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Synergistic Effects Among Cellulases during Enzymatic Cellulose Degradation, Visualized by In-situ Atomic Force Microscopy

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Cellulose, a biopolymer consisting of linked sugar molecules as polymer-units, is used by plants as a structural compound in their cell walls. It is the most abundant polymer of this kind on earth and by degrading it to sugar and then into ethanol it is a valuable resource of energy referred to as 2nd generation biofuels. These fuels do not only show a zero emission footprint since burned CO_2 is used again by plants to build up there cellulose livestock, it also triggers no food vs. fuel concerns as for 1st generation biofuels. In nature a variety of organisms use cellulose as energy resource via enzymatic degradation into sugar. The specialized enzymes, which can degrade cellulose, are called cellulases and are bio-catalytic protein units. An important and well investigated model organism in this research field is the fungus *Trichoderma reesei* which has also been used for this study.

Enzymatic cellulose decomposition is known for almost 70 years and has been investigated by various methods like electron microscopy and biochemical methods [3]. The process itself is empirical understood by combining results of electron microscopy and biochemical measurements but there are some missing links and unresolved questions related to it. While electron microscopy allows nanometer resolution, it cannot provide an appropriate environment for the enzymes due to vacuum conditions, thus inhibiting in-situ investigations. In contrast, biochemical methods allow time resolved characterization but lack microscopic information. Combing both processes would be a highly desirable way to answer fundamental questions and find solutions to the problems still attendant for the efficient fuel production out of cellulose.

In this study we have used atomic force microscopy in liquid environments, which allow dynamic in-situ characterization with single molecule resolution on a nanoflat cellulose surfaces (see Figure 1A). While most natural cellulose sources do not show sufficiently flat surfaces, we use a special method for the fabrication of nanoflat and reproducible cellulose substrates with tuneable crystalline / amorphous ratios (see Figure 1 B & C). The combination of such substrates with the supernatant of *Trichoderma reesei*, characterized via with liquid AFM, allow dynamic nanoscale observation of cellulose degradation on crystalline and amorphous areas, simultaneously (see Figure 2 A).

The core part of this contribution is the precise understanding of synergistic effects between different types of cellulases which plays a major role in decomposition of crystalline / amorphous mixed cellulose substrates. We first analyse the degradation behaviour of the supernatant followed by investigations of individual enzyme activities. Based on these results we can reconstruct the synergistic effects and gain new mechanistic understanding of the underlying degradation processes.

In particular, it is found that not only the ratio of individual enzymes in the supernatant plays a major role, but also the substrate morphology is of significant influence to the process. In more detail, two different degradation velocities are observed which depend on the structural properties of the substrate (see Figure 2 B) [1]. This is triggered by the individual enzyme activities with respect to the substrate structure and their mutual interaction which will be discussed in detail.

As a result of this in-situ AFM study in combination with biochemical experiments, it can be shown that exclusive tuning of the enzymatic ratio is not enough to achieve highest decomposition efficiencies. The substrates structure has to be taken into account as an essential part of the process.

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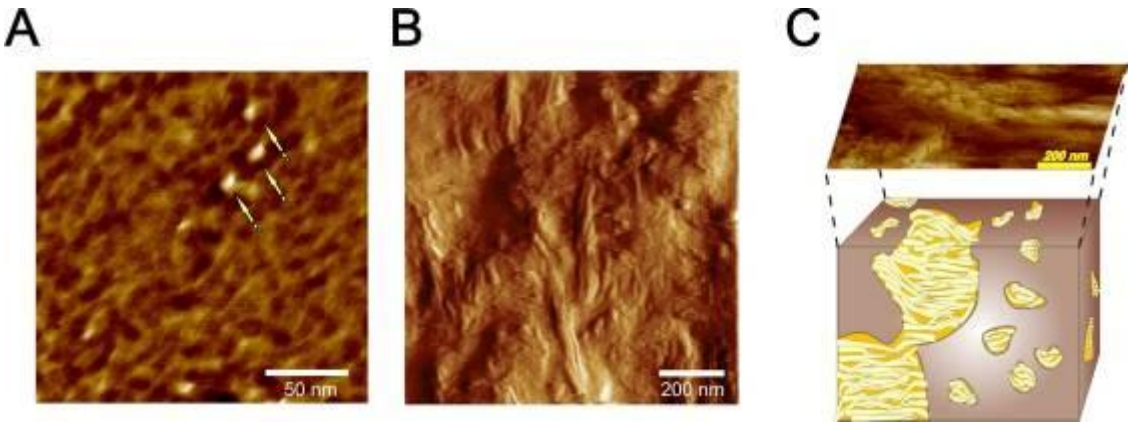

Figure 1 a.) Single enzymes (yellow arrows) on a cellulose surface [2]. b.) Cellulose surface during degradation, showing cellulose fibre-bundles (crystalline) embedded in an amorphous matrix as schematically shown in c.)

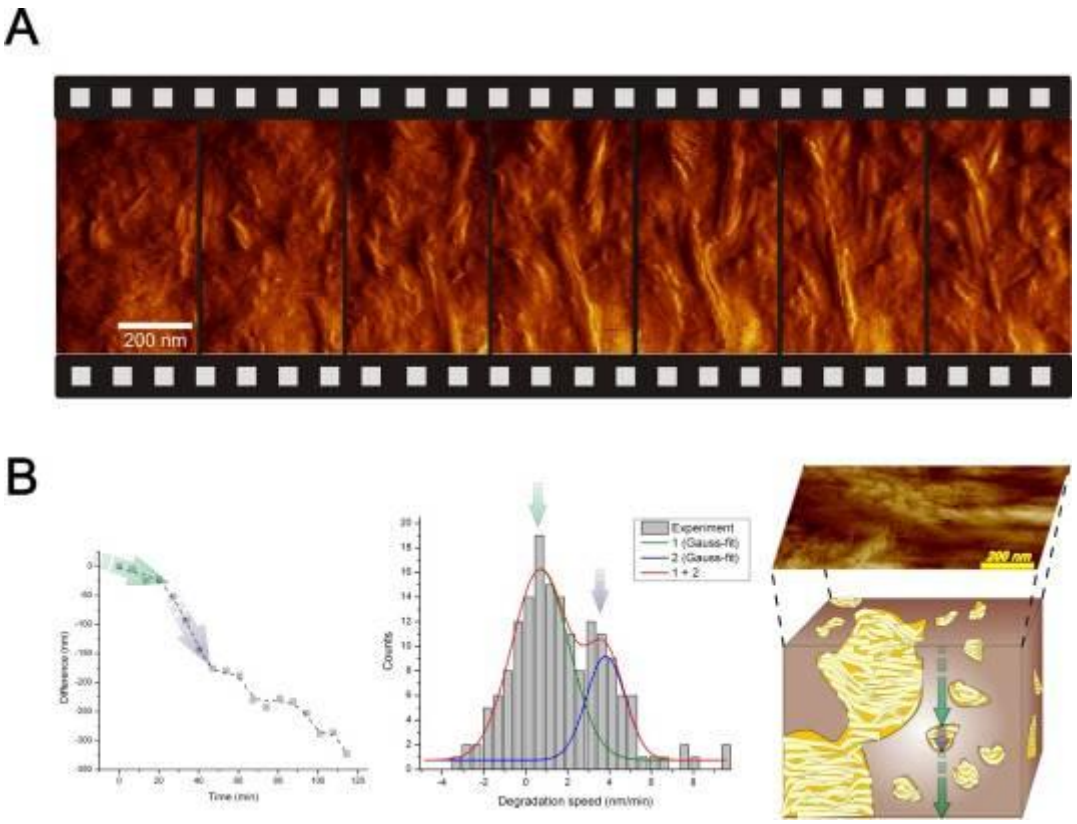

Figure 2 a.) Real time AFM in-situ observation of enzymatic cellulose degradation revealing cyclic appearance and decomposition of cellulose fibre-bundles. b.): representative degradation behaviour of one isolated spot (left), which reveals two different velocities. Statistical analyses (centre) confirm the presence of two distinct degradation rates which can be correlated to crystalline and amorphous areas (right) based on in-situ AFM experiments.