneous Saccharification and Fermentation

Protein Concentration Results



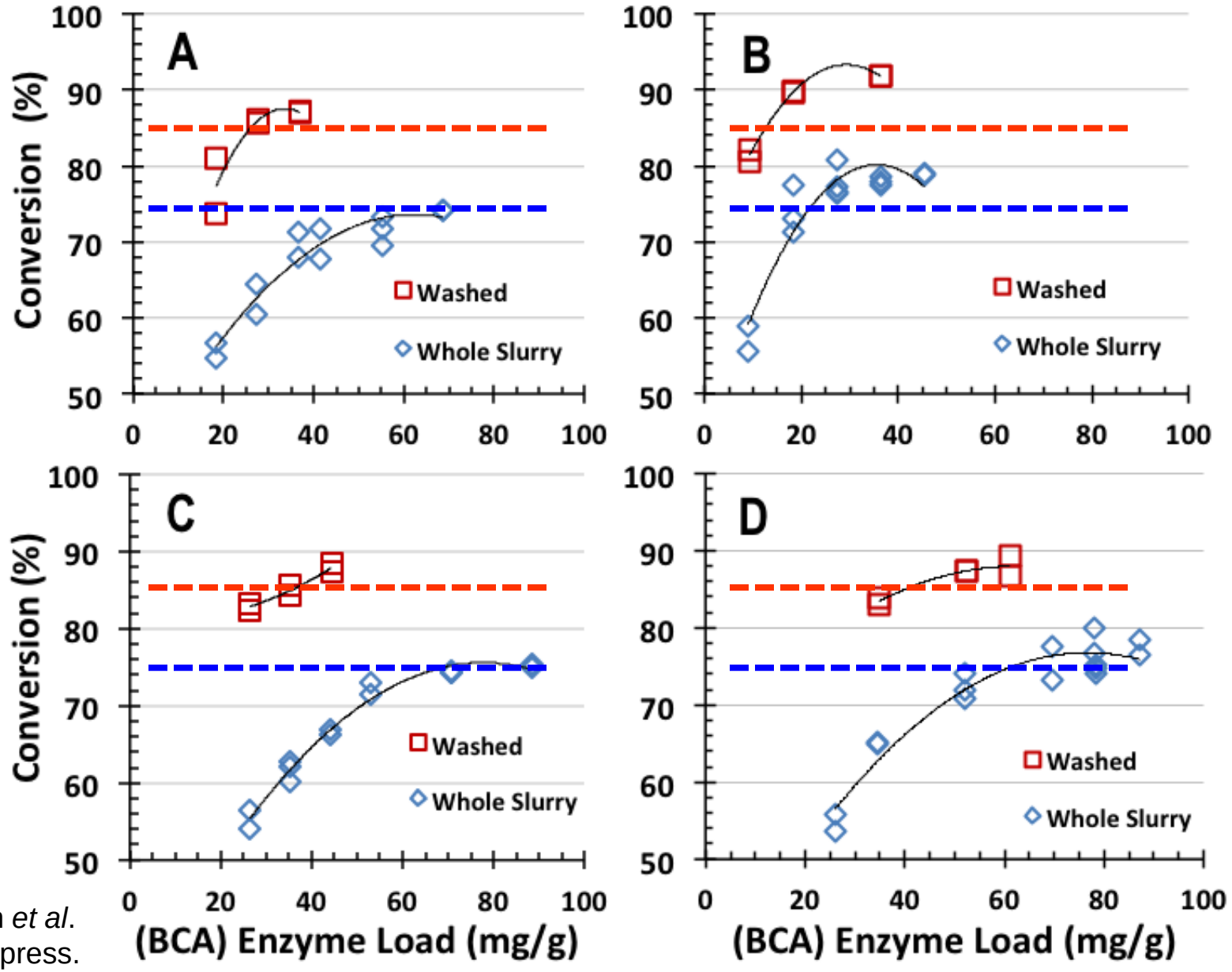
McMillan *et al*. 2011. In press.

Comparative Performance in SSF



McMillan *et al*. 2011. *Biotechnology for Biofuels*. In press.

Comparative Performance in EH



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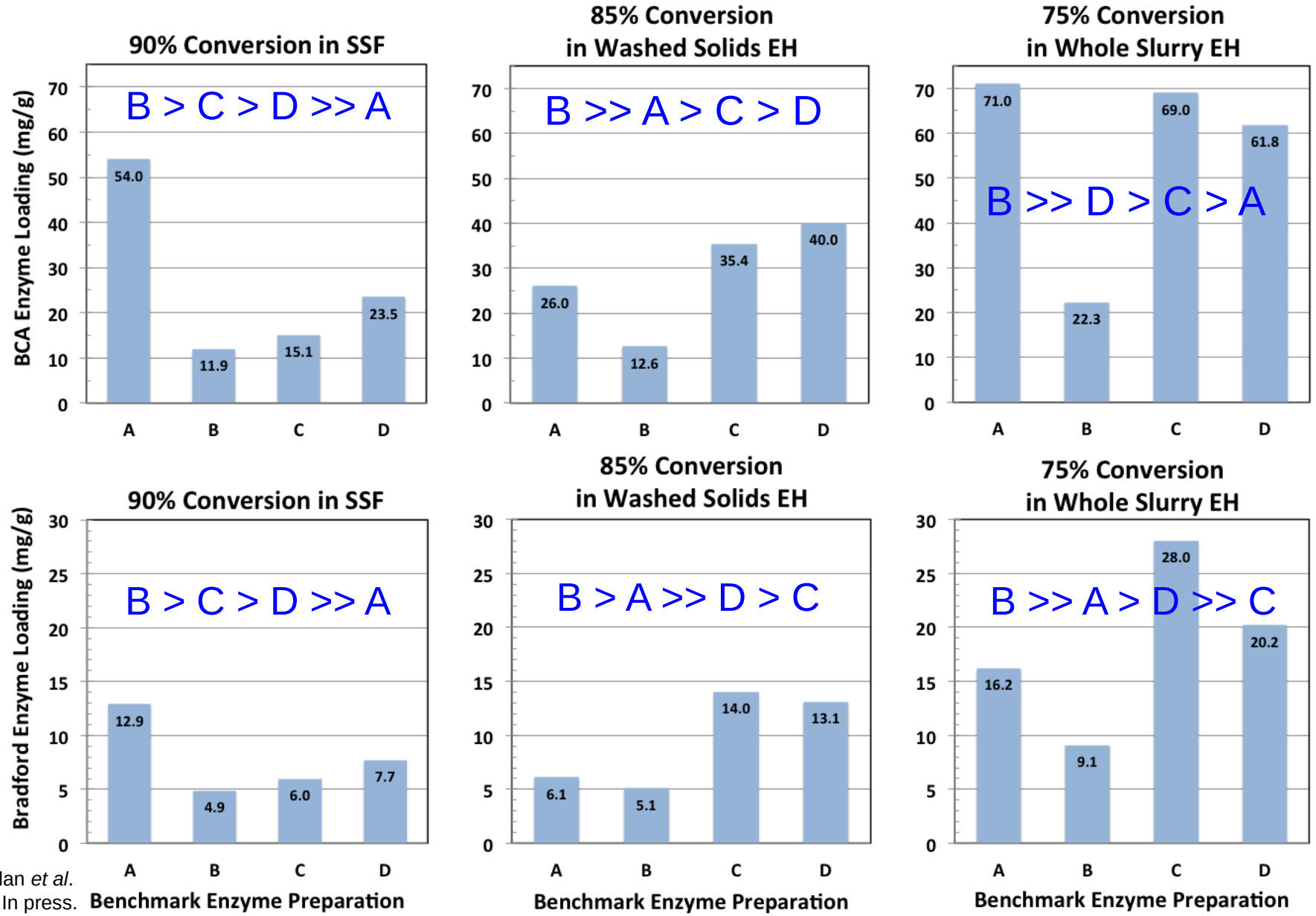
Overall Performance Comparison



McMillan *et al*. 2011. In press.

Comparative Testing Summary

- Apparent protein concentrations in the benchmark preparations were 2.5 to 5-fold lower using the Bradford assay compared to the BCA assay.
- Obtained cellulose to glucose yields were ≥ 90% in SSF, and ≥ 85% in EH on washed solids and ≥ 75% in PCS whole slurry.
- “Enzyme B” performed the best in all hydrolysis modes.
- The test conditions may not have been optimal for some (or all) of the enzymes.

Conclusions

- All benchmark enzymes effectively saccharified PCS cellulose to glucose albeit with significantly different dosage response curves.
- Higher conversion yields on washed solids than whole slurry show that overcoming sugar feedback inhibition remains a challenge.
- The dependence of relative and absolute performance levels on which hydrolysis mode and protein assay are used as bases for comparison (and the inability to correlate performance with activity levels) illustrates how important knowledge gaps remain.
- Wide variations in apparent enzyme protein concentrations using the different assays motivate the need to establish better consensus methods for quantifying enzyme protein.
- These results provide a quantitative baseline against which other enzyme systems can be assessed; better performing systems are likely available today since “starting point” enzymes were tested.

Acknowledgments

Funding

- The DOE EERE Office of the Biomass Program

Partners

- DOE Golden Field Office (Bryna Berendzen *et al*.)
- Project teams at Verenium, Novozymes, DSM and Danisco

Thanks for Your Attention!
Questions?