

# Multiple levers used to overcome recalcitrance of biomass

## Background

Biomass recalcitrance is a barrier to biological processing to fuels and chemicals, but the relative impacts of physical, chemical and genetic interventions to improve biomass processing have yet to be evaluated systematically.

## Approach

We tested total carbohydrate solubilization (TCS) with combinations of

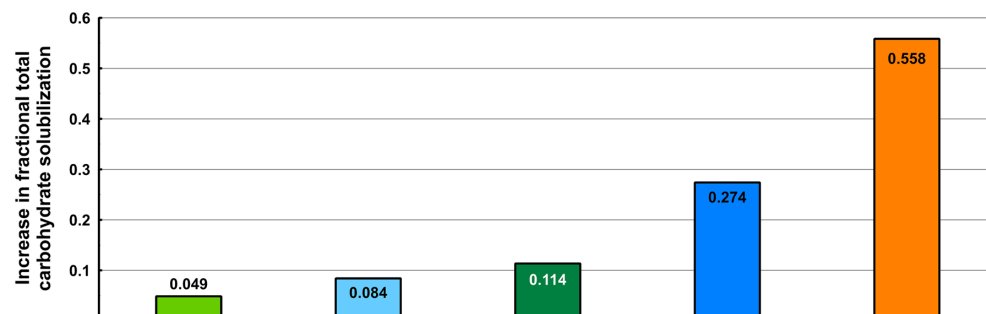
- Three biocatalysts (*Clostridium thermocellum*, *Caldicellulosiruptor bescii*, and commercial Novozyme enzymes),
- Feedstocks of varying recalcitrance in poplar and switchgrass, and
- Biological process augmentation of biological attack using mechanical cotreatment or cosolvent-enhanced lignocellulosic fractionation (CELf) pretreatment.

## Outcomes

- Recalcitrance was determined by both the feedstock and the biocatalyst. TCS with *C. thermocellum* was higher than the other biocatalysts.
- Increased TCS was observed for 8 of the 9 combinations of feedstocks and biocatalysts.
- Both CELf pretreatment and cotreatment via continuous ball milling enabled TCS of >90%.

## Significance

- Some form of non-biological augmentation will be needed to achieve high TCS for most feedstocks. However, this need not necessarily involve thermochemical pretreatment, nor must this occur prior to biological conversion.
- The relative magnitude of TCS increase was augmentation > biocatalyst choice > plant choice > plant modification > plant natural variants. Process augmentation can overcome most plant feedstock variation in recalcitrance.



Recalcitrance lever	Feedstock Natural variants	Feedstock modification	Feedstock choice	Biocatalyst choice	Augmentation
Feedstock(s) (SG = Switchgrass)	natural variants <i>Populus</i> GW9947 vs. BESC97*	3 lines of transgenic SG (+) vs. 3 lines of nontransgenic SG controls (-)	6 lines of SG (+ & -) vs. <i>Populus</i> GW9947 & BESC97*	6 lines of SG (+ & -) and <i>Populus</i> GW9947 & BESC97	COMT SG (+ & -) and <i>Populus</i> GW9947 & BESC97
Biocatalyst(s)	<i>C. thermocellum</i> & fungal cellulase SSF	<i>C. thermocellum</i> & fungal cellulase SSF	<i>C. thermocellum</i> & fungal cellulase SSF	<b><i>C. thermocellum</i> vs. fungal cellulase SSF*</b>	<i>C. thermocellum</i>
Augmentation	-	-	-	-	Cotreatment & CELf vs. unaugmented controls*

Relative impact of five recalcitrance levers on total carbohydrate solubilization. The increase in total carbohydrate solubilization for each lever in bold is calculated with other levers as indicated. The lever 'Augmentation' includes cotreatment and CELf as well as unaugmented plant controls. Solubilization results are after 120 h of incubation with equal initial glucan loading. An asterisk (\*) denotes statistically significant.