

Supplementary Material 4: Impact of hydration levels during enzymatic hydrolysis on the IR spectra

Spatiotemporal dynamics of cellulose during enzymatic hydrolysis studied by infrared spectromicroscopy

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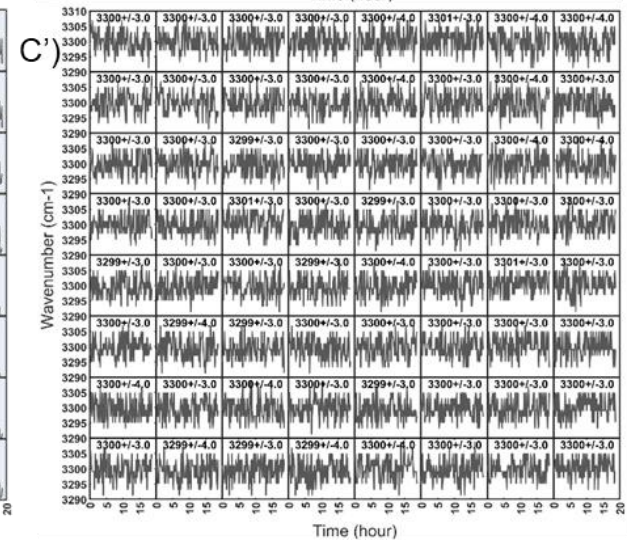
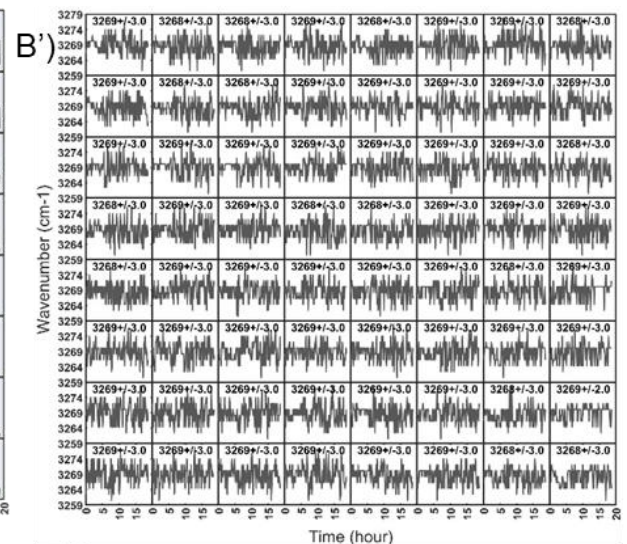
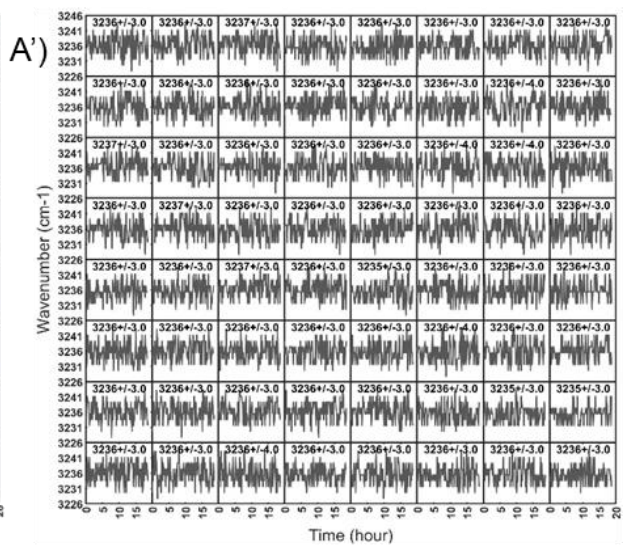
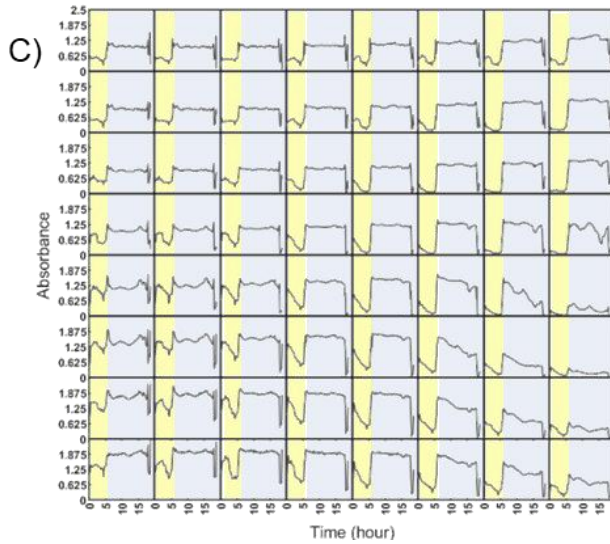
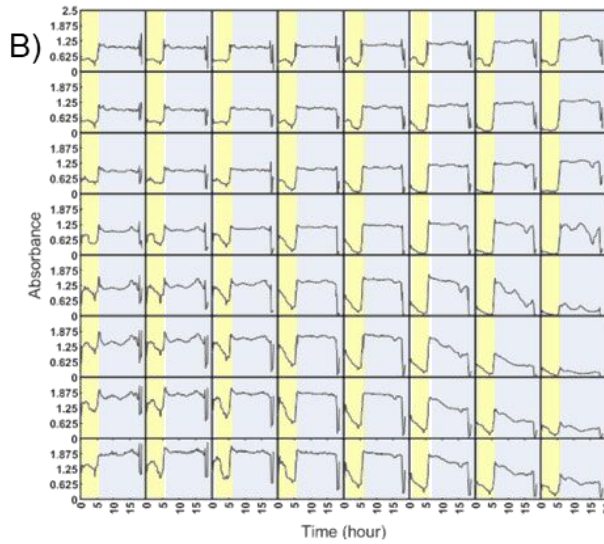
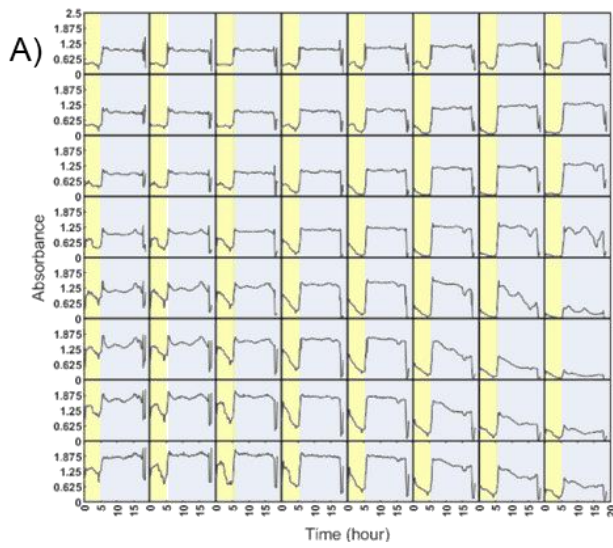
⁴Current: Genus IntelliGen Technologies, Windsor, Wisconsin

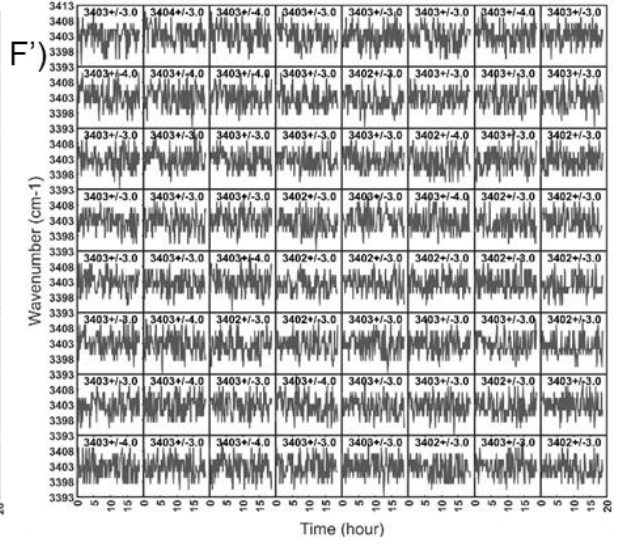
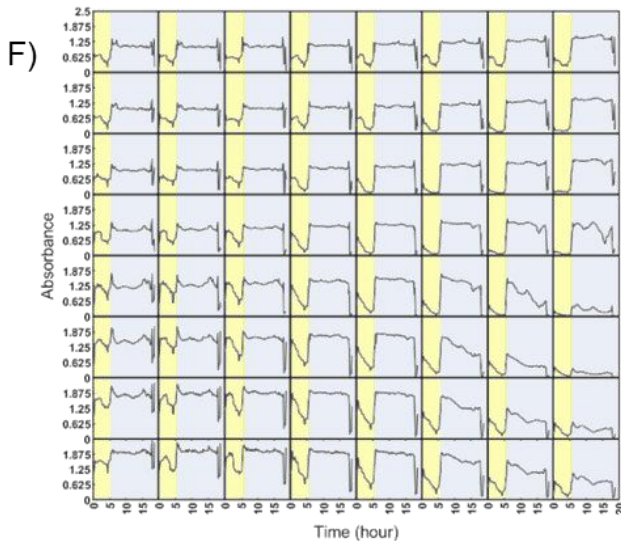
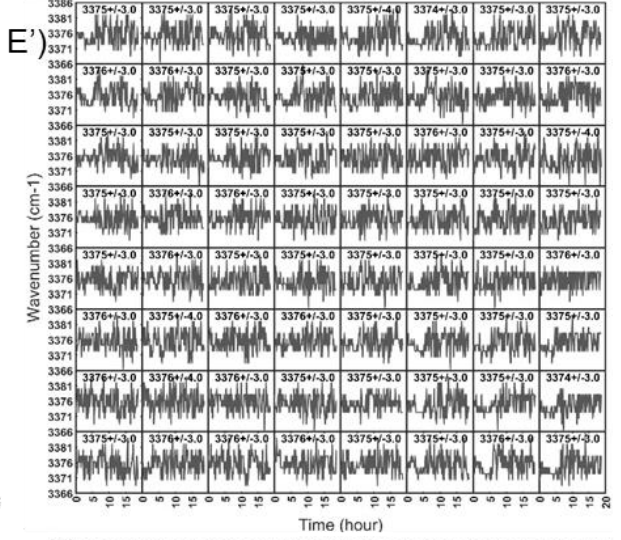
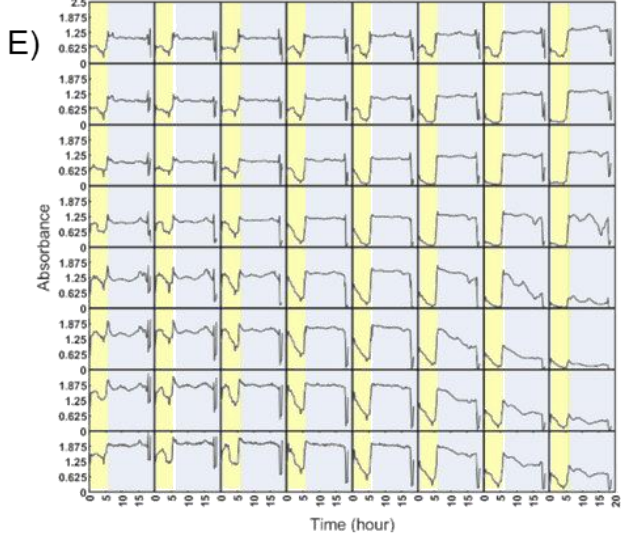
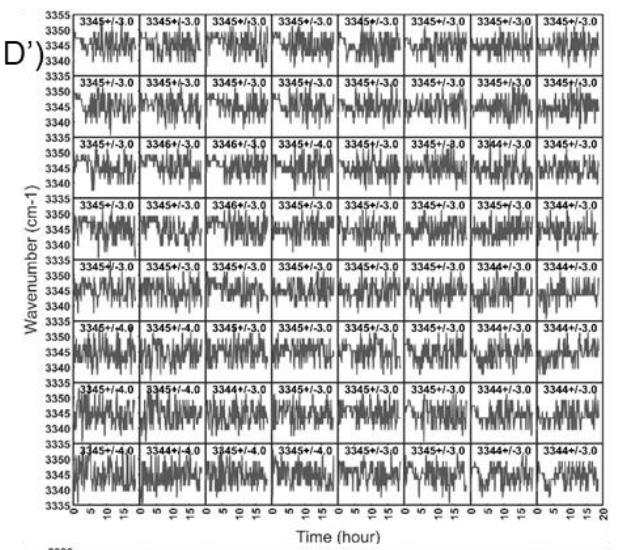
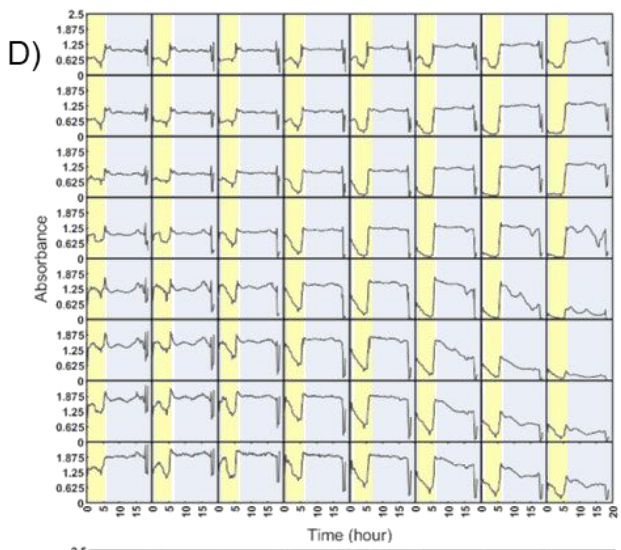
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The signal in some regions of the spectra were obscured by higher levels of moisture while other regions were less impacted. Figure S4. 1 shows time courses for absorption intensities and peak centers of the key hydrogen-bonding bands of cellulose (3326, 3369, 3300, 3345, 3376, 3403, and 3426 cm^{-1}) mapped over the region of interest. The plots confirm that temporal trends in signal intensity are captured within the first 5 hours, and that the absorbances saturate beyond 5 hours. The IR spectra in the cellulose fingerprint region ($\sim 900 - 1500 \text{ cm}^{-1}$), however, were less influenced by the added moisture as water does not absorb sufficiently in this frequency range. As such, changes in peak intensity over the entire observation period reflect changes due to enzymatic action (Figure S4. 2). As shown in Figure S4. 2, overall peak intensities of cellulose-associated IR bands decrease over the course of enzymatic hydrolysis, indicating general depletion of cellulose. Thus, the full time course data are used in the main manuscript to discuss changes to cellulose due to enzyme action.





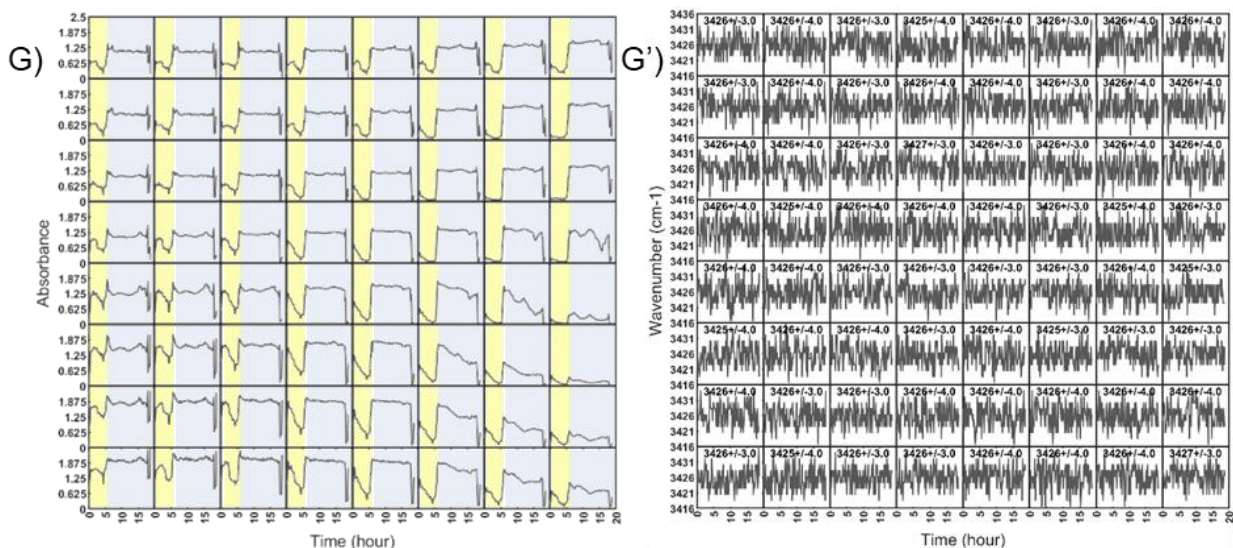
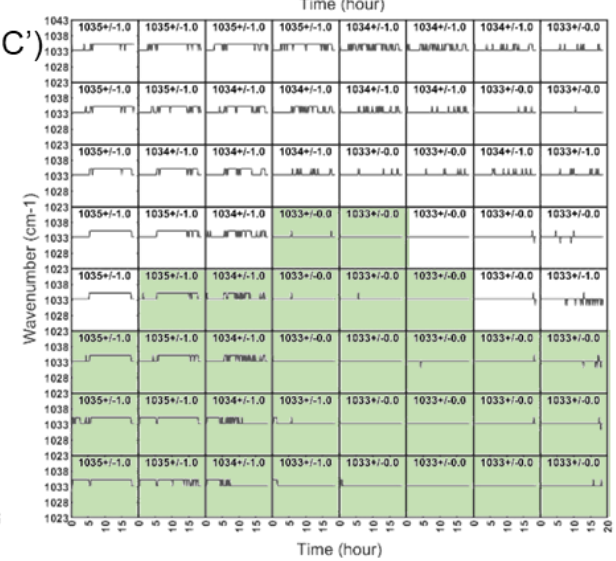
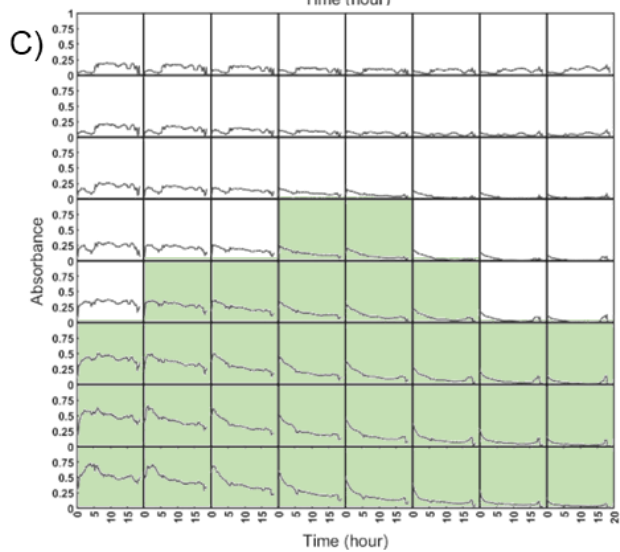
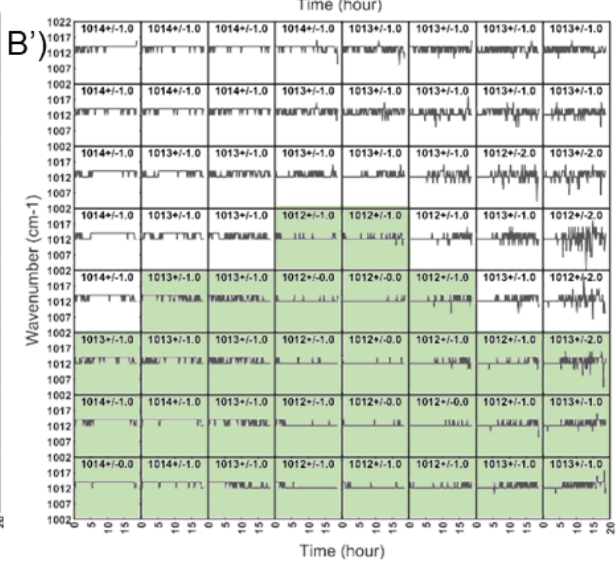
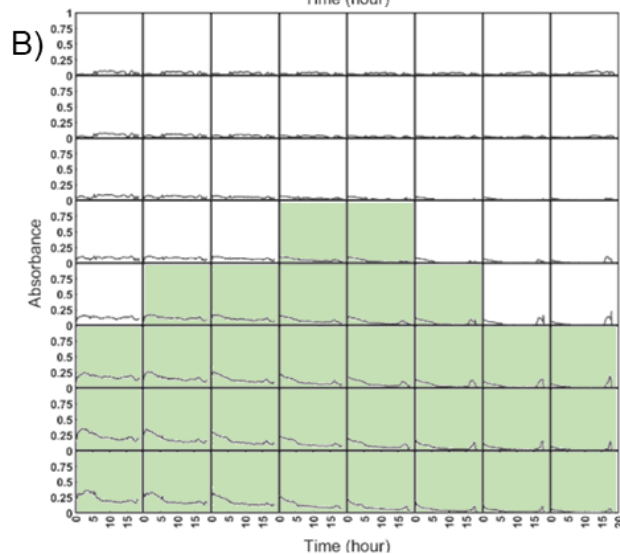
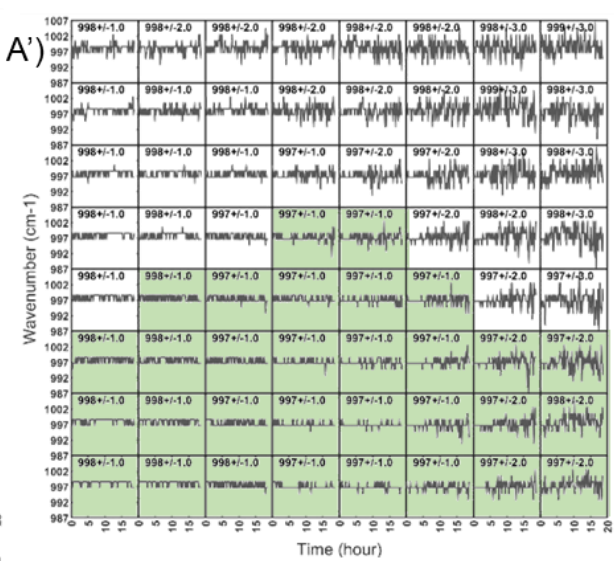
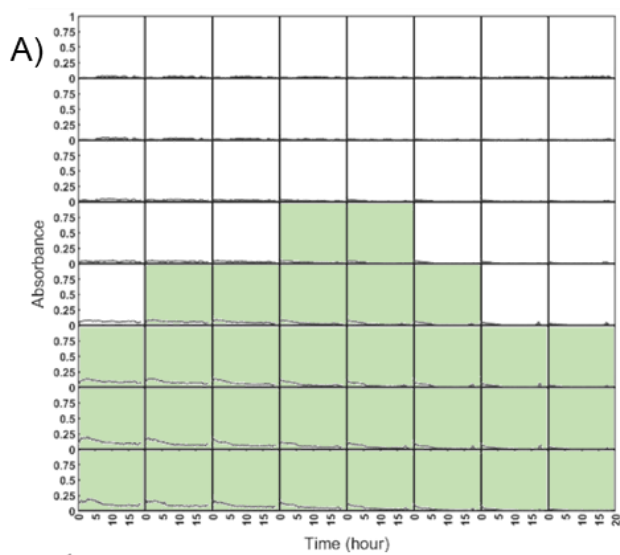
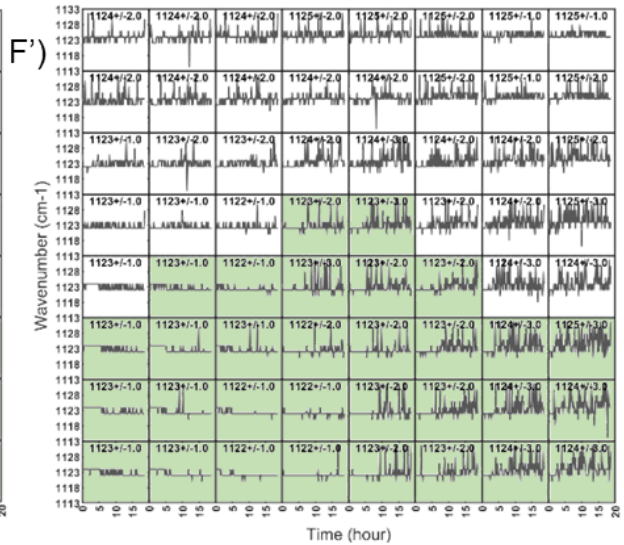
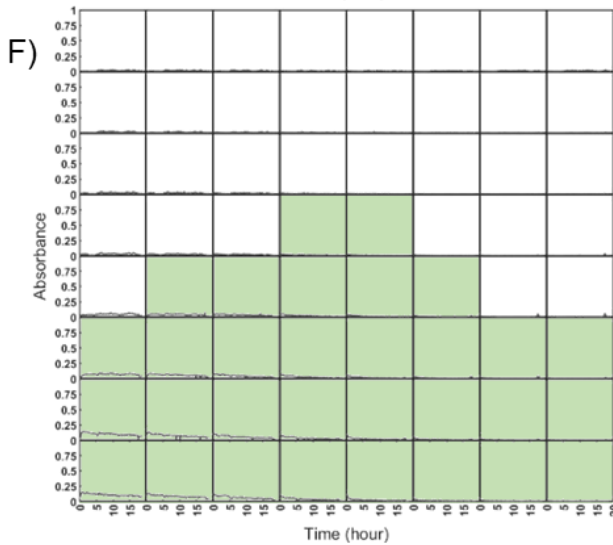
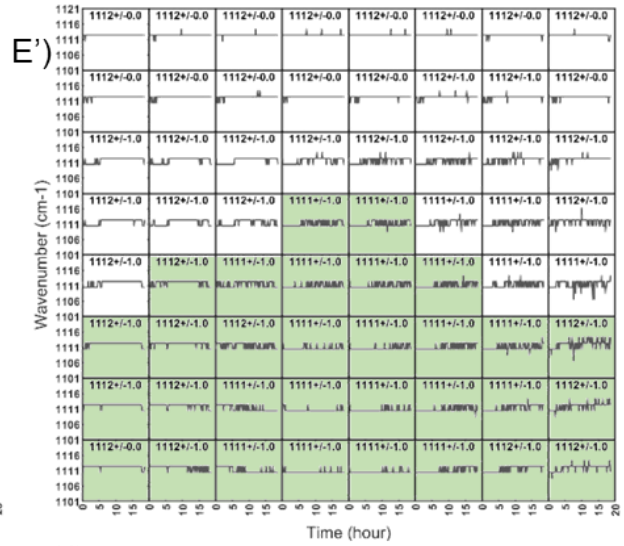
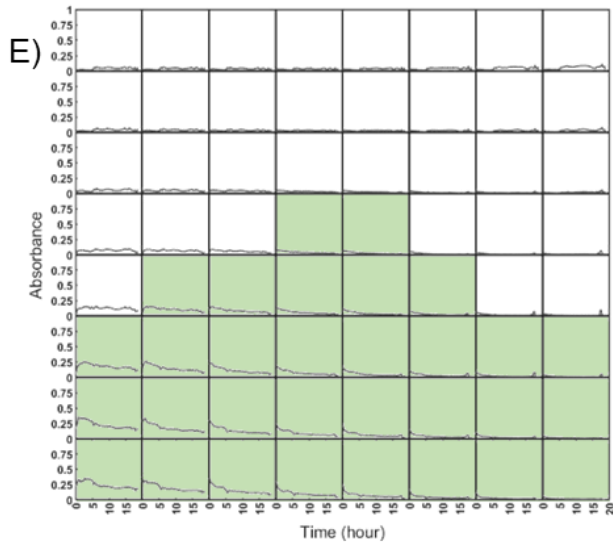
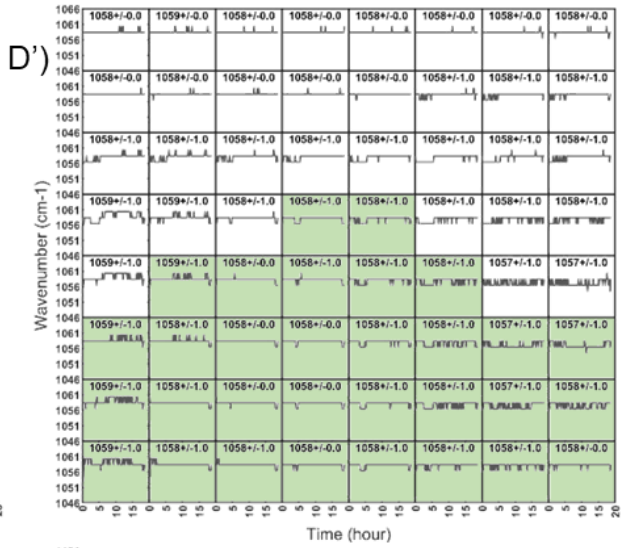
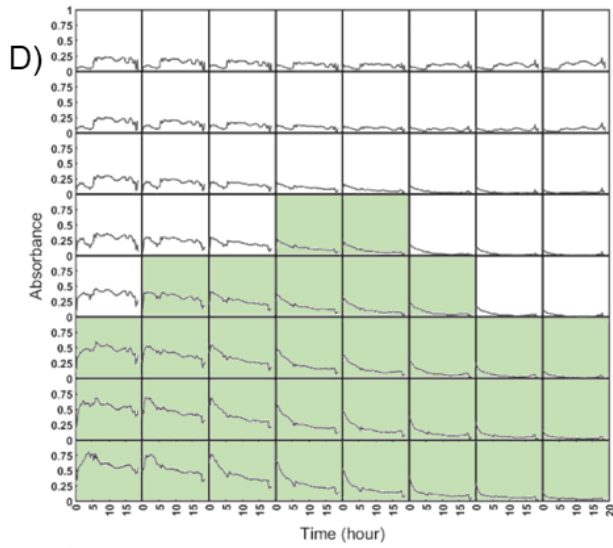


Figure S4. 1: Spatially mapped time course plots of IR band intensities (A – G) and peak centers (A' – G') centered around A) and A') 3226, B) and B') 3269, C) and C') 3300, D) and D') 3345, E) and E') 3375, F) and F') 3403, and G) and G') 3426. Plots are arranged in 8x8 grids, corresponding to sample locations where data were collected. In all plots, the y-axes range from 0 to 2.5 A.U; x-axes range from 0 to 20 h. In the peak intensity plots (A – G), the first 0-5h are shaded in yellow, 5-20h are shaded in blue. In the peak center plots (A' – G'), the average peak center and the standard deviation are shown at the top of each plot.





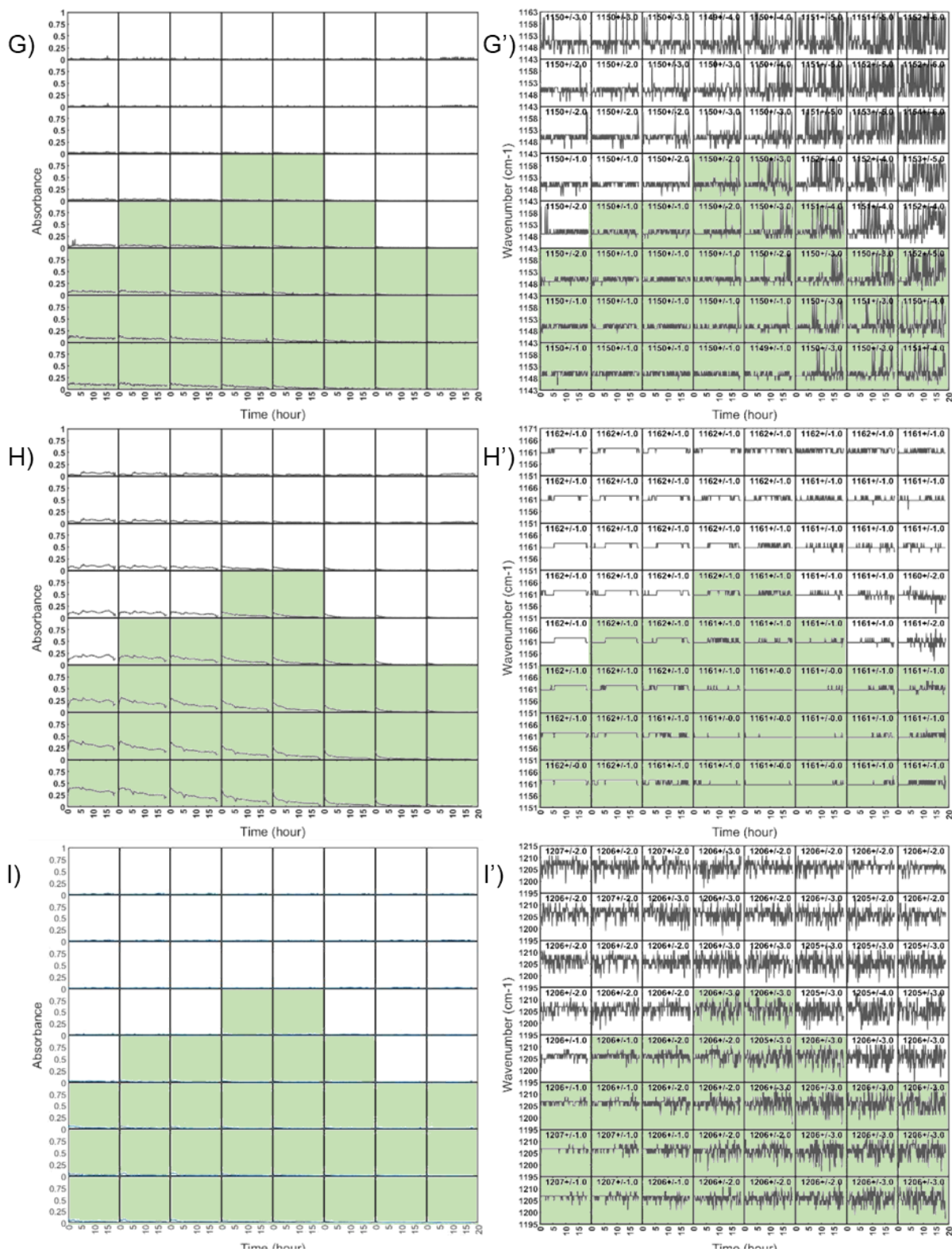


Figure S4. 2: Spatially mapped time course plots of IR bands centered at A) 998, B) 1013, C) 1034, D) 1059, E) 1112, F) 1123, G) 1150, H) 1161, I) 1205 cm⁻¹. Plots are arranged to correspond to the 8x8 grid shown in the main manuscript. In all plots, the y-axes range from 0 to 1 A.U.; x-axes range from 0 to 20 h. The region with the thickest initial cellulose sample is shaded in green.