

Enzymatic degradation of lipid films caused by lipase

The removal of lipids from surfaces is a common household task for detergents. In the following application note the enzymatic degradation of a lipid film is investigated in real time by using the Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring technique.

Experimental

A nano-sized (~100nm) lipid layer of triolein (triacylglycerol) was formed on a gold-coated quartz crystal by evenly distributing the triolein by a soft lens paper. The crystal was mounted into the measurement chamber and the lipid layer was let to stabilize in buffer solution before it was exposed to the lipase (lipid degrading enzyme). The changes in frequency and dissipation were continuously monitored during the enzymatic degradation of the lipid film. Frequency and Dissipation data were processed, by using the viscoelastic model described by Voinova¹, to extract physical properties such as thickness, viscosity and elasticity of the triolein film.

Results and discussion

The frequency data showed that the enzymatic degradation of the lipid film involved mass loss from the surface. The dissipation data indicated an initial softening (first minutes) of the film followed by later stiffening.

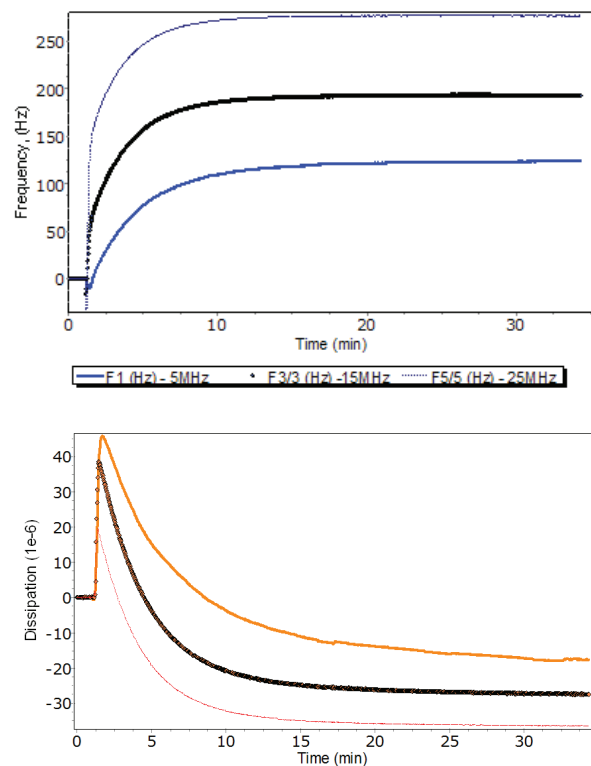
Viscoelastic modelling*

The frequency and dissipation data (Figure 1) was modelled with the viscoelastic model incorporated in QTools with a fixed lipid layer density of $0,95 \times 10^3 \text{ kg/m}^3$. The output from the viscoelastic model (Figure 2) showed an initial increase in thickness followed by a long-term decrease. The viscosity and elasticity also showed different initial responses compared to the response after longer times. Partly based on the results of the viscoelastic model, a mass ejection model describing the enzymatic degradation of lipids was proposed. Figure 3 shows the degradation model which is comprised of the following phases; (A) lipase molecules adsorb on the lipid film, (B) start of enzymatic activity, build-up of charged products and formation of lipid clusters at the lipid surface, film swelling (C) water solvation and ejection of clusters into the liquid phase, eventually complete degradation of the lipid layer.

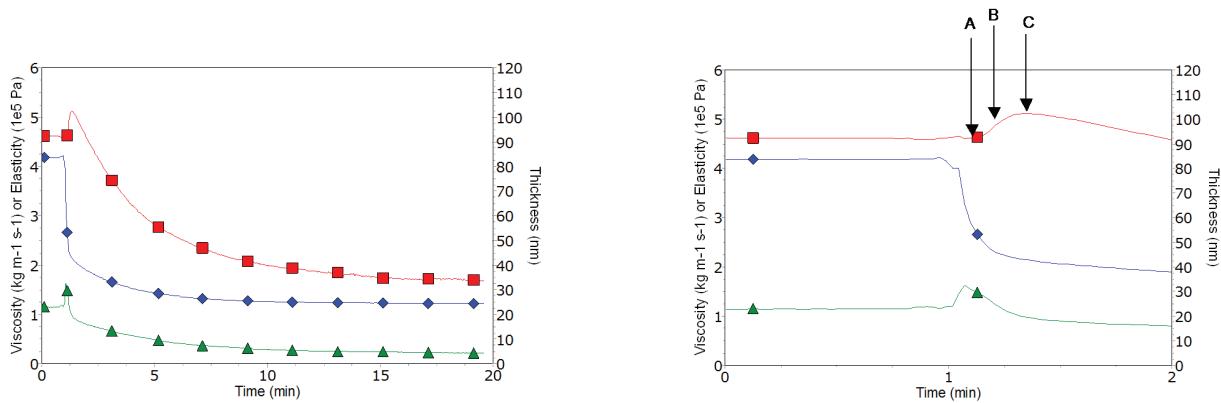
The results presented in this application note is a summary of

work published by Torben Snabe and Steffen Bjørn Petersen at the Section of Biostructure and Protein Engineering, Institute of Life Science, Aalborg University, Denmark^{2,3}.

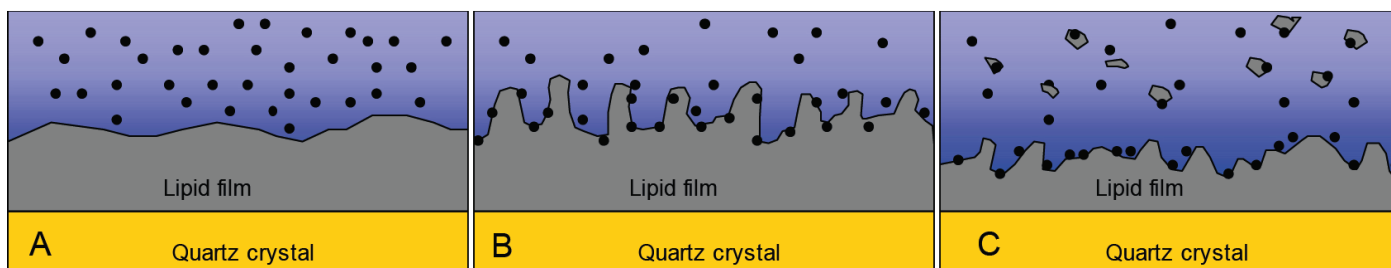
*) Viscoelastic modelling and curve fitting is included in the evaluation software QTools provided by Q-Sense.



[Figure 1]: Frequency and Dissipation response during enzymatic degradation of triolein.



[Figure 2]: Results from viscoelastic modelling. Viscosity (◆, blue), elasticity (▲, green) and thickness (■, red). The complete experiment is shown to the left and the first 2 minutes (indicated by a dashed box) is shown to the right. The different phases of the degradation model is indicated by arrow A, B and C.



[Figure 3]: Proposed model for enzymatic degradation of lipid films. Adsorption of lipase molecules (A), start of enzymatic activity, build up of charged products and formation of lipid cluster at the surface (B), water solvation and ejection of lipid clusters (C).

Conclusions

QCM-D is a very useful technique to study enzyme activity *in situ*. Real time variations in thickness and viscoelastic properties of the lipid film during degradation caused by lipase, are easily followed.

References & further reading

- [1] Voinova, M. V., Rodahl, M, Jonson, M, Kasemo, B. Viscoelastic acoustic response of layered polymer films at fluid-solid interfaces: continuum mechanics approach. *Phys. Scripta*, 59, 391-399 (1999).
 - [2] Snabe, T, Petersen, S. B., Lag phase and hydrolysis mechanisms of triacyl glycerol film lipolysis. *Chemistry and Physics of Lipids*, 125, 69-82 (2003).
 - [3] Snabe, T, Petersen, S. B., Application of infrared spectroscopy (attenuated total reflection) for monitoring enzymatic activity on substrate films. *Journal of Biotechnology*, 95, 145-155 (2002).
- Westwood, M, Roberts, D, Parker, R, Enzymatic degradation of poly-L-lysine-polygalacturonic acid multilayers. *Carbohydrate polymers*, 84, 960-969 (2011).
- Hardesty, J, O., Cascão-Pereira, L., Kellis, J, T., Robertson, C., R., Frank, C., F., Enzymatic proteolysis of a surface-bound α -helical polypeptide. *Langmuir*, 24, 13944-13956 (2008).