

Fig. S1

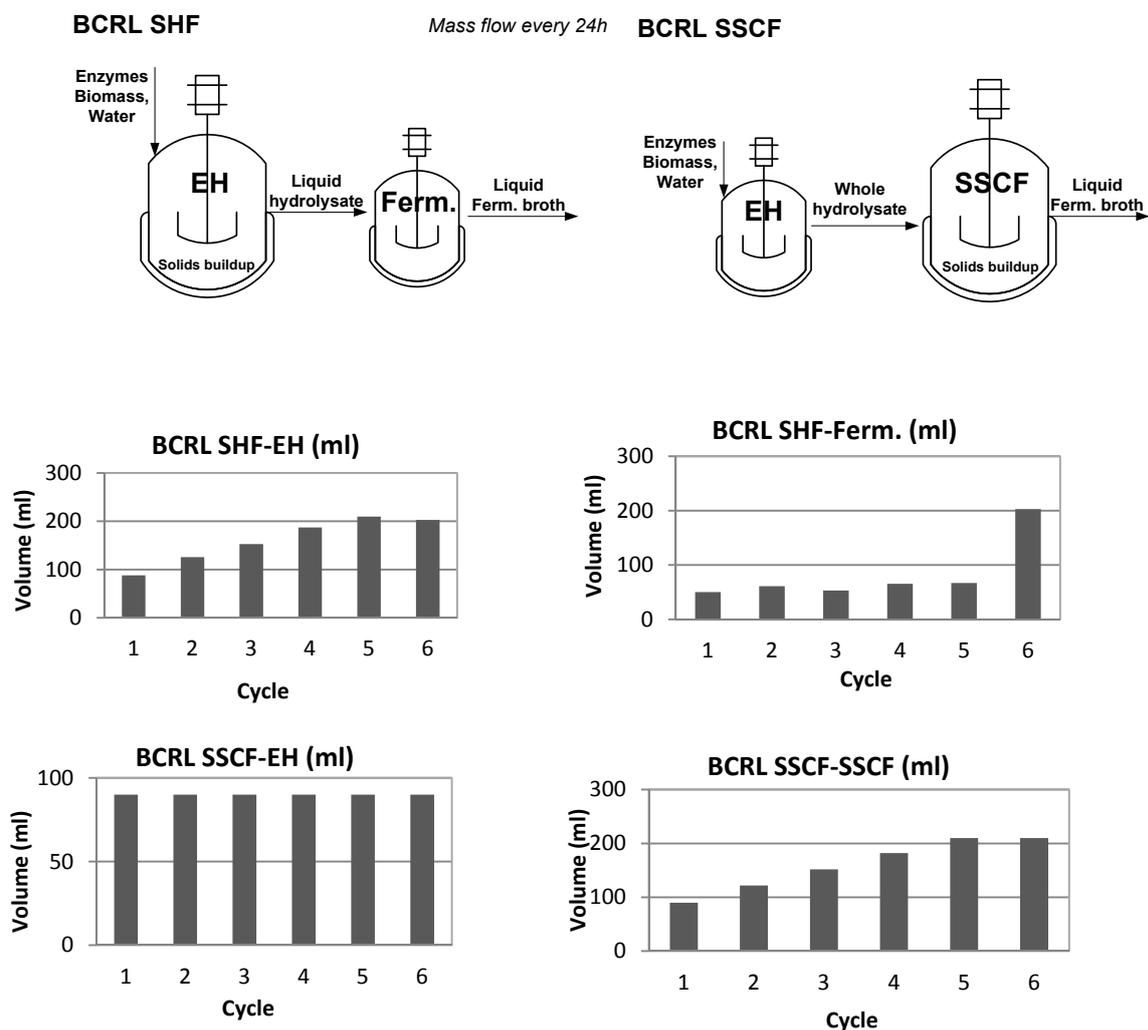


Fig.S1 Tank setup and volume change for BCRL SHF and SSCF processes. The last step is denoted as cycle 6. Basically, the volume increase happened in the enzymatic hydrolysis (EH) tank for the BCRL SHF process and in the SSCF tank for BCRL SSCF process due to the buildup of solid residues. The last step of fermentation for BCRL SHF process was carried out in the EH tank after 24h hydrolysis without solid-liquid separation.

Fig. S2

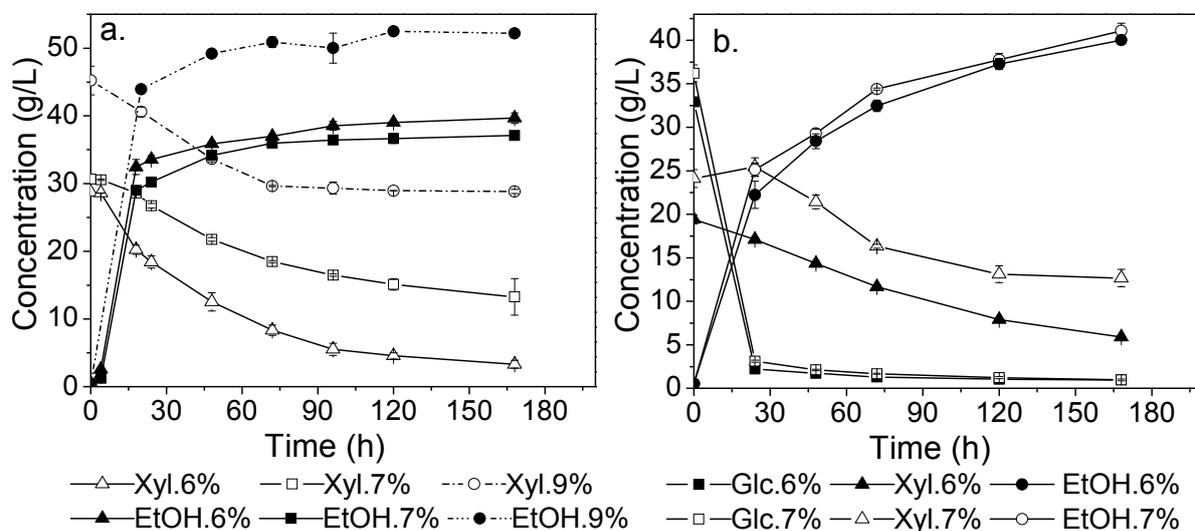


Fig.S2 SHF fermentations of AFEXTM-CS hydrolysates derived from 6%, 7% and 9% glucan loading enzymatic hydrolysis (a), and SSCF of AFEXTM-CS at 6% and 7% glucan loading (b).

Fig. S3

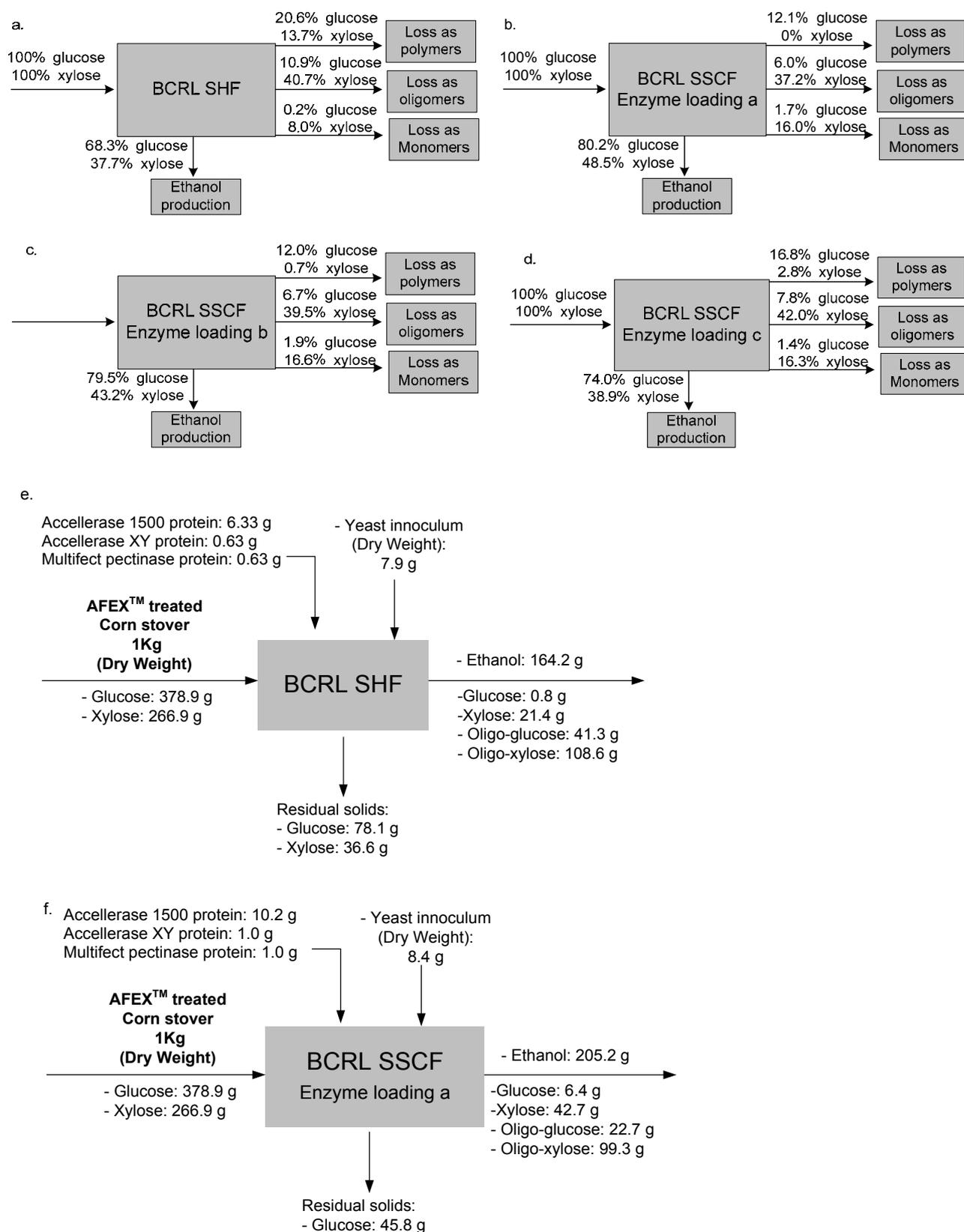


Fig.S3 Mass balance of BCRL SHF and SSCF processes

Fig. S4

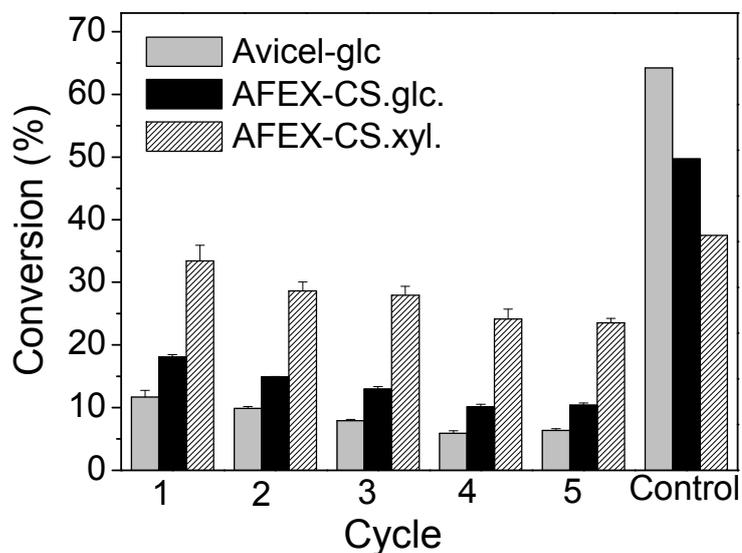


Fig.S4 Enzyme activities of proteins in the removed hydrolysate after enzymatic hydrolysis during fast SHF process. The assay was performed on Avicel and AFEXTM-CS. Monomeric glucose/xylose conversion is shown in the figure. The protein concentrations in each cycle hydrolysate were 3.2, 2.7, 2.6, 3.2 and 2.8 mg/ml. The control assumed all the enzymes were removed in the hydrolysate after cycle 1 (i.e., no enzyme recycle with unhydrolyzed solids stream). The protein concentration for the control was 5.2 mg/ml. The enzyme activity assay was conducted in micro-plates with working volume 1.5ml/well, substrate loading 1 mg/well, protein loading 30 ug/well, reaction time 12h.

Fig. S5

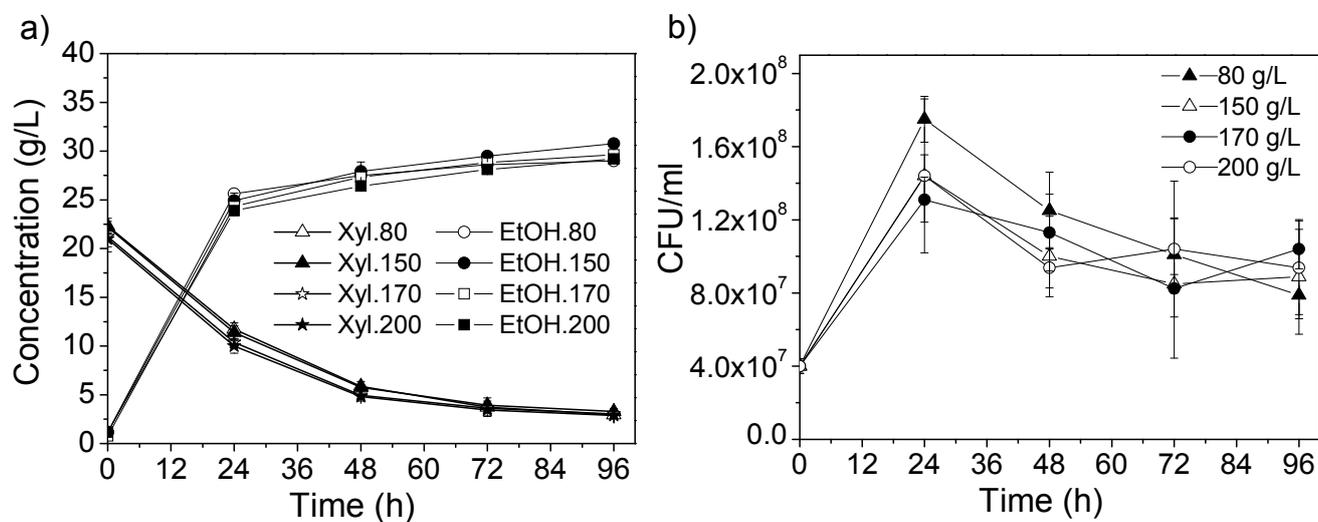


Fig.S5 Effect of enzymatic hydrolysis residual solids concentration on fermentation in hydrolysate. Solids concentrations investigated included 80, 150, 170, and 200 g/L. Fermentations were performed in 250ml shake flasks with 100ml working volume at 180 rpm, 32°C, pH 5.5 and initial OD 2.0. The initial glucose concentration was 43.8 ± 1.4 g/L and was consumed completely in 24 h for all of the cases. The solids concentrations in cycle 1-6 of the BCRL SSCF process were around 88, 125, 153, 170, 185, and 185 g/L, respectively.