Application Note # 181

Real-time monitoring of xylanase-mediated degradation of xylan on cellulose surfaces using MP-SPR

This application note highlights how Multi-Parametric Surface Plasmon Resonance (MP-SPR) can be used to monitor enzymatic degradation of xylan on cellulose model surfaces in real time under flow conditions. The parameters calculated from the MP-SPR data, such as layer thickness and surface mass density reveal how xylan degradation is xylanase-concentration dependent.

Introduction

Monitoring enzymatic degradation directly at solid–liquid interfaces remains challenging because reactions occur within structurally heterogeneous, hydrated, and often viscoelastic polysaccharide layers. In xylan–cellulose assemblies, the substrate is not a uniform film but a composite interface with variations in density, accessibility, and hydration. These features complicate conventional analytical approaches, which typically rely on bulk measurements and fail to capture interfacial processes in real time.

MP-SPR overcomes these limitations by probing refractive index changes at the sensor surface with high sensitivity, enabling continuous monitoring of nanoscale changes in adsorbed biopolymer layers. Because the technique operates under flow and does not require labeling, it is particularly suited to studying enzymatic degradation of surface-bound substrates where mass transport, adsorption, and catalysis are tightly coupled.

Materials and Methods

Gold MP-SPR sensors slides were previously spin coated with trimethylsilylcellulose (TMSC) to produce a uniform and smooth thin film. Successively, the TMSC is converted to pure cellulose exposing the film to acidic vapor. Prior to MP-SPR measurements, the film is swollen in aqueous media, reaching a thickness of ~20-30 nm, then equilibrated in buffer. Xylan deposition was performed by spin coating the sensor with Xylan solution at different concentrations on top of the cellulose layer.

Real-time enzymatic degradation of Xylan by Endo-1,4-β-xylanase was monitored through MP-SPR at 25°C using an angular scan range of 50 to 78°. The first step consisted in equilibrating the previously TMSC Xylan coated sensor with sodium phosphate buffer 100mM at flow rates of 25 µL/min for 30 min. In the second step, the enzyme solutions (concentrations range 0.002-2 U/ml) were injected into the system and allowed to degrade the substrate for either 8 min at a flow rate of 25 µL/min. In the third step, the substrates were again rinsed for about 30 min with sodium phosphate buffer.

BioNavis MP-SPR Navi™ DataViewer software was used for data processing. De Feijter equation was used to calculate the amount of digested xylan (mg/m²).

$$\Gamma = \frac{\Delta\theta k \cdot dp}{dn/dc}$$

Δθ: change in SPR angle
dn/dc: refractive index increment
Γ: xylan areal mass
k dp: calibration factor, constant for thin films

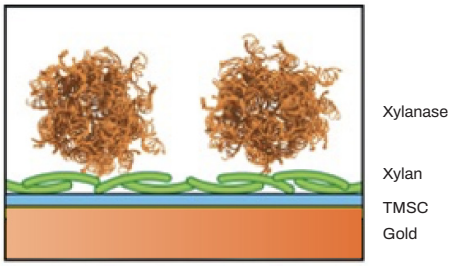

Figure 1. MP-SPR measures enzymatic degradation of a xylan layer in real time.

Enzymatic Degradation Process

MP-SPR measurements enabled direct, real-time monitoring of xylan degradation on cellulose-supported thin films through continuous tracking of resonance angle shifts. Upon injection of xylanase, a clear decrease in SPR response was observed at all the concentrations tested, corresponding to a reduction in surface-bound mass and layer thickness (Figure 2). The signal evolved dynamically during enzyme exposure, demonstrating that MP-SPR can resolve enzymatic hydrolysis at the solid–liquid interface without the need for labeling or endpoint analysis. This capability is strengthened by the use of ***ex situ*–fabricated xylan–cellulose thin films**, where the sensor is pre-coated prior to measurement, decoupling substrate preparation from the enzymatic assay. Such an approach provides a well-defined, multilayer, and morphology-controlled interface, ensuring that the recorded SPR signal reflects purely the **enzymatic degradation of a biomimetic surface**, rather than overlapping effects from simultaneous adsorption or film formation.

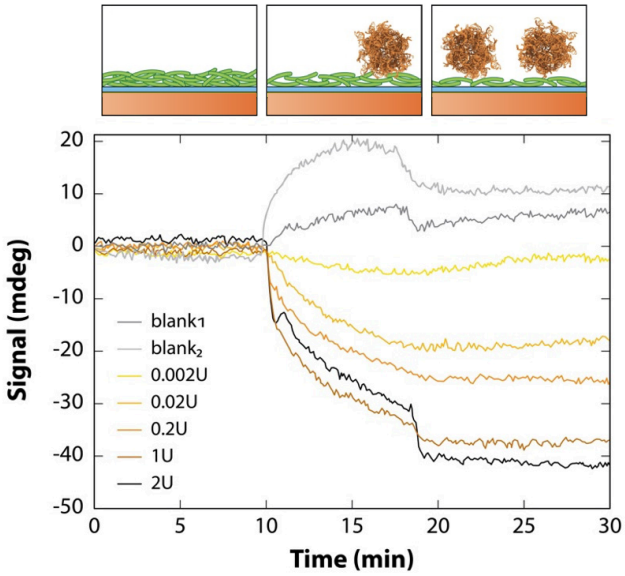

Figure 2. Concentration-dependent degradation of xylan–cellulose films measured by MP-SPR

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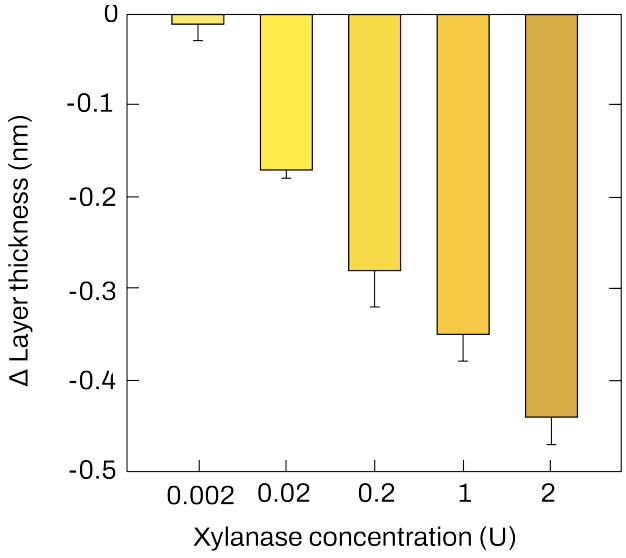

Figure 3. Higher enzyme concentrations producing reductions in the SPR signal, confirmed by the decrease in film thickness

Quantitative analysis of the MP-SPR data, based on optical modeling and the De Feijter relation, showed that the enzymatic treatment resulted in a decrease in xylan areal mass density ranging from approximately 0.01 ± 0.02 to 0.52 ± 0.04 mg/m^2. The magnitude of this decrease was strongly dependent on enzyme concentration (figure 2), with higher concentrations producing faster and more pronounced reductions in the SPR signal, confirmed also by the decrease in film thickness (Figure 3). This proves that MP-SPR provides a quantitative and concentration-sensitive readout of enzymatic activity directly at the interface.

The time-resolved MP-SPR response further revealed that degradation proceeds in a non-linear manner, characterized by an initial rapid decrease in signal followed by a slower phase approaching a plateau. This behavior indicates that enzymatic hydrolysis is not uniform throughout the film, but instead governed by accessibility of xylan chains and progressive depletion of readily degradable regions. The ability of MP-SPR to capture this transition highlights its suitability for resolving kinetic features of heterogeneous surface reactions.

A key observation from the MP-SPR measurements is that enzymatic degradation of xylan is incomplete. Even after extended enzyme exposure, the SPR signal does not return to the baseline corresponding to the cellulose-only surface. Instead, a residual signal remains, indicating the presence of a non-degradable or poorly accessible fraction of xylan. This residual layer cannot be detected by bulk analytical methods and underscores the advantage of MP-SPR in directly quantifying remaining surface material.

In addition to concentration effects, the MP-SPR data show that incubation time influences the extent of degradation, with longer exposure leading to increased mass loss. However, the rate of signal decrease diminishes over time, suggesting that enzymatic activity becomes limited by structural and diffusion constraints within the film. This reinforces the interpretation that the xylan layer is heterogeneous and partially shielded, with only a fraction readily accessible to enzymatic attack.

The SPR signal changes were interpreted primarily as mass loss due to enzymatic hydrolysis and desorption of soluble fragments, although the discussion acknowledges that structural changes within the remaining layer—such as compaction or variations in hydration—may also contribute to the optical response. The multiparametric capability of MP-SPR, combined with appropriate modeling, enables these effects to be evaluated alongside mass changes.

Conclusions

A well-defined cellulose thin film combined with a controlled MP-SPR workflow enables precise monitoring of enzymatic degradation at solid–liquid interfaces. The regeneration of cellulose from TMSC ensures a stable and reproducible substrate, while MP-SPR provides real-time, quantitative insight into changes in surface mass and thickness.

By integrating careful surface preparation with multi-wavelength SPR analysis under flow, MP-SPR emerges as an optimal technique for predicting degradation of different kinds of materials kinetics of such as xylan, in this case, delivering both sensitivity and mechanistic insight in a single, label-free experiment.

Original publication:

1. Schaubeder et al., Carbohydrate Polymers, 2024.
2. Schaubeder et al., , Carbohydrate Polymers, 2022.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 210A VASA & 220A NAALI

Sensor surface: Au

Software: MP-SPR Navi™ Control, DataViewer, LayerSolver™ for MP-SPR Navi™

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