The introduction of second generation fuels, based on enzymatic cellulose breakdown, might be one of the favorite prospects to encounter our ever growing need for new energy resources. The abundant bio-availability and accessibility of cellulose in our biosphere explains the extensive effort in today’s research to find applicable economic ways for the efficient degradation of cellulose. While the process of enzymatic degradation is known almost for a century the diverse structures of cellulose, its recalcitrant nature and the complex interplay of different enzymes during synergistic degradation elucidate the efficiency problems still present.

In recent years versatile in-situ studies using Atomic Force Microscopy (AFM) revealed strongly varying degradation mechanisms on different cellulose phases (crystalline/amorphous). This becomes highly relevant if they are side by side as found in nature. In order to emulate natural circumstances but still being able to control phase-related composition and its according dimensions, it is necessary to develop an artificial substrate which furthermore provides nanoflat surfaces to allow high-resolution AFM imaging.

In this study we present two new types of substrates to study the degradation of cellulose dynamically in liquid environments via high-resolution AFM. For the first approach, we introduce preparation protocols, entirely based on highly crystalline AVICEL cellulose, to fabricate a substrate with tunable content of amorphous and crystalline cellulose and a surface roughness below 10 nm. We used Raman spectroscopy, X-ray diffraction (Figure 2), scanning and transmission electron microscopy to demonstrate the tunable multiphasic nature of the cellulose substrates further denoted as “mixed amorphous-crystalline cellulosic model substrate” or shortly MACS.

Although well suited, there remains the issue of size control with respect to the crystalline areas in MACS. Therefore, a second concept is introduced, based again on a mixture of amorphous and nanocrystalline cellulose whiskers with typical dimensions of 300 nm to 30 nm. The combination of both phases in a tunable manner results in a nanoflat substrate further denoted as nanocomposite cellulose (TFNC).

Complementary in-situ AFM degradation studies with different enzyme types on MACS and TFNC confirm the expected behavior regarding affinity and activity of different cellulases (Figure 1) and demonstrate the close-to-nature character of both substrates. Hence, both approaches allow conclusions regarding the degradation of natural cellulosic materials, thus helping to understand the complex nature of the process.

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Fig. 1: Dynamic AFM measurements of enzymatic degradation of MACS cellulose using Trichoderma reesei cellulases. Due to the different degradation resistivity, highly crystalline particles (top left corner) are much less affected than the surrounding amorphous matrix.

Fig. 2: X-ray diffraction characterization of MACS substrates revealing the tuneability of the crystal content indicated by the evolution of the 101, 10-1, 021 and 002 related peaks of cellulose Iα together with the amorphous peak.

Fig. 3: Dynamic AFM measurements of TFNC substrates during enzymatic degradation revealing the excavation of crystalline parts (a --> b) followed by their full decomposition (b --> d).