Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Materials

Toward lower cost cellulosic biofuel production using ammonia based pretreatment technologies

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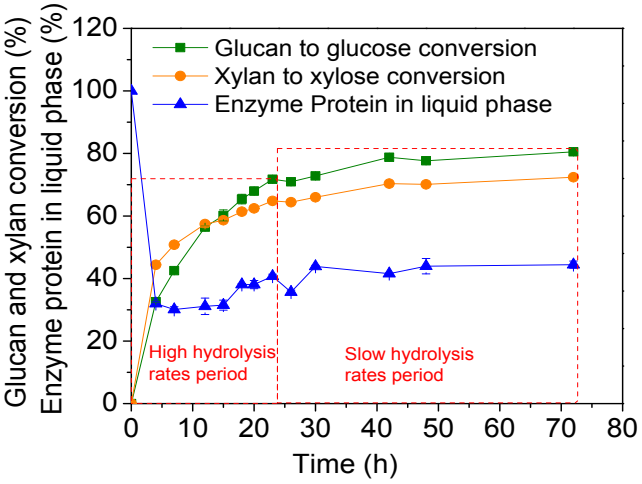
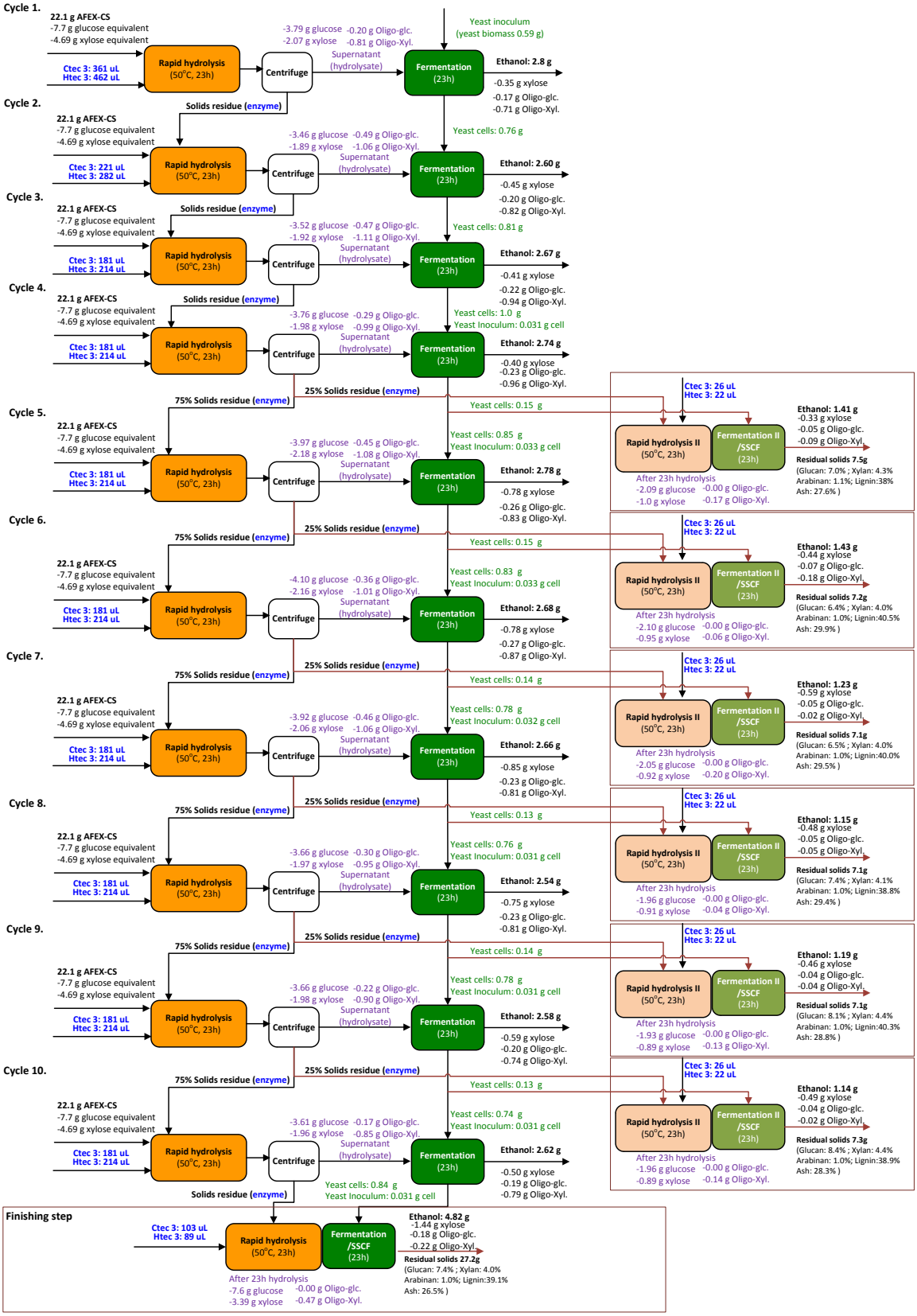
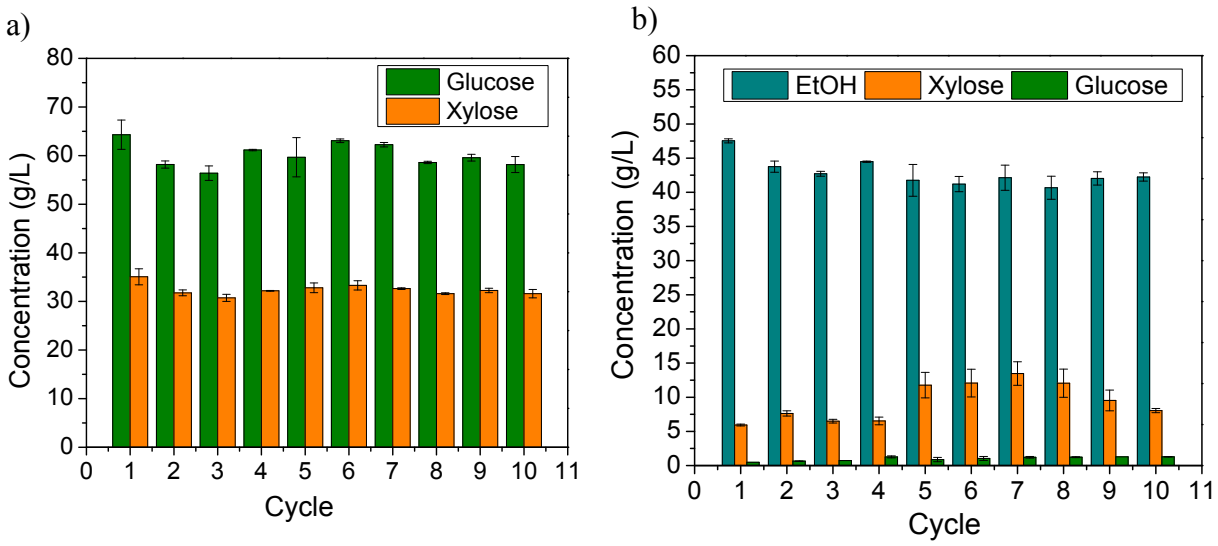
Supplementary Figure 1 Enzymatic hydrolysis profile on AFEX-CS at 7 wt.% glucan loading (22.1 wt.% solid loading) with an enzyme loading of 22 mg protein/g glucan showing rates slow down and enzyme adsorption profile.



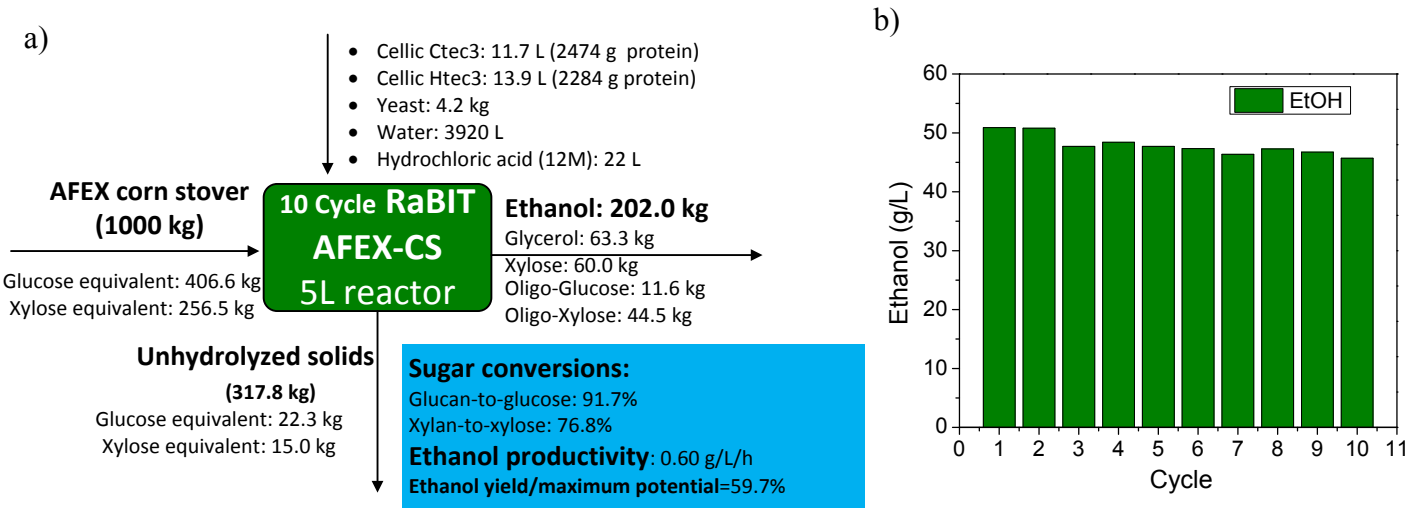
Supplementary Figure 2 Detailed mass balance of the ten-cycle shake flask RaBIT on AFEX-CS. The Fermentation II and the finishing step fermentation were performed in the hydrolysate with residual solids, which means they were actually simultaneous saccharification and co-fermentation (SSCF) and fermentable sugars were still being released during fermentation.



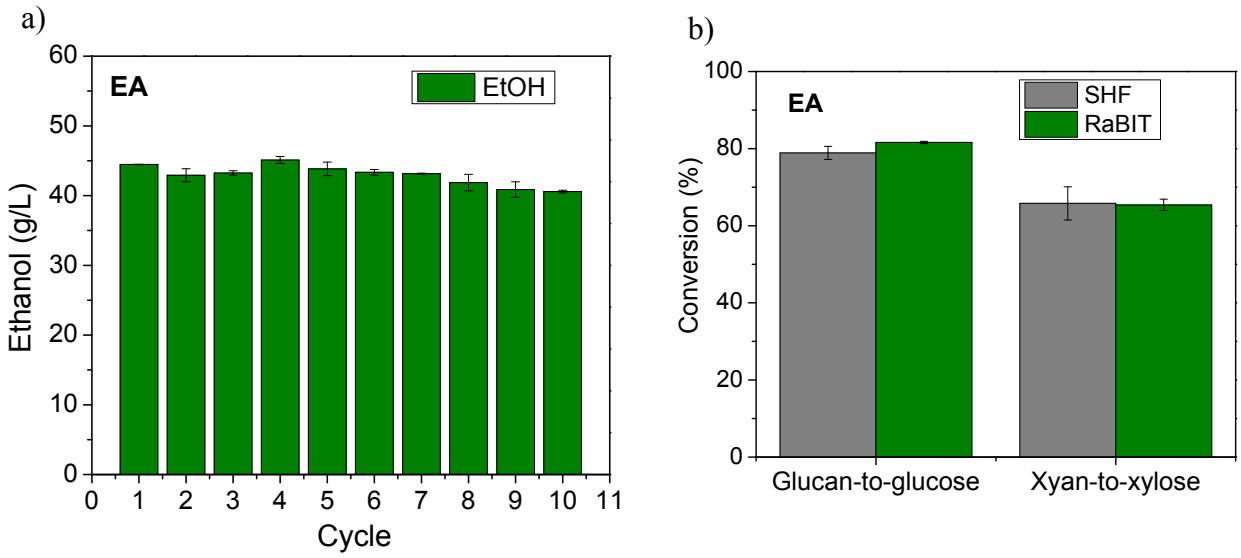
Supplementary Figure 3 Enzymatic hydrolysis and fermentation results of the ten-cycle shake flask RaBIT on AFEX-CS. a) sugar concentrations of each cycle in the main hydrolyzer showing consistent glucose and xylose released; b) ethanol and remaining sugar concentrations of each cycle in the main fermentater showing consistent ethanol (>40 g/L) produced.



Supplementary Figure 4 RaBIT performance on pelletized AFEX-CS in 5 L bioreactors. A glucan loading of 7.34 wt.% (20.0 wt.% solids loading) was applied. a) Mass balance of ten-cycle RaBIT in 5 L bioreactors; b) Ethanol concentrations produced from each cycle in the main fermenter.



Supplementary Figure 5 Ethanol concentrations produced in each RaBIT cycle on EA-CS in the main fermenter (a) and sugar conversion comparison between SHF and RaBIT (b).



Supplementary Table 1 Comparison of different substrates in enzyme recycling using RaBIT approach. Enzymatic hydrolysis were performed at 7 wt.% glucan loading in a 250 ml baffled flask with working loading of 100 g in total at 50 °C and 250 rpm. Avicel, Cellulose III, AFEX-CS, EA-CS and DA-CS were used as the substrates. To investigate the effect of lignin on enzyme recycling, AFEX-CS lignin residue obtained from enzymatically hydrolyzing AFEX-CS extensively was added to Avicel and Cellulose III at a loading of 4 g/7 g glucan (noted as Avicel + Lignin and Cellulose III + Lignin). Cellic Ctec3 were used for hydrolyzing Avicel, Avicel + lignin, Cellulose III and Cellulose III + Lignin at loadings of 11.9, 11.9, 6.0, and 6.0 mg protein/g glucan, respectively. Cellic Ctec3 and Htec3 were used for hydrolyzing AFEX-CS, EA-CS and DA-CS at loadings of 22.0 (Ctec3: 11.5 & Htec3: 10.5), 12.2 (Ctec3: 6.6 & Htec3: 5.6) and 15.4 (Ctec3: 11.8 & Htec3: 3.6) mg protein/g glucan. The enzymatic hydrolysis was performed for 23 h (Cycle 1) and then hydrolysate was centrifuged. Unhydrolyzed solids were recycled to the Cycle 2 hydrolysis. Enzymes were recycled together with unhydrolyzed solids to the Cycle 2. The amount of enzymes in supernatant, which were not recycled, was analyzed via 2D-quant method. The difference between total input enzyme and enzyme in the supernatant was counted as the enzyme recycled. The Cycle 2 enzymatic hydrolysis was set up as Cycle 1 expect that no enzyme was added for all cases expect the one shown in the last row of the table, in which 50% amount of the Cycle 1 enzyme was added. Experiments were performed in duplicates.

	Cycle 1		**Enzyme recycle from Cycle 1 to Cycle 2 via unhydrolyzed solids**		**Cycle 2 w/o enzyme addition**		**% sugar yield of Cycle 2**	
Substrate	Glucose (g/L)	Xylose (g/L)	Recycled enzyme (%)	Recycled enzyme due to moisture in solids (%)	Glucose (g/L)	Xylose (g/L)	Cycle 2 glucose concentration /Cycle 1 glucose concentration	Cycle 2 xylose concentration/ Cycle 1 xylose concentration
Avicel	29.4 ± 2.0	/	57.1% ± 0.8%	5.6% ± 0.0%	15.3 ± 0.8	/	52.0%	/
Avicel + Lignin	20.9 ± 2.1	/	89.5% ± 1.0%	2.7% ± 0.2%	15.5 ± 0.5	/	74.1%	/
Cellulose III	31.9 ± 0.9	/	50.4% ± 3.1%	5.3% ± 0.2%	8.9 ± 0.1	/	27.9%	/
Cellulose III + Lignin	21.9 ± 1.7	/	92.4% ± 0.7%	1.7% ± 0.2%	8.2 ± 0.6	/	37.6%	/
AFEX-CS	61.2 ± 1.4	33.7 ± 0.8	63.4% ± 1.5%	14.9% ± 0.1%	35.4 ± 0.3	22.3 ± 0.2	57.8%	66.2%
EA-CS	55.9 ± 0.5	32.3 ± 0.3	73.7% ± 4.7%	12.1% ± 1.4%	29.3 ± 1.2	23.7 ± 1.0	52.3%	73.3%
DA-CS	56.6 ± 0.1	2.2 ± 0.1	88.6% ± 0.2%	3.7% ± 0.1%	38.8 ± 0.4	1.4 ± 0.0	68.6%	61.9%
					Cycle 2 w/ 50% enzyme addition			
DA-CS	56.6 ± 0.1	2.2 ± 0.1	88.6% ± 0.2%	3.7% ± 0.1%	56.5 ± 0.1	2.2 ± 0.0	99.9%	98.5%