Table S1. Sugar concentrations in enzymatic hydrolysis of single-stage DDR and multi-stage DDR-SM corn stover substrates .

			Monomeric Sugars					Total Sugars				
Sample Description	Total solids at t=0 @ Sach	Density (g/ml)	Cellobiose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Arabinose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Arabinose (mg/ml)	Acetic Acid (mg/ml)
DDR @ 20mg/g	22.5%	1.08	4.1	93.8	71.3	1.0	3.8	103.1	86.8	3.9	7.8	2.2
DDR @ 20mg/g	25.0%	1.09	4.7	102.7	79.9	1.1	4.8	113.1	96.8	4.5	9.3	2.5
DDR @ 20mg/g	27.5%	1.10	6.1	117.5	87.3	1.2	5.3	131.9	108.8	5.2	10.6	3.4
DDR @ 20mg/g	30.0%	1.11	6.4	126.3	93.7	1.0	5.1	143.6	118.0	5.4	11.2	1.7
DDR-SM @ 20mg/g	22.5%	1.09	5.0	104.7	81.0	0.0	3.4	114.3	94.0	0.6	4.6	3.6
DDR-SM @ 20mg/g	25.0%	1.10	5.5	114.9	89.1	0.0	3.9	125.4	104.1	0.0	5.2	3.1
DDR-SM @ 20mg/g	26.5%	1.10	6.2	122.3	93.9	0.0	4.2	133.3	109.4	0.0	5.5	3.3
DDR-SM @ 20mg/g	28.0%	1.11	7.3	135.2	99.4	0.0	5.2	147.9	115.0	0.7	6.3	3.8
DDR-SM @ 10mg/g	22.5%	1.08	3.5	97.0	72.3	0.0	2.8	105.2	84.4	0.0	4.0	2.7
DDR-SM @ 10mg/g	25.0%	1.09	4.3	108.5	76.7	0.0	2.9	116.9	88.9	0.0	4.2	3.0
DDR-SM @ 10mg/g	26.5%	1.09	4.0	101.9	80.0	0.0	3.4	110.7	93.1	0.0	4.5	3.3

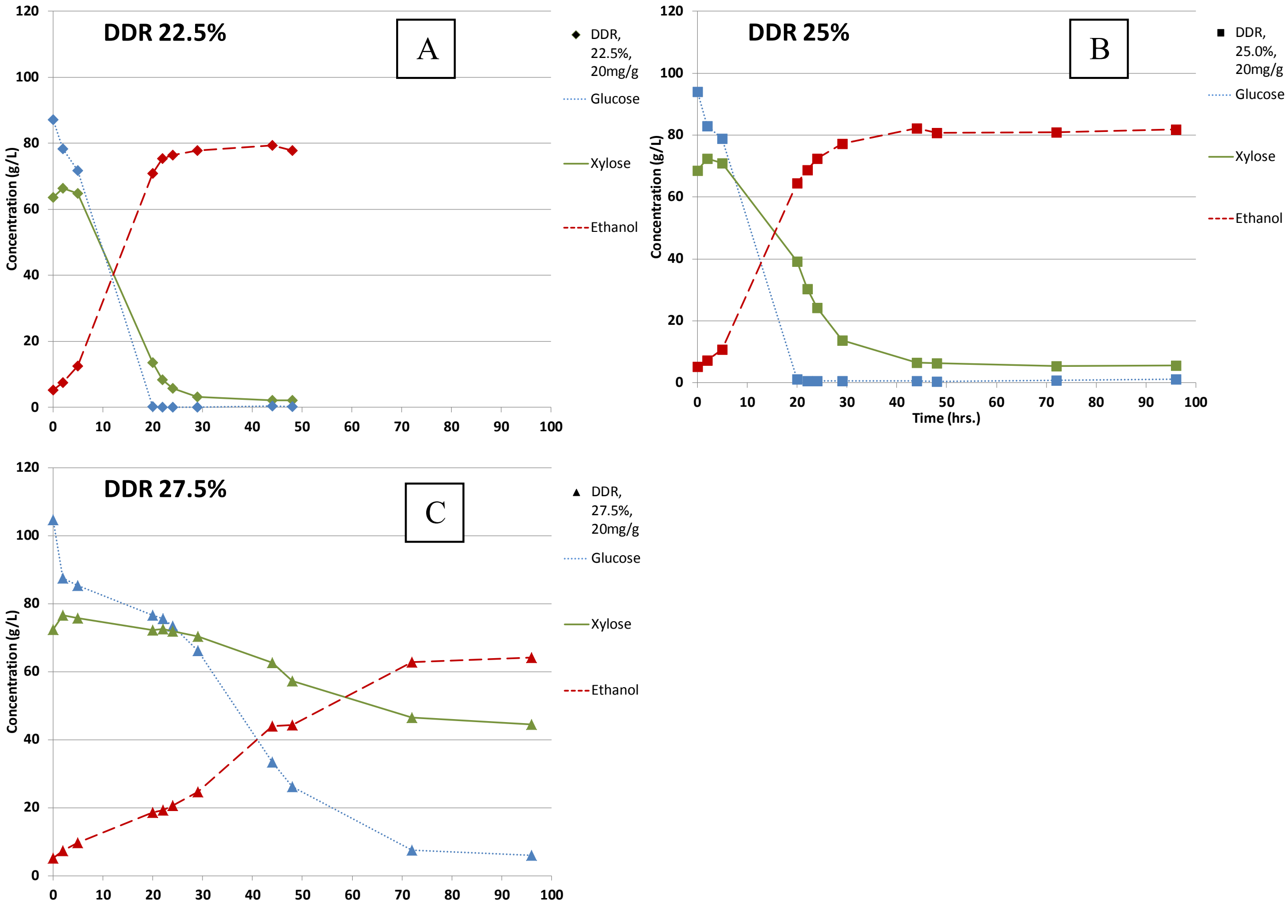

Figure S1. Glucose, xylose, and ethanol component concentration profiles for fermentations with the r*Zymomonas mobilis* strain 13-H-9-2, produced during fermentations (pH ~5.8 at 33°C) on enzymatically hydrolyzed single stage DDR substrates (produced using ~208 kWh/ODMT refining energy) at 22.5, 25, and 27.5 wt% starting total solids concentrations produced using 20 mg total protein/g cellulose enzyme loadings of Novozymes Cellic® CTec3 and HTec3 at 16 mg/g cellulose plus 4 mg/g cellulose ratio, respectively.

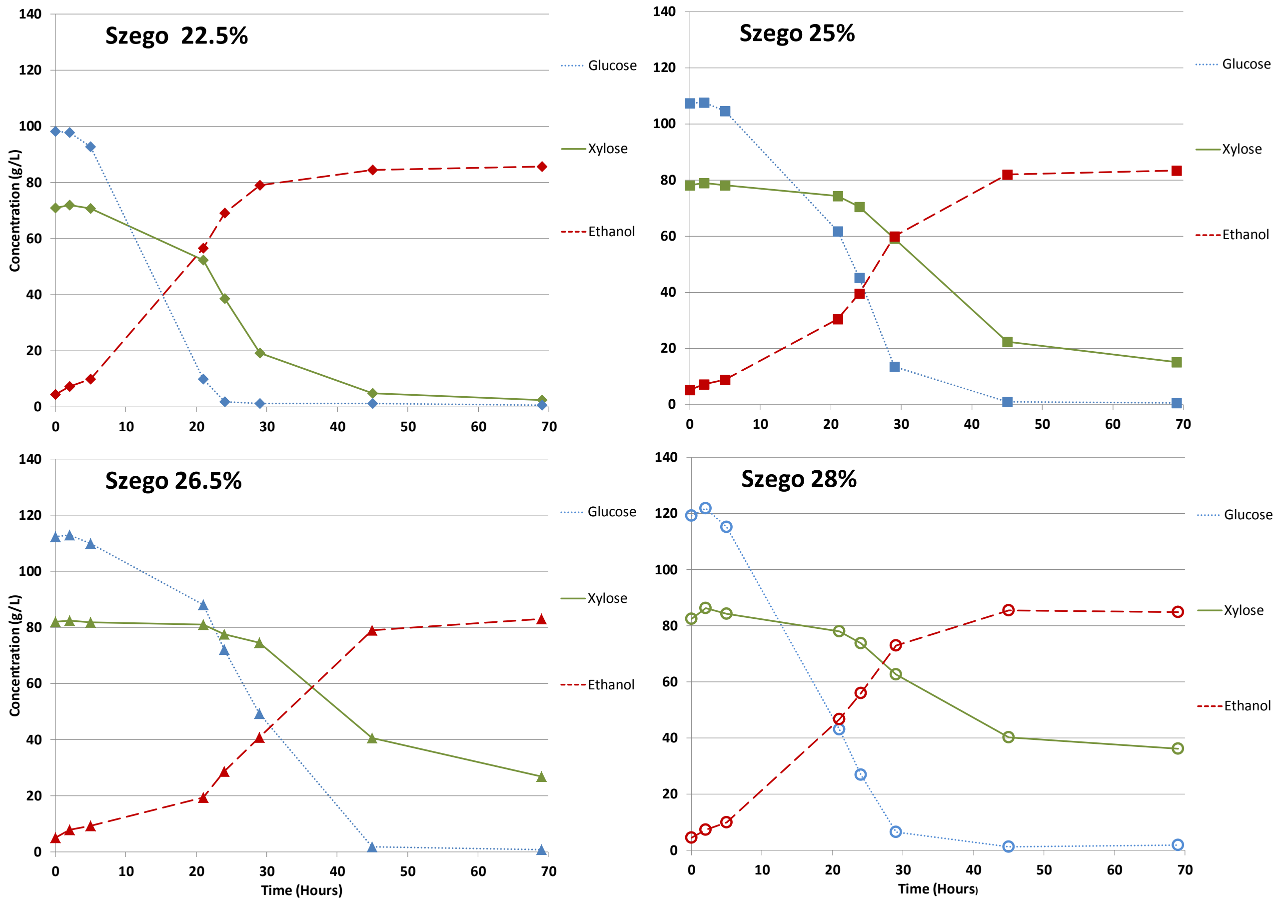

Figure S2. Glucose, xylose, and ethanol component concentration profiles for fermentations with the r<u>*Zymomonas mobilis*</u> strain 13-H-9-2, produced during fermentations (pH ~5.8 at 33°C) on enzymatically hydrolyzed multi-stage DDR-SM (disc refined plus Szego milled produced using a total of ~200 kWh/ODMT refining energy) substrates at starting total solids concentrations of 22.5, 25, 26.5 and 28 wt% and 20 mg total protein/g cellulose enzyme loadings of Novozymes Cellic® CTec3 and HTec3 at 16 mg/g cellulose plus 4 mg/g cellulose ratio, respectively.

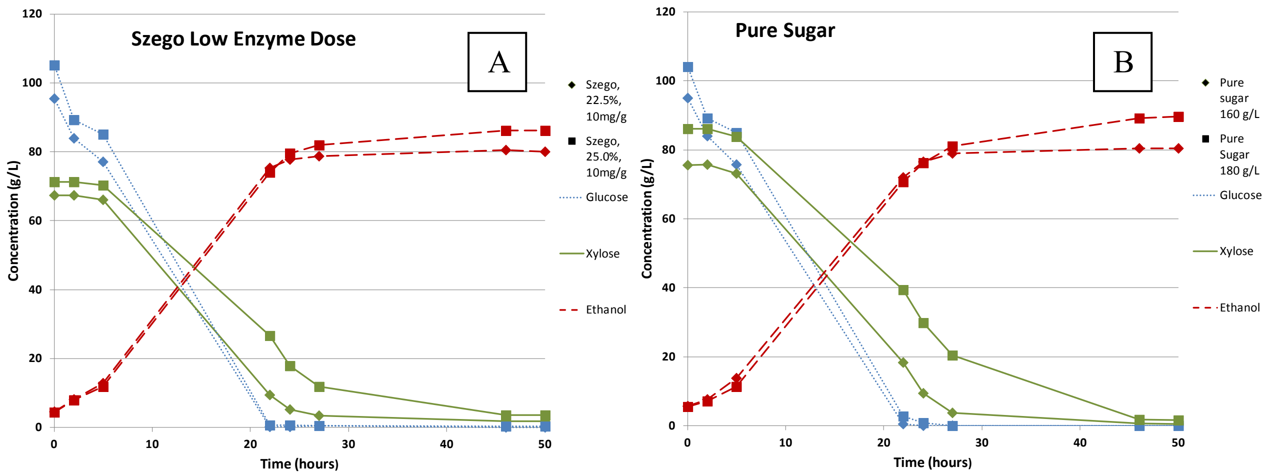

Figure S3. Glucose, xylose, and ethanol component concentration profiles for fermentations with the r*Zymomonas mobilis* strain 13-H-9-2, pH ~5.8, at 33°C on enzymatically hydrolyzed multi-stage DDR-SM (disc refined plus Szego milled at ~200 kWh/ODMT total refining energy) corn stover substrates at 22.5% and 25% starting total solids concentrations at 10 mg total protein/g cellulose enzyme loadings of Novozymes Cellic® CTec3 and HTec3 at 8 mg/g cellulose plus 2 mg/g cellulose enzyme ratio, respectively (panel A), and pure sugar control fermentations at 160 g/L and 180 g/l total fermentable sugars (glucose plus xylose) (panel B).

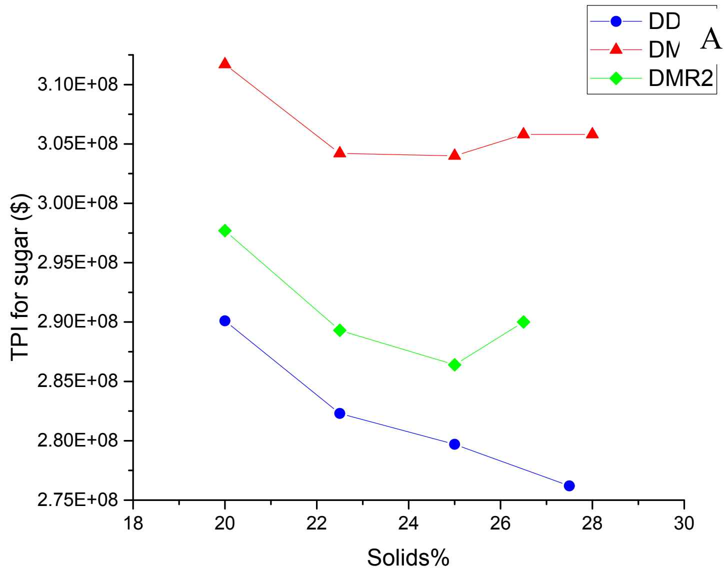

Figure S4. The effects of enzymatic hydrolysis solids concentrations on TPIs for sugars calculated for the single-stage DDR and multi-stage DMR processes.

Total project investment (TPI) equals the sum of total direct costs (TC) and total indirect costs (TIC) for a project. Figure S5 shows the effects of varying enzymatic hydrolysis solid concentrations on total project investment (TPI) for sugars. The single-stage DDR process has the lowest TPIs compared to the other two multi-stage DMR processes mainly because the DMR process requires a **second** stage Szego milling step, which adds extra capital costs to the TPI. In this work, we estimated that the Szego mill costs about $13MM for a 2000 tonne (dry) biomass per day mill, the same estimated costs for the disc refiners at the same throughput.

The TPIs for sugar production for the single-stage DDR process shows a decreasing linear trend with increasing solid concentrations in the enzymatic hydrolysis step. The minimum TPI costs calculated for sugar production for the DDR process were found to be 27.5% solids, with an approximate $15MM savings compared to that case where DDR substrates were hydrolyzed at a lower 20% solids. This savings at higher solids loadings is mainly due to the smaller size of hydrolysis reactors required at higher solids loadings in enzymatic digestion. It should be noted that in this report the mixing energy in the hydrolysis reactors are fixed at a constant rate, which is not likely to happen when varying solids concentrations in enzymatic hydrolysis solids. Future work will address this energy issue when measured or calculated mixing energy supported by experimental data becomes available. This research is currently being undertaken at NREL.

Enzyme loadings in the enzymatic hydrolysis step also make a large difference in TPI costs for sugar production. For both multi-stage DMR processes studied here, the TPI costs for sugars with enzyme loadings at 10 mg protein per gram of cellulose are about $15MM lower than that when the enzyme loadings are doubled at the same hydrolysis solids concentrations.

This is mainly because of the larger size of the enzyme production facility, and higher air flow and electrical energy requirements for compressors for the higher enzyme loading scenarios.

For both DMR processes at different enzyme loadings, increasing hydrolysis solids helps to reduce the TPI costs. However, at the lower enzyme loading scenarios investigated in this report, we found that when solids concentrations reached 26.5%, the sugar yields decrease from 80% to 70%, which eventually leads to an increase in TPI costs because more unhydrolyzed biomass is required to be burned, which requires an increase in the size of the expensive combustion boiler.

In summary, to further reduce the TPI costs for sugars, future research should be focused on reducing capital investment in mechanical refining, reducing enzyme loadings, and increasing hydrolysis solids, while maintaining high sugar yields.