Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Cellulose-Hemicellulose Interactions at Elevated Temperatures Increase Cellulose Recalcitrance to Biological Conversion

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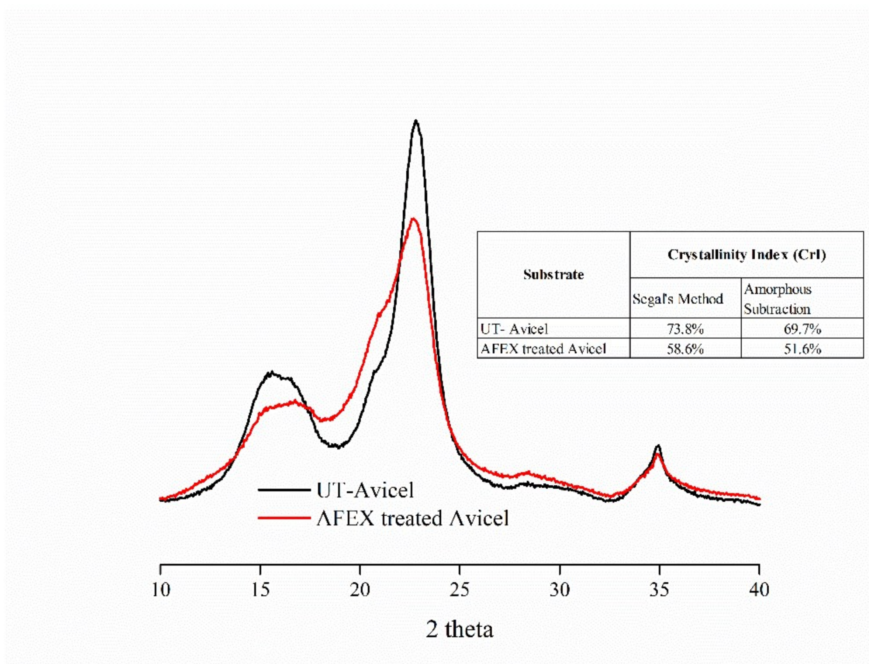

Figure S1. XRD and crystallinity index (CrI) values for untreated and AFEX treated Avicel cellulose

Effect of xylan types

A new batch of cellulose (Avicel® PH 101) and two different kinds of xylan (beechwood or birchwood) were used to investigate and confirm the negative effects of precipitated xylan on cellulose conversion. Hydrothermal pretreatment of Avicel cellulose alone and Avicel cellulose mixed (ACM) with beechwood or birch wood xylan (cellulose to xylan weight ratio - 2:1) was performed at 180°C for 30 min in a 1 L high pressure Parr reactor with total reaction weight of 800 g. Pretreated solids of the new Avicel alone (control), ACM with beechwood xylan, and ACM with birch wood xylan were determined to contain 95.5 wt.% glucan and 4.5 % xylan, 89 wt.% glucan and 10.7% xylan, and 80.85 wt.% glucan and 19 % xylan, respectively. Although the same amount of xylan (cellulose to xylan weight ratio = 2:1) was used in pretreatments for both ACM with beechwood xylan and ACM with birch wood xylan, the higher xylan amount in the solids for the latter (19% vs. 10.7%) suggested that xylan types impact the amount of xylan precipitated on the cellulose surface. Enzymatic hydrolysis of hydrothermally pretreated solids of the new Avicel cellulose control and ACM with xylans was performed with Accellerase®1500 cellulase protein loading of 15 mg/g glucan in the pretreated solids with data shown in Figure S2. Consistent with the data shown in Figure 2, Figure S2 clearly shows that precipitated xylan had a strong negative impact on cellulose conversion. However, although the amount of xylan in solids from hydrothermal pretreatment of both the new and old batches of ACM beechwood xylan was quite similar, i.e., ~ 10 wt%, the drop in 120 h cellulose conversion for pretreated solids of the new ACM with beechwood xylan was much greater (~44%) than observed for the old batch of ACM beechwood xylan (~ 27%). Therefore, it appears that cellulose structural properties affect its interaction with hemicelluloses in pretreatment and consequently cellulase conversion. However, this hypothesis needs further research. Figure S2 also shows that the drop in cellulose initial conversions for pretreated solids of ACM with birch wood were much higher (73%) than for solids of ACM with beechwood xylan (54 %), possibly due to higher precipitated xylan in the solids for ACM with birch wood (19 wt%) than ACM with beechwood (10 wt %). Nevertheless, the drop in final 120 h cellulose conversion was greater for the solids from pretreatment of ACM with beechwood xylan. Once again, this result suggests that these two xylan types have possibly different kinds of association with cellulose in pretreatment and, consequently, affect cellulose conversion by different degrees.

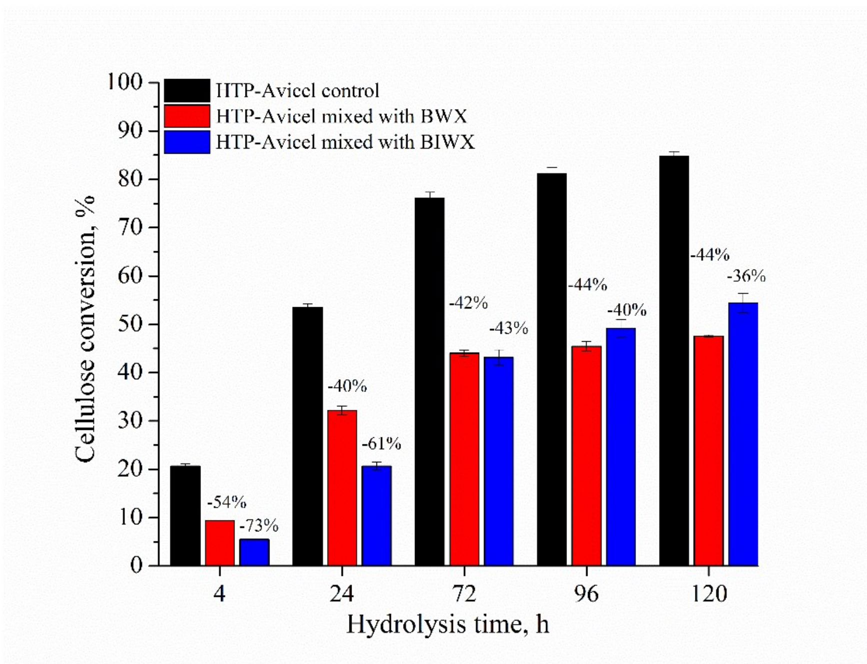

Figure S2. Cellulose conversion vs. time plot for enzymatic hydrolysis for hydrothermally pretreated (HTP; 180°C for 30 min) solids of the new batch of Avicel cellulose alone (control) and Avicel cellulose mixed with beechwood (BWX) or birchwood (BIWX) xylan. **Note-** Numbers on the graphs are relative drop in cellulose conversion.

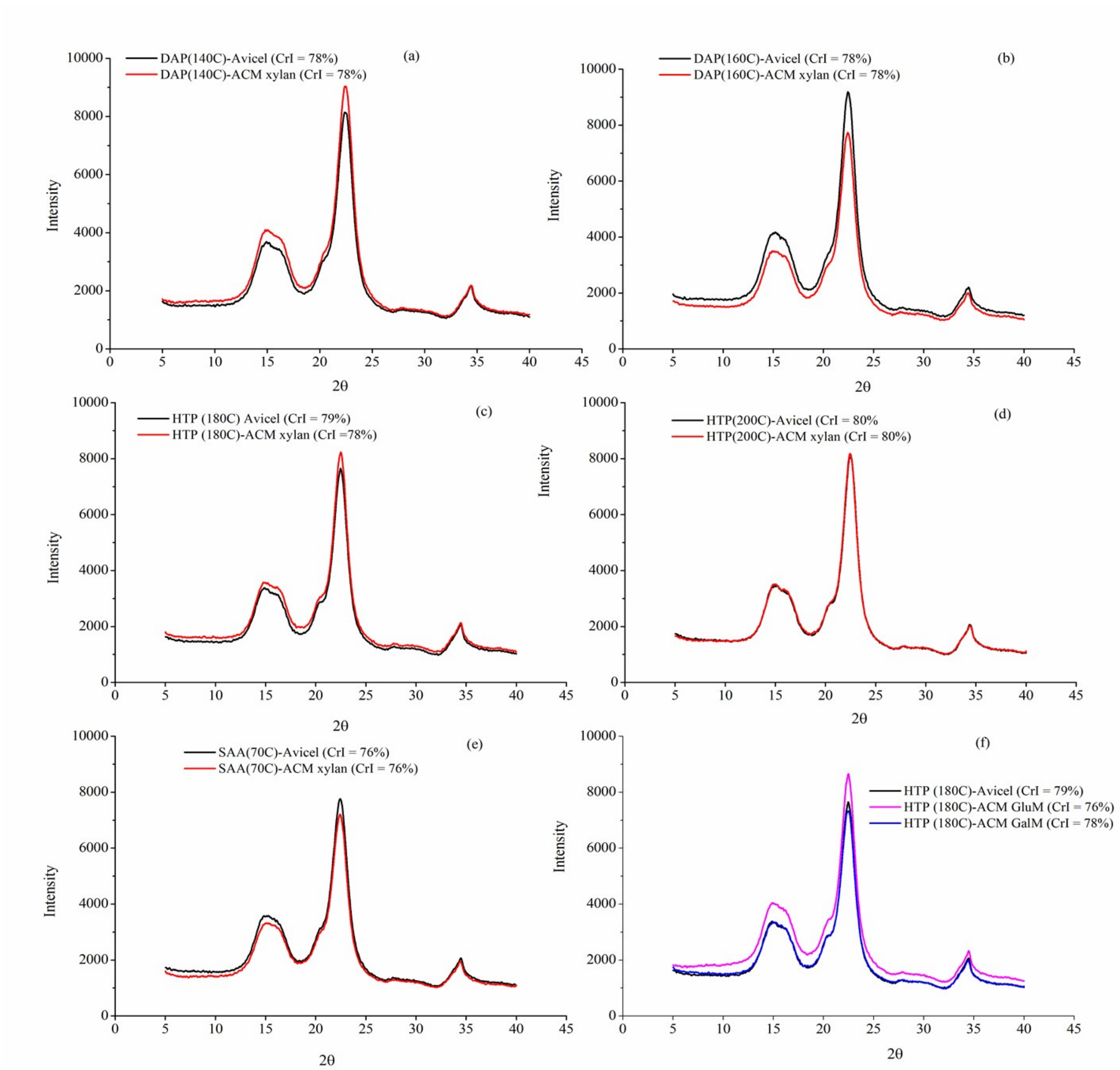

Figure S3. XRD spectra and crystallinity index (CrI) values, noted in parenthesis, for solids from pretreatment of Avicel cellulose alone (control) and Avicel cellulose mixed (ACM) with beechwood xylan (or glucomannan (GluM) or galactomannan (GalM); as noted on the graphs) prepared by (a) dilute acid pretreatment (DAP) at 140°C in 1 wt% sulfuric acid for 30 min, (b) DAP at 160°C in 0.5 wt% sulfuric acid for 10 min, (c) hydrothermal pretreatment (HTP) at 180°C for 30 min, (d) HTP at 200°C for 30 min, (e) soaking in aqeuous ammonia (SAA) at 70°C for 24 h, and (f) HTP at 180°C for 30 min.

Effect of xylanase supplementation to cellulase and high enzymes loadings on cellulose conversion

The effects of Multifect® Xylanase (MXy1- 7.5 mg/g glucan; MXy2- 30 mg/g glucan) supplementation of Accellerase®1500 cellulase (15 mg protein/g glucan) on cellulose conversion was evaluated for pretreated solids of Avicel cellulose alone or ACM with BWX (or guar gum galactomannan) prepared by hydrothermal, dilute acid, and SAA pretreatments. The data in Figure S4 show that for dilute acid and ammonia pretreated solids (Figure S4a, b, and e), cellulose conversion was significantly enhanced and was nearly equal to their respective controls by a low loading of supplemental xylanase (7.5 mg/g glucan). However, although hydrothermally pretreated solids contained about the same amount of xylan as solids from SAA (~6 to 10wt %), pretreated solids for the former required higher xylanase (30 mg/g glucan) supplementation to alleviate the negative impact of precipitated xylan (Figure S4c and S4d). In fact, cellulose conversion for solids prepared at 180°C for 30 min was not equal to the control even at a high xylanase loading, Figure S4c. This supports our hypothesis that temperature had a significant impact on cellulose-hemicellulose association strength. Furthermore, xylanase supplementation had only a marginal effect on cellulose conversion for solids from hydrothermal pretreatment of ACM with galactomannan (XGM in the pretreated solids ~5wt %), and the final 120 h yield was still much lower (>30%) than for the cellulose control (Figure S4f). These results suggest that non-structural galactomannan had stronger association with cellulose and/or more inhibitory to cellulase than non-structural xylan and other appropriate enzymes such as endo-mannanase and β-mannosidase are required to increase the conversion.

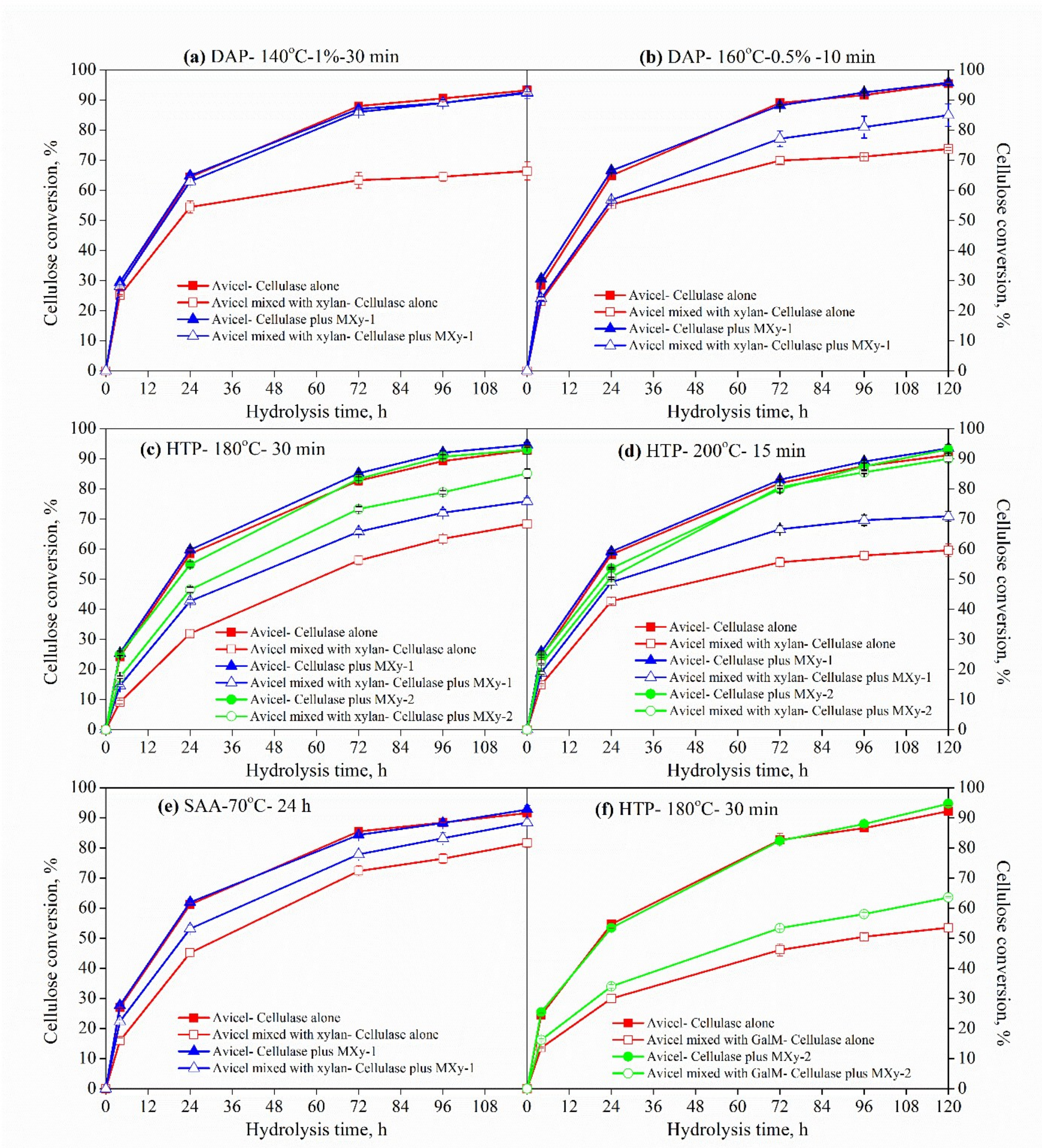

Figure S4. Effect of Multifect® Xylanase (MXy) supplementation of cellulase (15 mg/g glucan in pretreated solids) on cellulose conversion for solids from pretreatment of Avicel cellulose alone (control) and Avicel cellulose mixed with beechwood xylan (or galactomannan (GalM); as noted on the graphs) prepared by (a) dilute acid pretreatment (DAP) at 140°C in 1 wt% sulfuric acid for 30 min, (b) DAP at 160°C in 0.5 wt% sulfuric acid for 10 min, (c) hydrothermal pretreatment (HTP) at 180°C for 30 min, (d) HTP at 200°C for 30 min, (e) soaking in aqeuous ammonia (SAA) at 70°C for 24 h, and (f) HTP at 180°C for 30 min at cellulase protein loadings of 15 mg/g glucan. **Note**- MXy-1 and MXy-2 represent xylanase protein loadings of 7.5 and 30 mg /g glucan in pretreated solids, respectively. Experimental conditions were as shown in Table 1 and discussed in materials and methods.

Enzymatic hydrolysis was also performed at a high cellulase plus xylanase protein loading of 60 mg plus 60 mg per g glucan with data shown in Figure S5. Cellulose conversions for all the pretreated solids of ACM with xylan over time were equal to the controls and showed negligible impact of exogenous, precipitated hemicelluloses (Figure S5a-e). Yet again, the initial cellulose conversions for solids from hydrothermal pretreatment of ACM with GalM were still lower (>15%) than for the control (Figure S5f), again indicating the stronger negative impact of galactomannan on cellulose conversion than xylan. The final 120 h conversions shown in Figure S5f, however, were about the same for both solids.

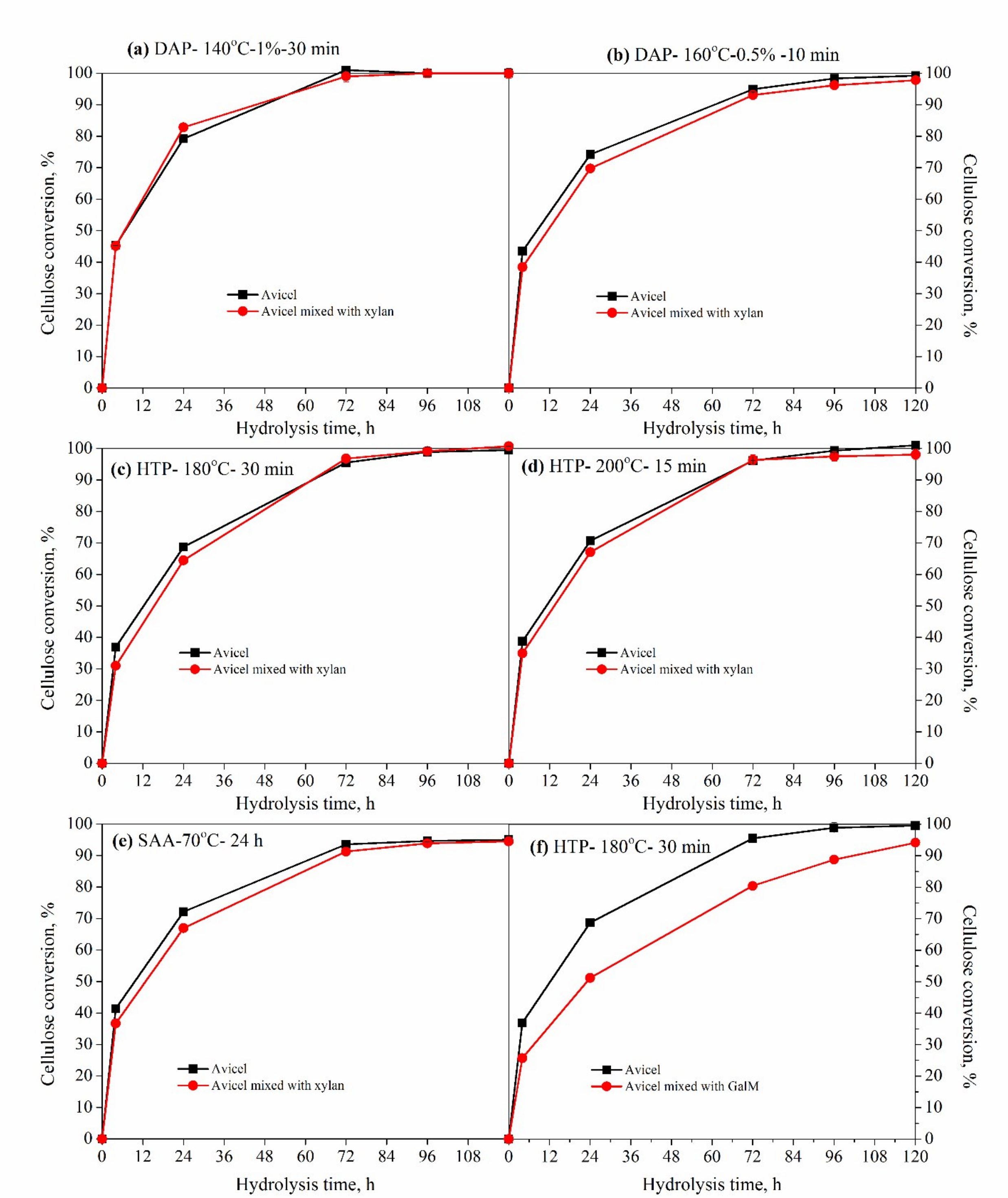

Figure S5. Cellulose conversion vs. time plots for enzymatic hydrolysis performed at Accellerase®1500 cellulase plus Multifect® xylanase protein loadings of 60 mg plus 60 mg/g glucan in the pretreated solids for solids from pretreatment of Avicel cellulose alone (control) and Avicel cellulose mixed with beechwood xylan (or galactomannan; as noted on the graphs) prepared by (a) dilute acid pretreatment (DAP) at 140°C in 1wt% sulfuric acid for 30 min, (b)

DAP at 160°C in 0.5wt% sulfuric acid for 10 min, (c) hydrothermal pretreatment (HTP) at 180°C for 30 min, (d) HTP at 200°C for 30 min, (e) soaking in aqueous ammonia (SAA) at 70°C for 24 h, and (f) HTP at 180°C for 30 min. **Note**- Experimental conditions were as shown in Table 1 and discussed in materials and methods.