Experimental report

13/12/2019

Proposal: 9-13-718 **Council:** 4/2017

Title: Enzymatic deconstruction of semidilute hemicellulose solutions

Research area: Soft condensed matter

This proposal is a continuation of 9-13-638

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Samples: Solutions/gels of arabinoxylan

Instrument	Requested days	Allocated days	From	To
D11	2	2	21/06/2018	23/06/2018

Abstract:

Enzymes that degrade lignocellulose are extensively studied by biochemists for developing eco-friendly transformation processes of plant biomass into fuels or plastics. However, little is known about the exact deconstruction course of "real" biomass materials. One fundamental objective is to characterize the action of the enzymes in conditions where the local concentration of polymer is elevated. We propose to contribute to this objective by following with SANS the deconstruction of semidilute arabinoxylan (AX) solutions in which enzymes are dispersed at time zero. We plan to determine precisely the variations of the characteristic lengths of the polymer as hydrolysis occurs. We will then analyze these variations using different scenarios for enzyme's generic behavior in concentrated polymeric systems.

EXPERIMENTAL REPORT for Proposal 77853

Enzymatic deconstruction of semidilute hemicellulose solutions

Experiments performed from 21/06/2018 to 23/06/2018

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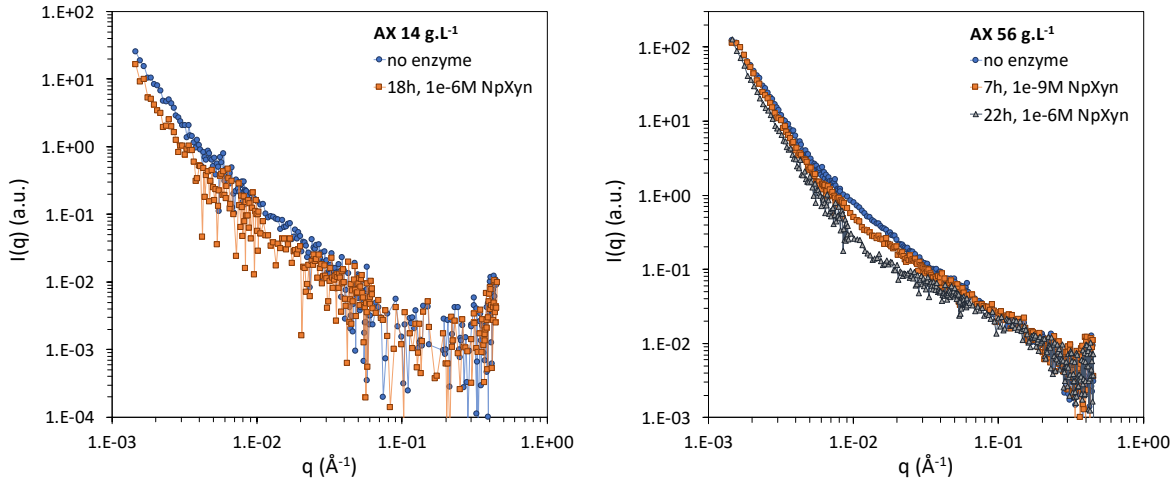
Context and motivation

. A crucial question for developing eco-friendly processes of enzymatic deconstruction of plant biomass is to understand how enzymes behave in an excess of their substrate, i.e. at concentrations where - as in a natural lignocellulosic substrate - the polymer concentration is such that the spatial constraints experienced by the enzymes possibly affects their kinetics and the nature of the products released. To answer that question, one of our approaches consists in following the action of one specific type of enzymes (hemicellulases) in pure model solutions of one hemicellulose polymer (arabinoxylase AX = backbone of xylose residues substituted with 0 to 2 arabinose residue(s)), at concentrations ranging from the dilute to the semi-dilute and concentrated regimes. At each concentration, our purpose is to follow the deconstruction process by characterizing the evolution of the structural and dynamical properties of the polymer solutions, the change in size distribution of the polymer chains (using SEC-MALS for instance), while of course measuring the kinetics of the enzymatic reaction (number of cleavages per unit of time).

. Proposal 77853 was motivated by some test experiments performed in 2016 on D11 that showed an unequivocal effect of enzymes action of the structural properties of semi-dilute AX solutions at two concentrations (see proposal). We found in particular that the AX solutions are heterogeneous and contains large and poorly hydrated regions surrounded by regions where the AX chains are under good solvent conditions and form an entangled network. The enzymatic degradation had a strong effect on the SANS profile at intermediate length scales, which we hypothesized could be due to different scenarios of attack. In order to go deeper into our analysis, the objective of proposal 77853 was to complement these results with experiments performed more systematically, in a wider range of polymer concentrations, enzyme concentrations, and reaction times.

Enzymatic deconstruction

. During our stay at ILL, we were able to acquire SANS profiles for three additional AX concentrations, thus nicely completing our first set of data obtained from D11 in 2016. Different concentrations of enzymes were also used: micro- and nanomolar. Some of these - excellent - results are given in Fig.1.



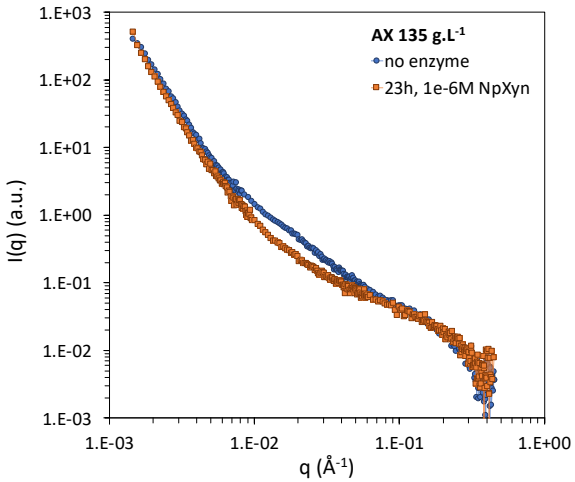


Figure 1. Examples of SANS results obtained with semidilute arabinoxylan (AX) solutions at various AX concentrations before (circles) and after enzymatic cleavage (squares and triangles). Before cleavage, the AX has an average Mw of 200 kDa and a C* comprised between 1-2 g/L. The enzyme (*Np*Xyn) is an endo-1,4-β-xylanase that supposedly cleaves randomly the main chain of the AX polymer.

. We find again that the enzymes action is visible at intermediate length scales only, with a clear drop in scattered intensity. As already shown (see proposal), it is possible to estimate a characteristic length ξ of the structure from a fit of the data to a Lorentzian scattering law at intermediate length scale. We are now in the process of building a more complete model that considers the SANS profile in its whole and model the data at all size ranges in a more precise way. The quantitative information obtained from the modeling will be compared to the kinetics and SEC-MALS experiments that were planned to characterize the enzymatic reaction; some of them are still needed to be performed.

. Note that we were not able to perform a 'clean' kinetics experiment where the change in the SANS spectra could be followed in-situ and at many times, as it was planned initially. This is essentially because our workplan was quite ambitious and that such an experiment is very difficult to plan correctly. Also SAXS on synchrotron instruments is probably more appropriate for this, with exposure times that are much less. However the danger in this case lies in the radiation damages that can occur with such polysaccharides solutions (the reason why we chose SANS in the first place).

Reversibility

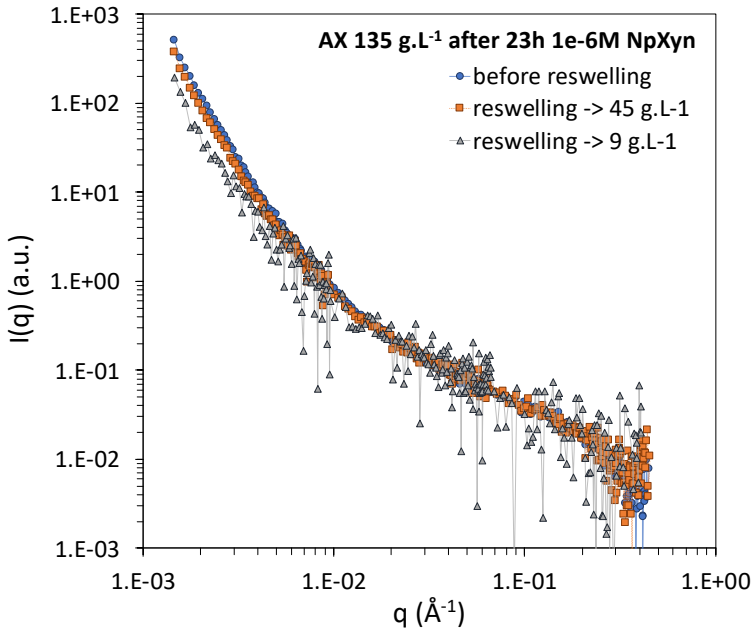


Figure 2. After 23h of hydrolysis, a 135 g.L^{-1} AX solution is reswollen by addition of successive volumes of H2O buffer until the concentration reaches 45 and 9 g.L^{-1}. After a shift along the y-axis, the shape of the three different spectra are virtually identical at intermediate and high q, while they differ a bit in the low-q region.

. One way of discriminating the different scenarios of enzymatic attack that were hypothesized in our proposal was to look at the changes in the shape of the SANS profile after the addition of water into the hydrolyzed samples. We successfully performed these experiments and find that there is virtually no difference between the SANS spectra at intermediate length scale (Fig. 2). This suggests that the entangled network that surrounded the poorly hydrated regions in the samples has been totally deconstructed by the enzymes before reswelling. The only length scale that is measured in this range would be the average size of the small polymer chains that populate the hydrated regions of the sample after hydrolysis.

Arabinofuranosidase

. For the majority of the experiments (Figs.1-2), we used a xylanase enzyme that cleaves the main chain of the polymer at random positions. During our stay at D11, we had the opportunity to perform some tests with an arabinofuranosidase (Abf). This other type of hemicellulase does not attack the backbone of the AX but rather cleaves the arabinose residues that are attached to the backbone.

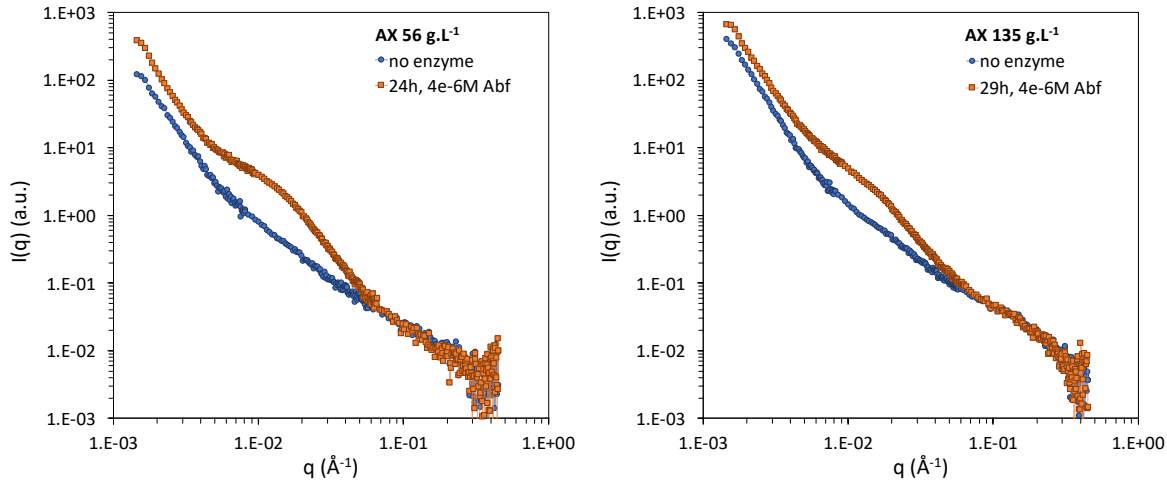


Figure 3. Action of an arabinofuranosidase (Abf) on 56 and 135 g.L-1 AX semidilute solutions. Abf does not cleave the main chain of the polymer and consequently does not change the contour length of the chains. Instead it cleaves the arabinose residues that are attached to the backbone of the chain. This is known to have the effect of reducing the hydrophilicity of the chains thus provoking chain association and aggregation.

. The consequences of this enzymatic activity on the SANS profiles are given in Fig.3 where we clearly see the appearance of a new scattering contribution at intermediate length scale. This is consistent with the fact that a turbidity arises in the samples subjected to Abf, as we observed visually. This is explained by the fact that the cleavage of the arabinose residues reduces the hydrophilicity of the chains and leads to the association and aggregation of the AX chains. The results of Fig. 3 suggest that this aggregation takes place at length scales $2\pi/q_p \approx$ 30-50 nm (with q_p the position of the 'peak' in the intermediate q-region), and this whatever the AX concentration of the samples. This is certainly an interesting result here but that still needs to be analyzed and interpreted through modeling and/or further experiments (SAXS, dosage of arabinose release at each concentration, etc.).