Quantifying Biofilm-Surface Interactions Using Quartz Crystal Microbalance with Dissipation

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Statement of Purpose: Understanding the formation of biofilms at biologically-relevant interfaces has been of intense interest for quite some time. Microscopy methods are typically used to interpret the growth and adhesion of biofilms on surfaces. Microscopy methods are excellent for evaluating cell number and cell shape at interfaces, however in order to achieve a more comprehensive understanding of biofilm formation, one would like to be able to probe the interaction that the biofilm has with the underlying surface. Thus, we have attempted to use a surface sensitive technique in an attempt to quantify biofilm-surface interactions.

Methods: All experiments were performed using A quartz crystal microbalance with dissipation system (Q-Sense) was used to quantify bacterial attachment and growth. QCM-D sensors were coated with stainless steel (Stainless Steel Type: 2343, Equivalent chemistry to steel 1.4436). Leuconostoc mesenteroides cultivated on sucrose was incubated above the steel-coated sensors. Epiflourescence was also used to visualize the formation and growth of the biofilm layer.

Results: While incubating the steel-coated sensors under the cultivated environment, both the change in vibrational frequency (Δf, changes in mass) and change in energy dissipation (ΔD, changes in viscoelasticity) were simultaneously measured. To verify the relationship between the responses in Δf and ΔD and the adhering bacteria, the biofilm-modified steel-coated sensor crystals were 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 microscopy 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 (See Figure 1). 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. 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 this theory[1]. Others 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 microenvironment of the biofilm.

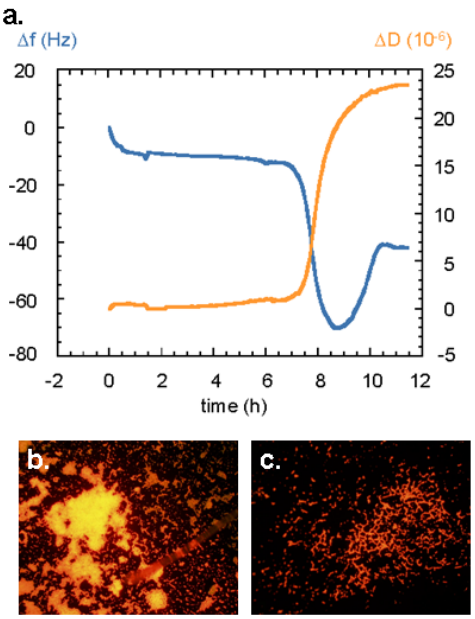

Figure 1. a. Real-time QCM-D data acquired during the formation of a biofilm on steel surface. The change in surface mass is plotted in orange and the change in viscoelasticity is plotted in blue. **b.** fluorescence image of the biofilm layer after 6 hours. **c.** fluorescence image of the biofilm layer after 12 hours.

Conclusions: 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. 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 example 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.

References:

1. Costerton et al. (Microbial Biofilms, Annual Review of Microbiology, vol 49:711-45, 1995