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# Enzymatic hydrolysis of cellulose: Effect of surface structure

The reduced availability of nonrenewable energy resources, as well as environmental concerns, has resulted in high interest in converting cellulose into biofuels. In the study reviewed here, four types of cellulose were investigated using Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring.

## Introduction

Biofuels can be produced from carbohydrates, including those derived from cellulose and other polysaccharides. However, enzymatic conversion of for example sugar cane or corn into biofuels is orders of magnitude more efficient than conversion of cellulose. An underlying issue is the fact that some of the sources of carbohydrates are better used in the production of food. This is not the case with cellulose. Therefore, there is intense research to increase understanding of the enzymatic conversion of cellulose into biofuels. The substrate's chemistry, heterogeneity, crystallinity and surface area strongly affect the kinetics of the enzymatic conversion. In this study, four different kinds of cellulose model films were analyzed with QCM-D to unveil details of their enzymatic hydrolysis upon incubation with cellulase enzyme mixtures, cellulases (Figure 1).

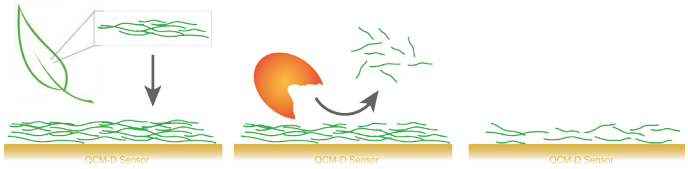
Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring is a surface-sensitive, real-time technique that provides real-time information on mass and structure of thin molecular films.

## Experimental

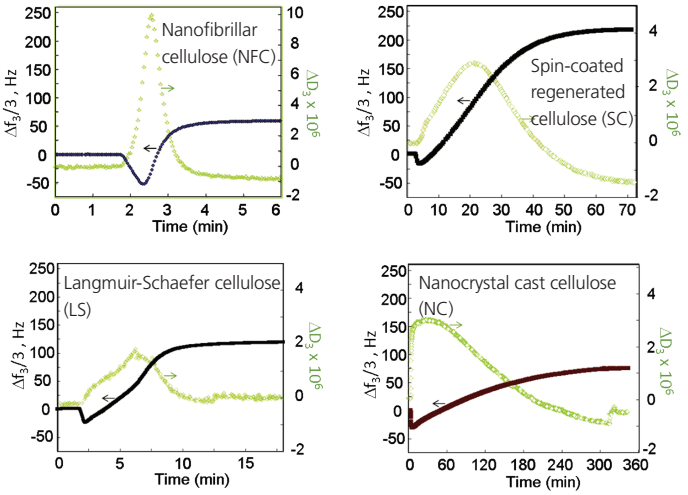
A cellulose nanofibril film (NFC) was studied and compared with three other cellulose model films (Figure 2). The films had different chemical and structural properties, and were prepared on QCM-D sensors to compare enzymatic hydrolysis. A commercially available enzyme mixture, optimized for efficient hydrolysis, was used. The four cellulose-coated sensors were subjected to the enzyme solution, and the effects of temperature (20-40°C) and enzyme concentration (0.01-0.5% (v/v)) on hydrolysis of the substrate were analyzed.

## Results and discussion

For the NFC film, faster rates of hydrolysis were observed at higher temperatures. However, compared to the effect of enzyme concentration, the incubation temperature was of less importance, as far as the overall hydrolysis rate is concerned. The higher temperature (40°C) was used for further experiments. Also, the extent of enzyme adsorption, dynamics of binding and substrate degradation were compared using QCM-D. The main




**[Figure 1]:** Illustration of how cellulose can be used to coat the QCM-D sensor and then be digested by a cellulase. The result can be followed by QCM-D.




**[Figure 2]:** Changes in frequency (f) and dissipation (D) for the four model cellulose films subjected to cellulase. Data is shown for the third overtone. Filled squares correspond to frequency and open diamonds correspond to dissipation. The nature of the cellulose films explains the differences in the dissipation curves.

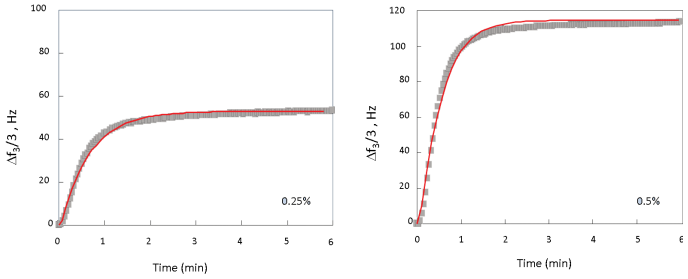
difference between these films was the degree of crystallinity and the crystalline form of the cellulose. The degradable mass varied for the four samples because of differences in film preparation and varying particle size of the cellulose materials. As cellulases were injected, a typical fast decrease in frequency was detected. This frequency decrease relates to the mass increase, as cellulases

are rapidly bound to the cellulose-coated surface. After incubation, the frequency increased, indicating a mass release from the surface when cellulose hydrolysis started. These phases could be detected with QCM-D for all model films studied, although the respective kinetics differed significantly. The more crystalline the cellulose structure, the slower the degradation kinetics.

The energy dissipation shown by QCM-D measurements increased during cellulose hydrolysis, as a result of increased softness (viscoelasticity) of the films. The explanation proposed is that the cellulose layers were cleaved, and the film became more hydrated and swollen. The observed maximum ΔD value for the NFC model film occurred very early and was very large compared to the ΔD values for the other three model films (Figure 2). The high ΔD value indicates that intact nanofibrils were liberated from the surface in an early phase, so that they protruded into the bulk solution. A characteristic of the NFC film is the high rate of hydrolysis. This is judged by the frequency profiles shown in Figure 2. The experimental results for the hydrolytic phase for the NFC model film were modeled using Equation 1 describing the hydrolytic activity.

$$\Delta f = A + \frac{B - A}{\left(1 + e^{\frac{V_{50} - t}{C}}\right)} \qquad \textbf{[Equation 1]}$$

Figure 3 presents the dynamics of hydrolysis as the cellulases start to act, both experimental data and best fit. This figure clearly shows the concentration dependence of film hydrolysis.




**[Figure 3]:** The change in frequency shows the hydrolytic activity for the NFC model film. Experimental values are plotted using filled squares and the best fit is indicated with a solid line. The phase modeled is that occurring after the initial cellulase adsorption step. Two different enzyme concentrations were used: a) 0.25% v/v and b) 0.5% v/v. Figures used with permission from the authors.

## Conclusions

QCM-D was successfully used to investigate enzymatic degradation of four different cellulose model films. This study shows that the structure and chemical properties of these films influence cellulose hydrolysis. The QCM-D study also shows that hydrolysis is affected by enzyme concentration and temperature. This deeper understanding of the nature of cellulose films and the effect of cellulases on such films is a step towards making cellulose a more realistic competitor to carbohydrates as a raw material for new biofuels.

## Acknowledgements

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**References and further reading:**

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