KEY WORDS: cellulose, enzymatic hydrolysis, second-harmonic generation (SHG), multi-photon excitation fluorescence (MPEF), coherent anti-Stokes Raman scattering (CARS)

Liberation of fermentable soluble sugars from cellulosic biomass during the course of enzymatic hydrolysis is the major obstacle to large-scale implementation of biorefineries due to high cost of enzymes. Enzymatic hydrolysis of cellulosic biomass is often incomplete and, therefore, it is of great importance to understand the limitations of the process. Among the limitations of enzymatic hydrolysis, structural properties of cellulose have an effect on enzymatic hydrolysis efficiency. Currently, there is a lack of direct methods for visualization and quantification of spatial polymer distribution in cellulosic biomass and monitoring of interactions between cellulose degrading enzymes and the substrate. In the current work, we used nonlinear microscopy to visualize cellulosic materials during the process of enzymatic hydrolysis. The overall aim was to contribute to understanding of relation between enzymatic hydrolysis and structural properties of cellulosic materials at the micro scale.

Enzymatic hydrolysis during the course of several hours was studied on three cellulosic materials: cellulose fiber, nano-crystalline cellulose (NCC) and commercial micro-crystalline cellulose (Avicel) particles. These materials were imaged with nonlinear optical microscope, employing multi-photon excitation fluorescence (MPEF), second-harmonic generation (SHG) and coherent anti-Stokes Raman scattering (CARS) modalities. Hydrolysis of cellulose fiber and NCC particles was more prominent compared to the particles of Avicel. Changes of the shape of cellulose fiber and NCC particles were observed during hydrolysis. We have investigated possible relation of the change in shape to the structure of the cellulosic materials prior to enzymatic hydrolysis and underlying structural changes during enzymatic hydrolysis.