

## Electronic Supplementary Information

### Development of an Ammonia Pretreatment that Creates Synergies Between Biorefineries and Advanced Biomass Logistics Models

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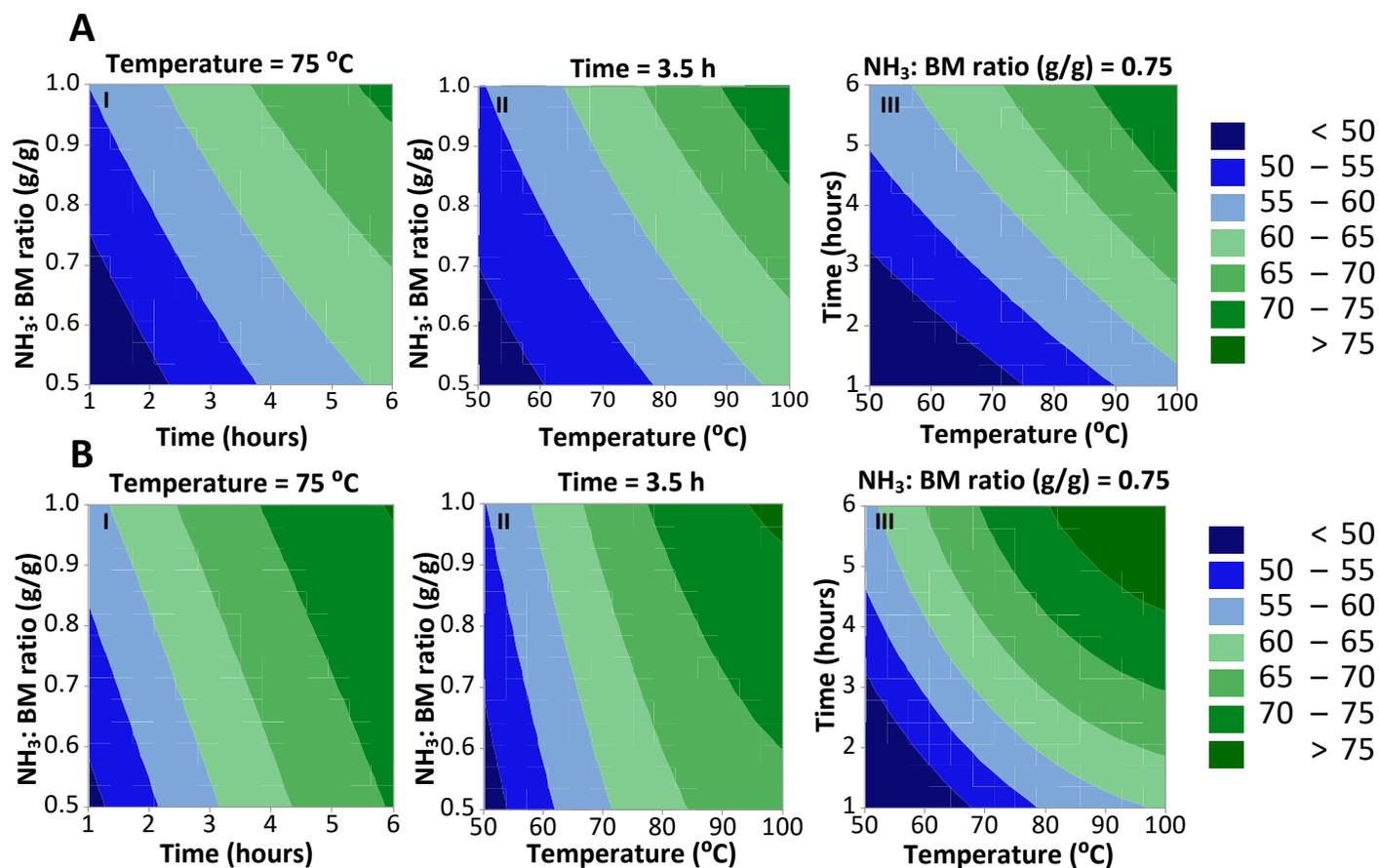


Fig. S1 Contour plots show the influence of pretreatment parameters (temperature, residence time and ammonia: biomass (NH<sub>3</sub>:BM (g/g)) loading on 72h of enzymatic conversion of glucan to glucose (A) and xylan to xylose (B) for pelletized sugarcane bagasse. Enzymatic hydrolysis experiments were carried out with 15mg protein/g glucan and 1% glucan loading (w/w, glucan). The enzymatic cocktail used was composed of 68 wt.% CTec3, 22 wt.% HTec3 and 10 wt.% Multifect Pectinase, on a protein basis, as previously optimized for AFEX-pretreated sugarcane bagasse.<sup>5</sup>

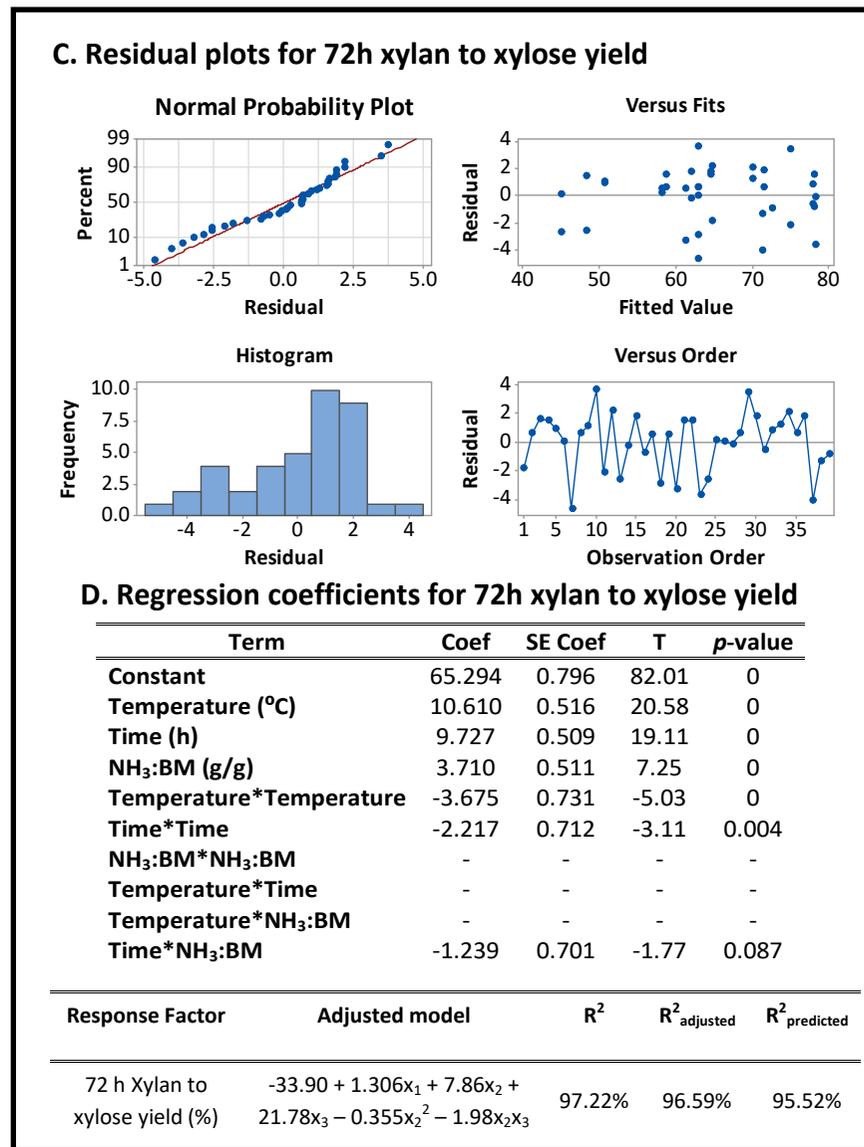
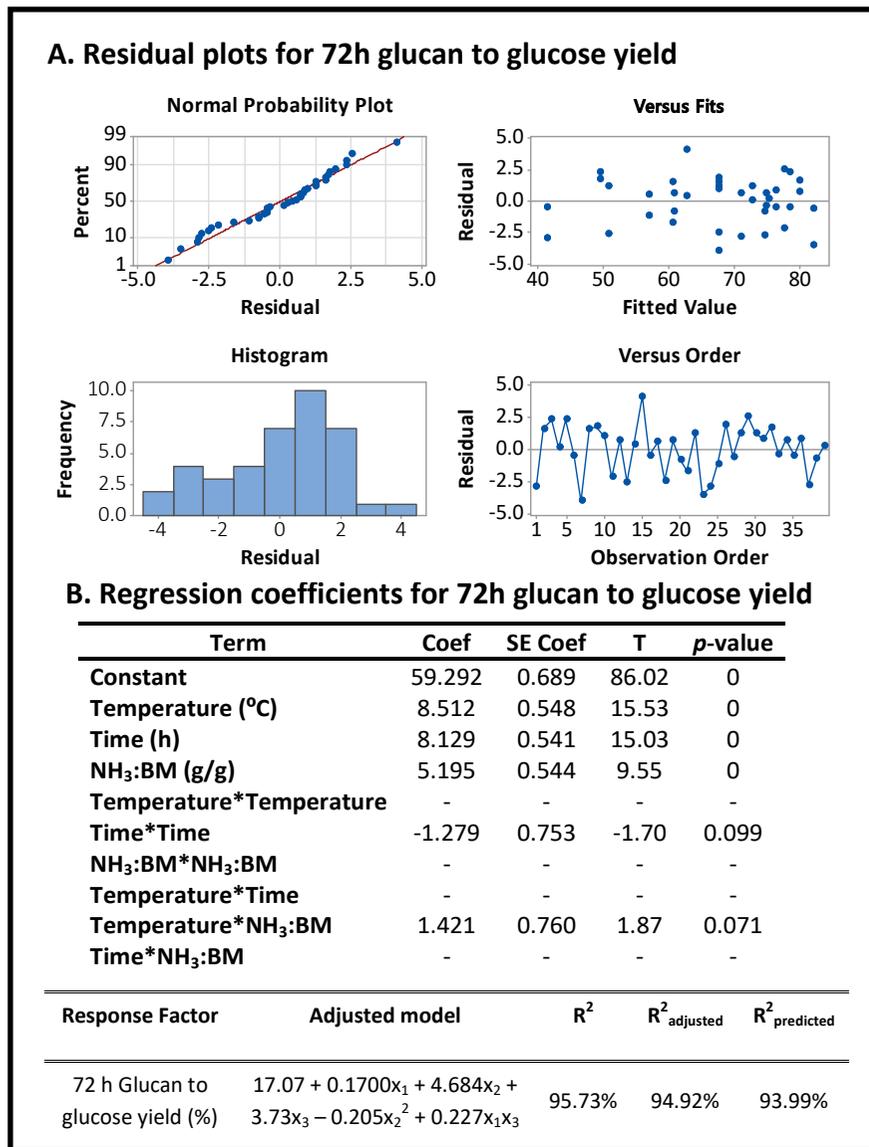


Fig. S2 The statistical residual plots and regression used to validate ANOVA assumptions in evaluating the effect of COBRA pretreatment operational conditions on 72h glucan and xylan to glucose and xylose, respectively.

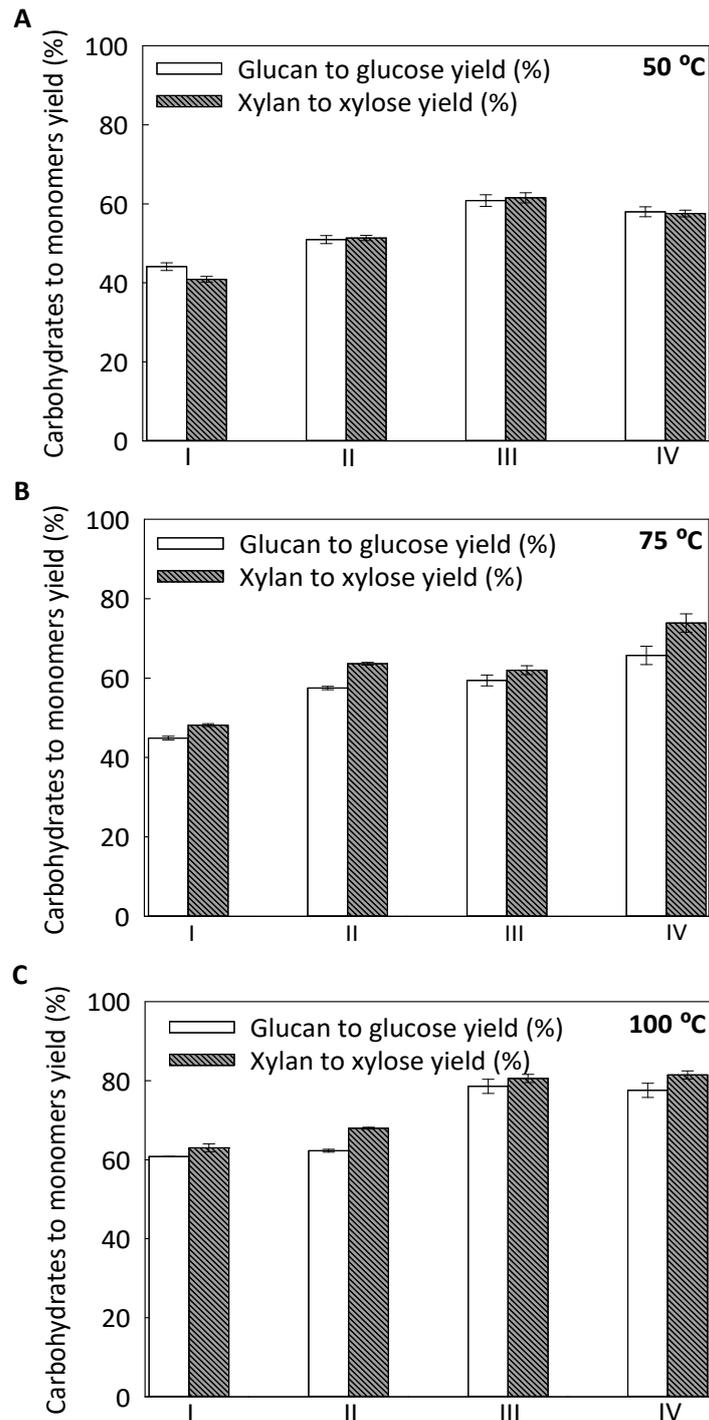


Fig. S3 Effect of COBRA pretreatment conditions on 72 h glucan and xylan conversion. **A** – 50 °C; **B** – 75 °C; **C** – 100 °C; I – 1h and 0.75:1 [NH<sub>3</sub>:BM (g/g)]; II – 3.5 h and 0.5:1 [NH<sub>3</sub>:BM (g/g)]; III – 6 h for 0.75:1 [NH<sub>3</sub>:BM (g/g)]; IV – 3.5h for 1:1 [NH<sub>3</sub>:BM (g/g)]. Enzymatic hydrolysis experiments were performed at 1% glucan loading and 15 mg protein/g glucan enzyme loading using an optimized enzymatic cocktail for AFEX-pretreated sugarcane bagasse as described by Mokomele et al.<sup>5</sup> All COBRA conditions studied were found to affect the release of fermentable sugars during enzymatic hydrolysis. COBRA-pretreated sugarcane bagasse at 75 °C for 3.5 h at 0.75:1 [NH<sub>3</sub>:BM (g/g)] loading allowed up to 55-60 % glucan and xylan

enzymatic hydrolysis into their monomers. Significant improvements in polysaccharide susceptibility to enzymatic hydrolysis were observed at 100 °C, NH<sub>3</sub>:BM loading of 1:1 (g/g) for 3.5 h of reaction time resulting in 78.6 ± 1.8 % glucan and 80.6 ± 1.0 % xylan conversion yields. While higher temperatures have been reported to promote de-esterification reactions, higher ammonia loading promotes the cleavage of ester bonds between the arabinoxylan and lignin as well as the conversion of cellulose CI into CIII increasing the biomass susceptibility to enzymatic attack.

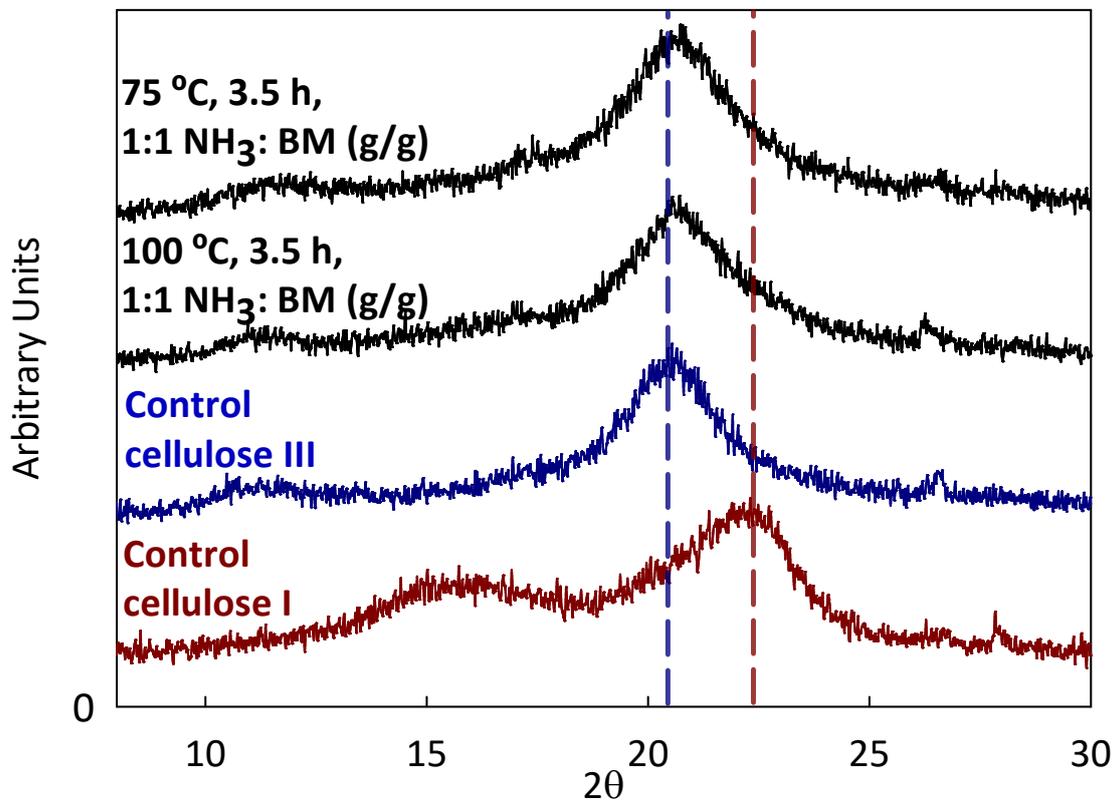


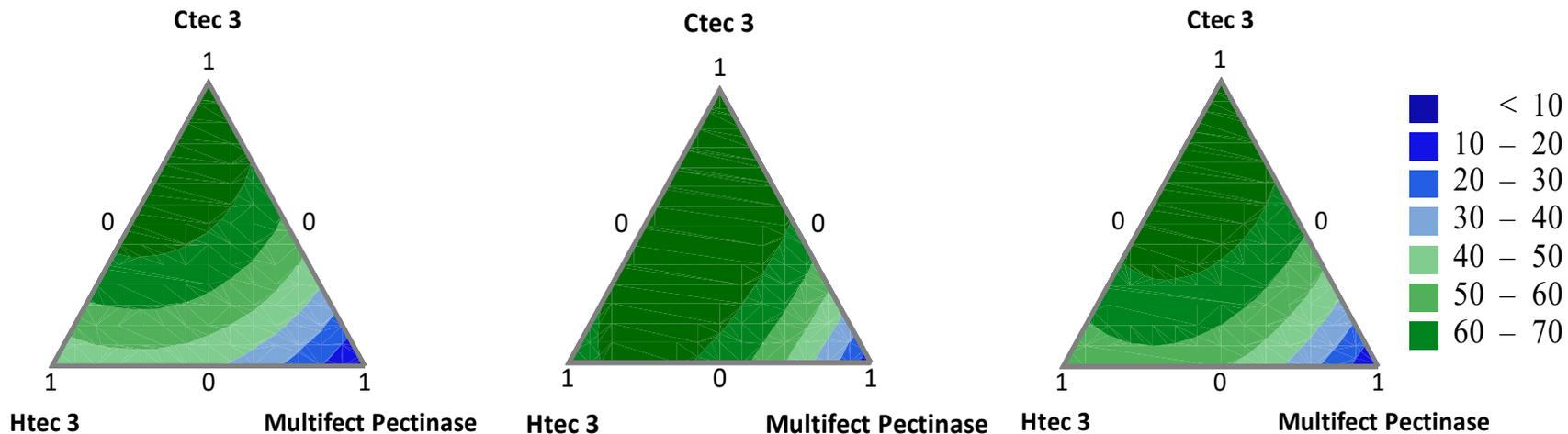
Fig. S4 Effect of COBRA temperature on sugarcane bagasse crystallinity. COBRA pretreatment temperature does not affect the formation of CIII, as it is formed at both 75 °C and 100 °C using 1:1 [NH<sub>3</sub>: BM (g/g)] for 3.5 h.

**Glucan to glucose yield (%)**

**Xylan to xylose yield (%)**

**Combined sugar yield (%)**

**A**



**B**

Response factor	Adjusted Model	Cocktail Mixture (x <sub>1</sub> :x <sub>2</sub> :x <sub>3</sub> )	Glucose yield (%)	Xylose yield (%)	Combined sugar yield (%)
Combined sugar yield	74.13X <sub>1</sub> +39.76X <sub>2</sub> +6.37X <sub>3</sub> +69.19X <sub>1</sub> X <sub>2</sub> +72.37X <sub>1</sub> X <sub>3</sub> +73.71X <sub>2</sub> X <sub>3</sub>	72%:23%:6%	78.0	74.8	77.1

Fig. S5 Contour plots showing the effect of combinations of commercial enzyme mixtures consisting of Cellic CTec3, Cellic HTec3, and Multifect Pectinase on the glucan to glucose yield, xylan to xylose yield and combined sugar yield during 1 % glucan loading enzymatic hydrolysis performed at 50 °C, 250 rpm for 72 h (A). Composition of the optimized enzymatic cocktail on wt.% basis, and the corresponding glucose, xylose and combined sugar yield (B).



Fig. S6 Example of sugarcane bagasse pellets.

Table S1. Summary of *Saccharomyces cerevisiae* 424A (LNH-ST) fermentation performance in hydrolysates produced from sugarcane bagasse subjected to various pretreatments and operational conditions. The enzymatic hydrolysates from COBRA, COBRA – LE and EA were produced with 6 % glucan loading (w/w, glucan), 15 mg of protein/ g glucan for 96h of hydrolysis time, whilst AFEX and StEx-derived liquors were produced with 25 mg protein/ g glucan for 96 h. Prior to fermentation, all enzymatic liquid streams were supplemented with 0.25 % (w/w) of corn steep liquor. Fermentations were carried out with an initial inoculum concentration of 1.072 g/L at 30 °C, pH 5.5, and a shaking speed of 150 rpm for 120 h.

Pretreatment	Operational conditions	Initial concentration		Consumption (%)		Metabolic Yield (%)	$Y_{p/s}$ (g <sub>EtOH</sub> /g <sub>sugar</sub> )	EtOH		Process Yield (%)
		n		Glu	Xyl			Concentration (g/L)	Yield (kg/ 100 kg SCB)	
		Glu (g/L)	Xyl (g/L)							
COBRA	100 °C, 3.5 h, 1:1 [NH <sub>3</sub> :BM (g/g)]	57.8	36.9	98.7 ± 0.0	83.5 ± 0.5	86.8 ± 0.4	0.443	38.9	25.5 ± 0.6	69
	75 °C, 4 h, 1:1 [NH <sub>3</sub> :BM (g/g)]	57.5	38.1	98.7 ± 0.1	81.7 ± 0.5	84.3 ± 0.4	0.430	37.8	24.8 ± 0.7	67
	75 °C, 4 h, 0.75:1 [NH <sub>3</sub> :BM (g/g)]	53.7	36.6	98.7 ± 0.1	85.8 ± 2.0	89.9 ± 0.9	0.459	38.7	25.4 ± 1.1	68

COBRA-LE	100 °C, 3.5 h, 1:1 [NH <sub>3</sub> :BM (g/g)]	60.9	36.2	99.0 ± 0.1	89.5 ± 0.4	97.5 ± 1.8	0.497	46.1	26.5 ± 0.1	72
	75 °C, 4 h, 1:1 [NH <sub>3</sub> :BM (g/g)]	58.5	37.3	98.9 ± 0.1	84.3 ± 0.1	96.6 ± 0.3	0.493	44.0	25.4 ± 0.4	69
EA	120 °C, 0.5 h, 6:1 [NH <sub>3</sub> :BM (g/g)]	54.8	36.3	99.2 ± 0.0	93.6 ± 1.0	91.2 ± 1.0	0.465	41.1	25.1 ± 0.4	68
AFEX*	140 °C, 1 h, 1:1 NH <sub>3</sub> :BM (g/g)	59.0	37.01	100	96	91.7	0.461	44.2	25.6	69
StEx*	200 °C, 0.1 h	69.7	26.1	98	37	87.0	0.362	34.6	16.2	44

ETOH – Ethanol; Glu – Glucose; Xyl – Xylose; AFEX – Ammonia Fiber Expansion; StEx – Steam-Explosion; \*The ethanol yields from AFEX and StEx were obtained by Mokomele et al.<sup>5</sup> Metabolic yield was calculated based on glucose and xylose consumed relative to theoretical yield (0.51 g per g of glucose or xylose g consumed during fermentation);  $Y_{p/s}$  – Ethanol yield was determined based on ethanol produced (g) per total sugar (glucose + xylose) prior fermentation (g); Ethanol yield – Ethanol yield per 100 kg of untreated sugarcane bagasse input; Process ethanol yield was calculated based on sugar yield from enzymatic hydrolysis, sugar consumption and metabolic yield during fermentation, on the bases of 100 kg of untreated sugarcane bagasse input.

Table S2 – Optimized lignocellulosic mega-biorefinery locations in the state of São Paulo, Brazil, their respective SCB processing capacity and information about the average delivered SCB price for each mega-biorefinery in the form of bales and pellets.

Cluster	Mega-Biorefinery Location	Mega-Biorefinery Capacity, Mg SCB/Day	Average Delivered SCB Biomass Price, \$/Mg SCB		One-Way Average Transportation Distance*, km/Mg SCB
			Bales	Pellets	
0	São Domingos	20,000	62.00	77.86	67.27
1	Virgolino de Oliveira- Unidade José Bonifácio	19,920	67.04	80.13	96.73
2	Biosev - Unidade Vale do Rosário	19,240	61.48	77.63	64.23
3	Alto Alegre - Unidade Floresta	18,990	71.33	82.06	121.80
4	Moreno	19,620	64.18	78.84	80.01
5	Raízen - Unidade da Barra	19,920	65.93	79.63	90.24

\* For transportation cost estimation purposes, the one-way average transportation distance was multiplied by two, to determine the cost of a round-trip.

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