Supplementary Figures and Tables

Densifying Lignocellulosic biomass with alkaline Chemicals (DLC) pretreatment unlocks highly fermentable sugars for bioethanol production from corn stover

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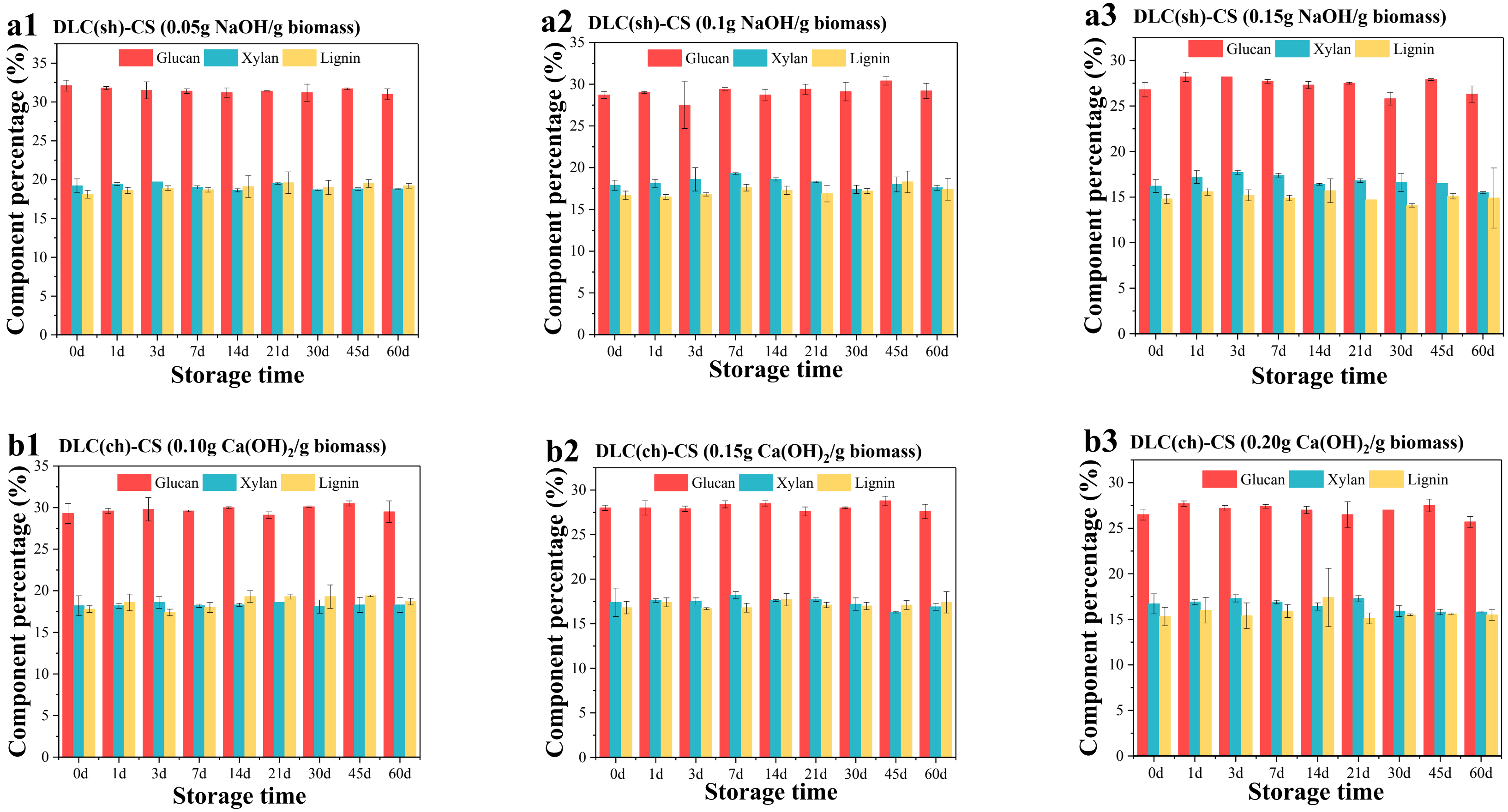


Fig. S1. Effect of storage time on composition of DLC(sh)-CS and DLC(ch)-CS. The untreated corn stover used in this experiment consists of 33.2% glucan, 20.3% xylan, and 21.8% lignin. The DLC-CS was stored on a clean bench to avoid microbial contamination and to investigate the effect of base in the DLC-CS on the composition of DLC-CS. DLC: Densifying Lignocellulosic biomass with Chemicals. CS: corn stover. “sh” in DLC(sh)-CS: sodium hydroxide; “ch” in DLC(ch)-CS: calcium hydroxide.

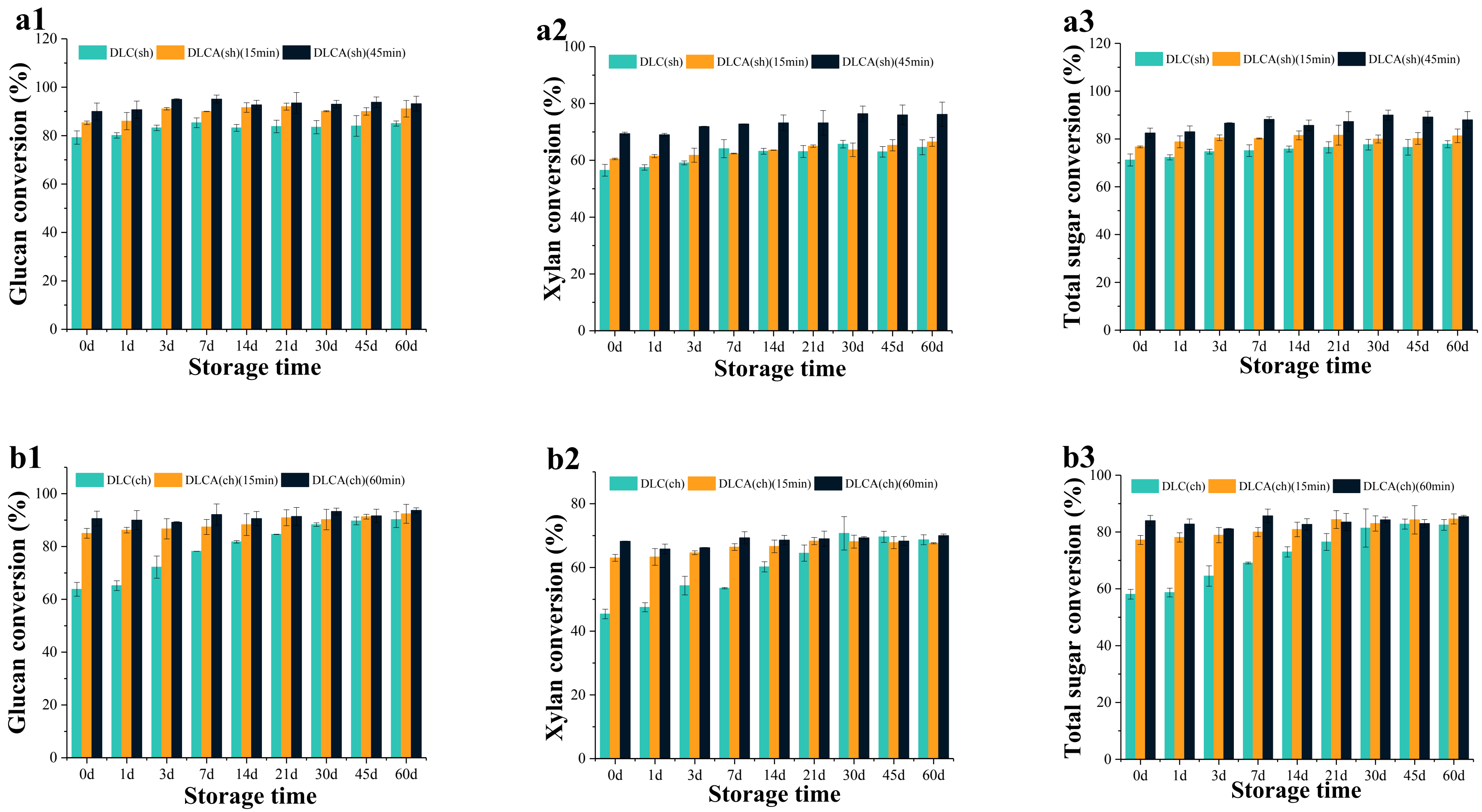
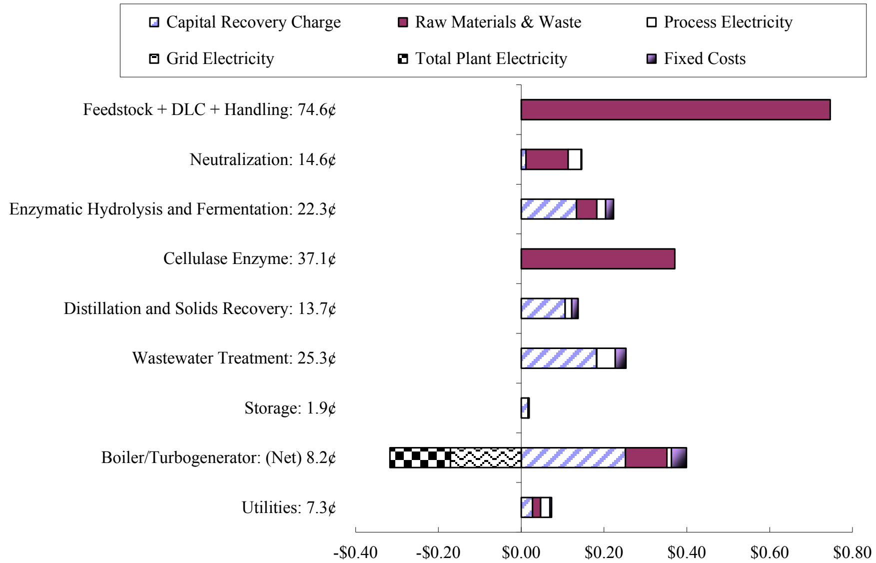


Fig. S2. Effect of storage time on steam autoclave treatment of DLC-CS for digestibility improvement. DLC(sh)-CS containing 0.1 g sodium hydroxide/g dry biomass, and DLC(ch)-CS containing 0.15 g calcium hydroxide/g dry biomass were stored at room temperature for different time and then were either used directly for enzymatic hydrolysis or further steam autoclaved before enzymatic hydrolysis. Sugar conversions of enzymatic hydrolysis are presented in the figure. The steam autoclave treatment conditions used for DLC(sh)-CS were 35% solid loading, 121 °C for 15 min or 45 min. The steam autoclave treatment conditions used for DLC(ch)-CS were 25% solid loading, 121 °C for 15 min or 60 min. Enzymatic hydrolysis was performed at 3%(w/w) solid loading and 50 °C for 24 h using commercial enzyme Cellic Ctec 2 with a dosage of 10 mg protein/g glucan.

a) DLCA(ch)



b) DA

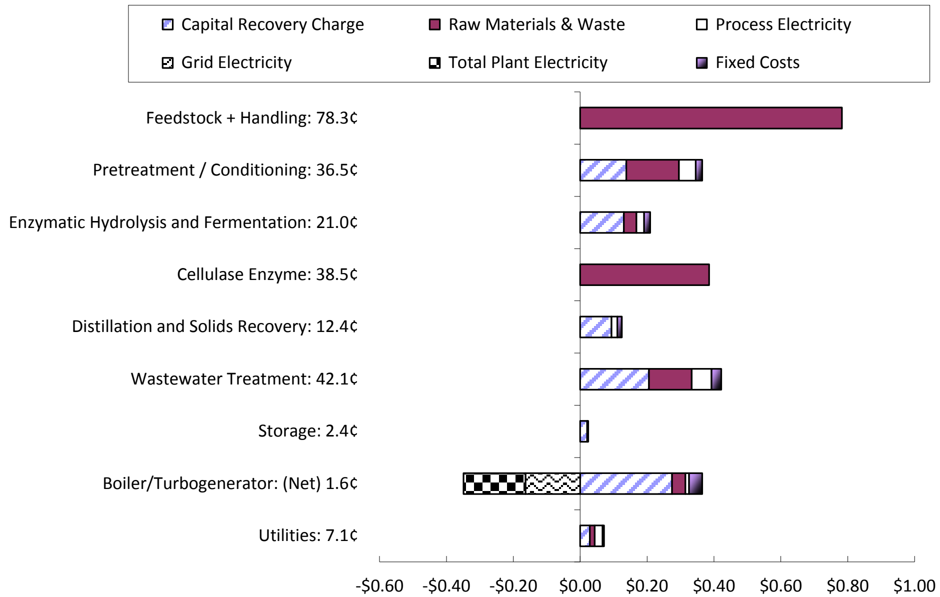


Fig. S3. Techno-economic analyses (TEA) of ethanol production from corn stover based on a) DLCA(ch) pretreatment and b) dilute acid (DA) pretreatment. The TEA was performed based on NREL model[1] with modifications. Specifically, the price of corn stover at field was set as $29.6/tonne and transportation cost was included in the feedstock handling; the enzyme price was fixed to $4.24/kg protein; the prices of electricity, calcium hydroxide, and sulfuric acid were set as $0.05/kwh, $51.8/tonne, $51.8/tonne, respectively, with the labor fee also adjusted to accommodate China's situation. The mass balance data used for DLCA(ch) pretreatment was generated by this study. The mass balance data used for DA pretreatment was obtained from the work reported by Uppugundla et al[2] with the assumption that 90% of the xylan in the pretreatment liquor was also converted to ethanol with the same ethanol metabolic yield as reported in that work, which would be an ideal situation.

Table S1 Effect of storage time on microbial contamination of DLC-CS

	Chemical dosage g/g biomass	Storage time (days)								
		0	1	3	7	14	21	30	45	60
Untreated-CS	0	+++	+++	+++	+++	+++	+++	+++	+++	+++
DLC(sh)-CS	0.05	++	+	+	-	-	-	++	++	+++
DLC(sh)-CS	0.1	-	-	-	-	-	-	-	-	-
DLC(sh)-CS	0.15	-	-	-	-	-	-	-	-	-
DLC(ch)-CS	0.1	+	+	+	-	-	-	-	++	++
DLC(ch)-CS	0.15	-	-	-	-	-	-	-	-	-
DLC(ch)-CS	0.2	-	-	-	-	-	-	-	-	-

DLC-CS was stored in a storage room. + contaminated very slightly, ++ slightly contaminated, +++ contaminated, - not contaminated

Table S2 Working mass of a pretreatment reactor

	Pretreatment solid loading	Working mass kg/m^3
loose corn stover (LCS)	20%	70.0
	30%	73.7
DLCA(sh)-CS	20%	179.5
	30%	250.0
	40%	350.0
DLCA(ch)-CS	20%	179.5
	30%	250.0
	40%	350.0

Working mass of a pretreatment reactor reflects the amount of biomass filled in a pretreatment reactor.

Pictures showing the same amount of biomass in a beaker

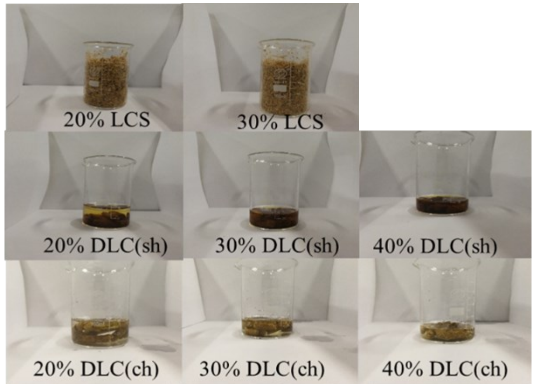


Table S3 Chemical analysis of liquid stream or water extract of pretreated corn stover

Pretreatment methods	Hydroxymethylfurfural （HMF） (g/L)	Formic acid (g/L)	Furfural (g/L)	Levulinic acid (g/L)	Acetic acid (g/L)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Oli-glucose (g/L)	Oli-xylose (g/L)	Phenols (g/L)
AL	0.00±0.00	0.3±0.2	0.0±0.0	1.4±0.0	2.6±0.1	0.6±0.0	0.4±0.0	0.0±0.0	0.9±0.0	6.7±0.1	5.28±0.28
DLC(sh)	0.00±0.00	0.2±0.0	0.0±0.0	1.3±0.0	2.7±0.0	0.3±0.0	0.1±0.0	0.2±0.0	0.4±0.0	3.9±0.0	2.19±0.01
DLCA(sh)	0.00±0.00	0.5±0.0	0.0±0.0	0.3±0.0	2.4±0.0	0.1±0.0	1.4±0.0	1.1±0.0	1.5±0.0	4.7±0.0	2.47±0.05
Lime	0.00±0.00	0.1±0.0	0.0±0.0	1.3±0.0	2.4±0.0	0.3±0.0	0.0±0.0	0.6±0.0	1.3±0.0	7.2±0.1	2.92±0.07
DLC(ch)	0.00±0.00	0.1±0.0	0.0±0.0	1.2±0.0	2.6±0.0	0.2±0.0	0.0±0.0	0.1±0.0	0.7±0.0	4.8±0.2	1.64±0.07
DLCA(ch)	0.00±0.00	0.2±0.3	0.0±0.0	1.2±0.0	2.2±0.0	0.0±0.0	0.5±0.0	2.0±0.0	1.4±0.0	5.2±0.1	1.46±0.04

Liquid streams of ALand lime pretreated corn stover were used for analysis, which was pretreated at 10%(w/w) solid loading. AL: dilute alkali pretreatment; Lime: lime pretreatment. Water extracts of DLC-CS or DLCA-CS (autoclaved DLC-CS) were used for analysis with extraction performed at 10%(w/w) solid loading. All experiments were done in triplicate and each value is expressed as mean ± S.D.

References

1. D. Humbird, R. Davis, L. Tao, C. Kinchin, D. Hsu and A. Aden, Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol, *Technical Report NREL/TP-5100-47764*, National Renewable Energy Laboratory Golden, Colorado, 2011.
2. N. Uppugundla, L. da Costa Sousa, S. P. Chundawat, X. Yu, B. Simmons, S. Singh, X. Gao, R. Kumar, C. E. Wyman and B. E. Dale, *Biotechnol. Biofuels*, 2014, **7**, 72.