Analysis of interactions with hydrated films using the Insplorion Acoulyte for combined NPS and QCM-D measurements

The combination of two label-free, surface sensitive measurement techniques based on different physical principles - Insplorion's NanoPlasmonic Sensing (NPS) and Q-Sense quartz crystal microbalance with dissipation monitoring (QCM-D) - enables detailed studies of the dynamics of hydrated films. Here, this is demonstrated by investigating the enzymatic hydrolysis of spin-coated polyester thin films using two enzymes that differ in their hydrolytic mechanism.

Introduction

The accumulation of persistent polymers in aquatic and terrestrial environments is a widespread concern. Biodegradable polyesters have significant potential to replace traditional persistent materials and to thereby reduce the environmental accumulation of plastics. Polyester biodegradation involves hydrolysis of its ester bonds by enzymes. This enzymatic hydrolysis is considered the rate-limiting step in overall polyester biodegradation. Hence, clarifying the mechanistic details of the dynamic interaction process between the hydrolysing enzyme and the polyester film is of great importance. Here, it is demonstrated that combined NPS and QCM-D measurements can be used to help study this process. The complementarity in the measured quantities ("dry" (optical) vs. "wet" (acoustic) mass) as well as the different probing depths of the two techniques (60-250 nm for QCM-D and ca. 30 nm for NPS) is used to obtain detailed information on the hydrolysis process.

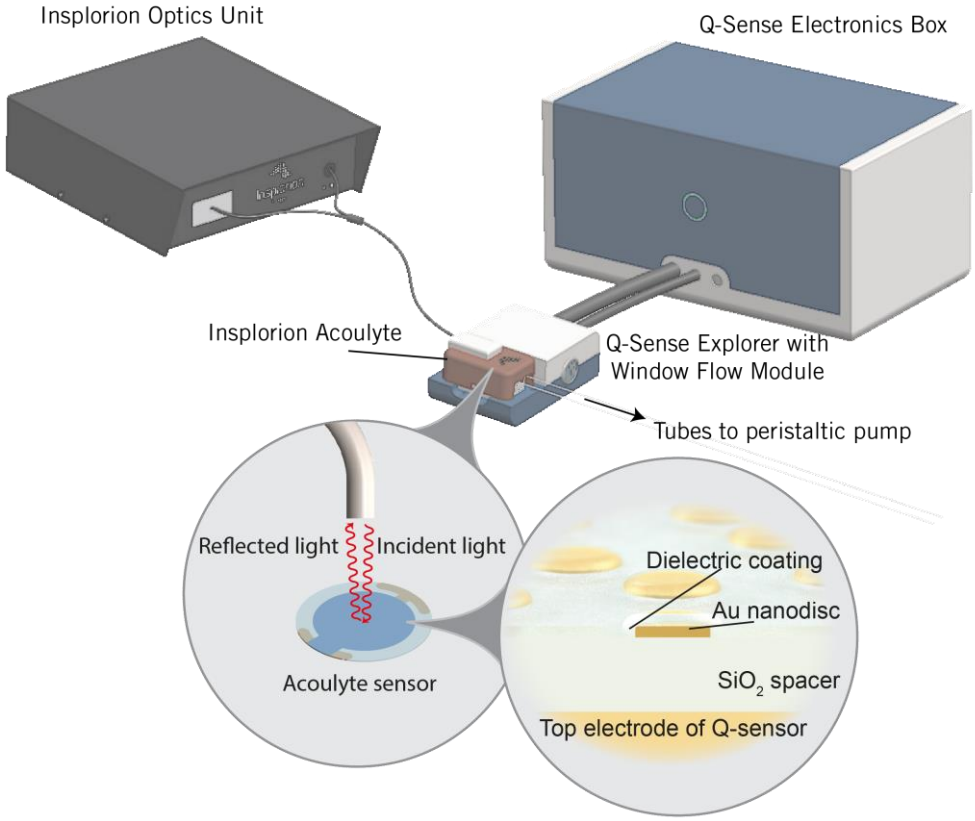

Figure 1. The Insplorion Acoulyte module mounted on a Q-Sense E1/Explorer instrument.

Experimental Procedure

The experiments were performed using the Insplorion Acoulyte system with Si3N4 coated sensors.
In a first step, thin films of the polyester (poly(butylene adipate (i.e., PBA) were spin-coated onto the sensors. In a second step, the enzymatic hydrolysis of these films by two different esterases *Rhizopus oryzae* lipase (RoL) and *Fusarium solani* cutinase (FsC), was studied.

Results

Figure 2 shows the changes in the NPS signal Δλ and the QCM signal (-Δf/13 for the 13:th overtone) during enzymatic PBA hydrolyses. The shape of the curves describing the PBA film mass during enzymatic hydrolysis was different for the two enzymes. RoL hydrolysis was a two-stage process. During the first stage, enzymatic hydrolysis resulted in the incorporation of water into

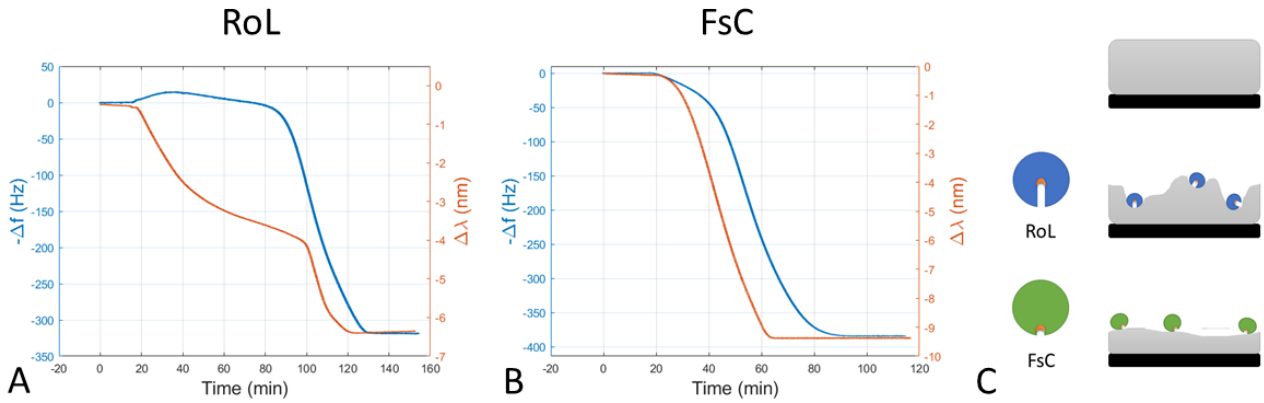

Figure 2. Frame A and B shows the hydrolysis process for RoL and FsC. Blue curves correspond to frequency shift, -Δf/13 for the 13:th overtone and red curves to centroid shift, Δλ. Frame C shows the proposed schemes for the different hydrolysis processes.

the polyester film, as evidenced from the decrease in the 'dry mass' (i.e., Δλ) while the 'wet mass' (i.e., -Δf) slightly increased. During the second stage, the wet film was removed from the sensor surface, as indicated by the rapid decreases in both Δλ and -Δf. Hydrolysis by FsC did not show this two-stage behavior. Instead, both the 'wet mass' and the 'dry mass' of the thin PBA film decreased more monotonously (as seen from similar changes in Δλ and – Δf over time in Figure 2).

These observations point at different film hydrolysis pathways for the two esterases. *RoL* as a lipase requires a hydrophobic surface to reach maximal hydrolytic activity, resulting in a preference for erosion vertically into the film rather than laterally on the film surface. By contrast, FsC as a cutinase can readily hydrolyze ester bonds in/on polar substrates, thus resulting in lateral hydrolysis on the polyester surface. RoL-mediated hydrolysis of the PBA therefore progressed through a water-rich polyester film intermediate, which was not formed during FsC-mediated hydrolysis (Scheme in Figure 2C).

Conclusions

Here, the Insplorion Acoulyte was used to study differences in the dynamics of enzymatic polyester hydrolysis by *Rhizopus oryzae* lipase and *Fusarium solani* cutinase. Combined NPS and QCM-D measurements helped to decipher enzyme-specific hydrolysis pathways that lead to differences in the hydration states of polyester film intermediates during the hydrolysis.

This study was performed by Michael Zumstein and Michael Sander with coworkers at ETH. The concept was originally studied in:

Reference

[1] M.T. Zumstein et al. *Enzymatic Hydrolysis of Polyester Thin Films at the Nanoscale: Effects of Polyester Structure and Enzyme Active-Site Accessibility*. DOI:10.1021/acs.est.7b01330
[2] M.T. Zumstein et al. *Enzymatic Hydrolysis of Polyester Thin Films: Real-Time Analysis of Film Mass Changes and Dissipation Dynamics.* DOI:10.1021/acs.est.5b04103
[3] M.T. Zumstein et al. *High-Throughput Analysis of Enzymatic Hydrolysis of Biodegradable Polyesters by Monitoring Cohydrolysis of a Polyester-Embedded Fluorogenic Probe.* DOI: 10.1021/acs.est.6b06060

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