

# EARLY DETECTION OF BIOFILM FORMATION ON STEEL SURFACES

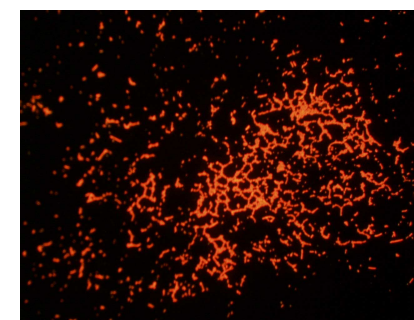
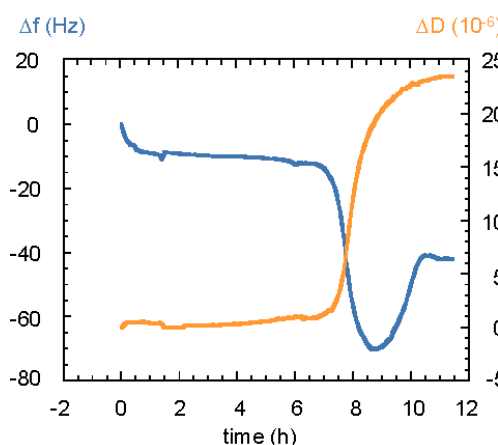
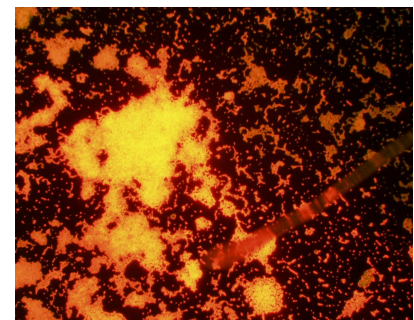
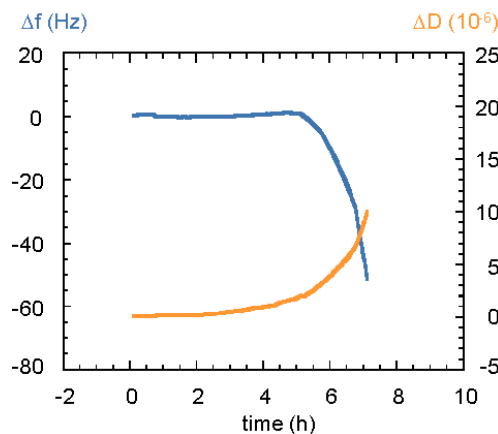
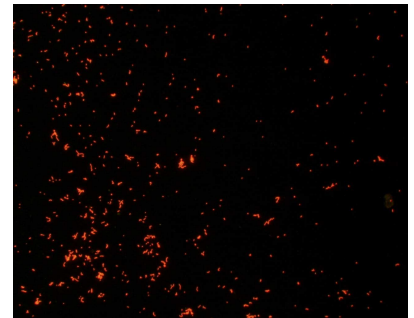
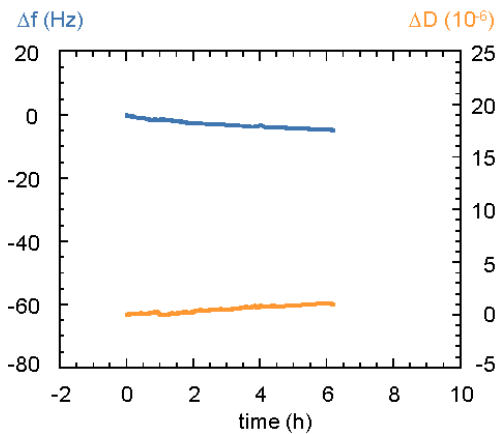
## INTRODUCTION

In this application note QCM-D has been used to detect early biofilm formation on stainless steel surfaces. The results show that the technique is sensitive to bacterial attachment and growth. The ability to measure viscoelastic properties by dissipation measurements were proven useful for detection of slime formation from the formation of *Leuconostoc mesenteroides* cultivated on sucrose.

## RESULTS

To verify the relationship between the responses in  $\Delta f$  and  $\Delta D$  and the adhering bacteria, a series of experiments were performed where the measurement was interrupted at different time points and the crystals were subsequently analyzed with epifluorescence microscopy. The relationship seemed clear: the number of cells on the surface was highest when the frequency reached its lowest value (**Figure 1**). At the same time the pictures gave information about potential infection by other microbes than *L. mesenteroides*, which is sometimes the case.

Early experiments gave an indication of a phenomenon in the frequency curve, which turned up several times; when the cells grow the frequency curve reaches a minimum after a while, before it raises a bit again. This appearance was not recognized in the dissipation curve, instead the dissipation just flattened out to a new level.



**Figure 1.** Series of interrupted experiments and correlation to photographs for fructose measurements with *L. mesenteroides*. The frequency shift indicates mass attachment and the dissipation shift indicates if the adsorbed mass is soft (high dissipation) or rigid (low dissipation).

As the cells adhere to the surface and divide, the frequency decreases due to mass uptake, and the dissipation increases due to water trapped in the formed film. When the bacteria attach to the surface, they start to produce slime (exopolysaccharides, EPS). If this secretion of slime to the surroundings of the cell occurs from all sides, even the side contacting the surface beneath, it would lead to a movement of the cell upwards and out of the detection range as shown in **Figure 2**. According to literature studies, it is hard to discover what happens to the biofilm cells adjacent to the surface. If the cells prefer sticking to a surface rather than swimming freely in a solution, a move out into the slime matrix could be regarded as a drawback as the surface then disappears beneath them. However, there is a fact supporting the theory illustrated in Figure 2: Costerton et al. (Microbial Biofilms, Annual Review of Microbiology, vol 49:711-45, 1995) have shown that, in some biofilms, most of the cells are located just beneath the interface between the film and the liquid. This indicates that the bacteria do not necessarily seek the underlying surface as long as they are situated within the stable micro-environment of the biofilm. If the return of the  $\Delta f$  curve to higher frequencies is an effect of a slime forming stage during biofilm formation, it is consistent with the constantly increasing D-factor.

Even if bacteria do leave the surface when slime production is high the amount of bacteria seen in the photographs in Figure 1 should not be that much lower. The explanation for this phenomenon is probably that the secreted EPS hinder the Acridine Orange (that the epifluorescence microscopy detects) to reach the bacteria. The polymers within the EPS are hydrophilic and may prevent Acridine Orange to penetrate the film to the same extent as with less or no EPS present in the film.

### CONCLUSIONS

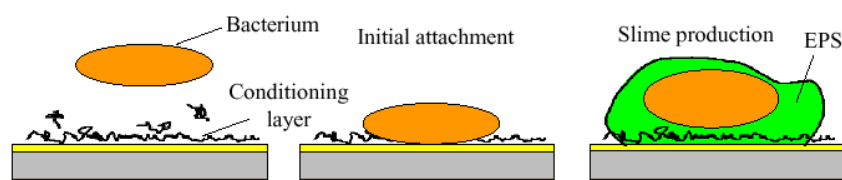
Since QCM-D measures in real time and offers detection of both mass and structural changes of biofilms during formation, a better understanding of the initial steps of biofilm formation can be achieved. In this particular ex-

ample there is a lag phase of about 4 hours, followed by rapid bacteria growth. After about 6 hours the bacteria start producing slime, which changes the frequency/dissipation ratio; the biofilm becomes softer.

A benefit of QCM-D is that substrates such as steel can be used without any need for calibration and without affecting the sensitivity of the instrument.

### REFERENCES

This example is a part of a Master thesis by Hans Green conducted in 2001 within a 5:th framework European Union Project, QLRT-1999-01389, "*Better management of process water systems by biotechnological identification and treatment of detrimental microorganisms and metabolites*", - Biotech Control



**Figure 2.** A possible explanation of the lower frequency after slime production. The last cartoon represents the last diagram in Figure 1.