Application Note

# Monitoring Bacterial Biofilm Growth and Removal

**Bacteria readily adhere and grow on surfaces to form biofilms, a major precursor to human infection. In this application, QCM instrument qCell T is used to monitor Pseudomonas aeruginosa biofilm growth and removal in real time.**


## Summary

Monitoring of bacterial biofilm growth is of significant interest in the medical community as it may hold the key to treating and preventing a wide variety of infectious human diseases. Of the limited techniques available, the quartz crystal microbalance (QCM) offers reliable measurement of bacteria growth and removal in real time. In this study, bacteria were loaded inside a QCM device (qCell T) where they adhered and grew on a gold-coated quartz sensor. Changes in the quartz sensor's oscillation frequency indicated the bacteria's stable attachment and growth on the sensor surface. By monitoring the sensor's response over the course of several hours, the formation of bacterial biofilms was investigated under stagnant and laminar flow regimes. The removal of these biofilms was also measured by adding a cleaning solution, further establishing the QCM as a unique platform for both monitoring bacterial growth and testing the effectiveness of chemicals as biofilm removal agents.


## Background

It is well established that bacteria form complex intercellular communication networks to facilitate the formation of polymer matrices known as biofilms [1]. A form of survival mechanism, biofilms allow bacteria to adapt to a wide-range of environmental stimuli, making them highly resistant to extreme conditions and antimicrobial agents. In the human body, biofilms are a frequent precursor to infections [2]. The development of new platforms for investigating bacterial growth and removal from surfaces is critical for preventing human infection. The QCM is an excellent approach for these studies, due to its ability to monitor small mass changes in a highly sensitive, localized environment.

## Strategy

Pseudomonas aeruginosa was selected as the target bacterial species due to its prevalence in the hospital setting and reputation as one of the most challenging species to treat among nosocomial infections [3]. Bacterial cells were loaded into the system's flow cell using a peristaltic pump. The bacteria were allowed to adhere and grow on the sensor surface, inside the flow cell, over the course of several hours (Figure 1). Changes in both frequency and damping were recorded to monitor bacterial accumulation on the surface under different flow regimes. After several hours of growth, a surfactant was added to remove the bacteria from the surface. The sensor's return to the original baseline indicates the successful removal of the bacteria. The effectiveness of new antimicrobial compounds can be determined by testing a wide-range of bacterial species and evaluating their responses to the agents using this system.

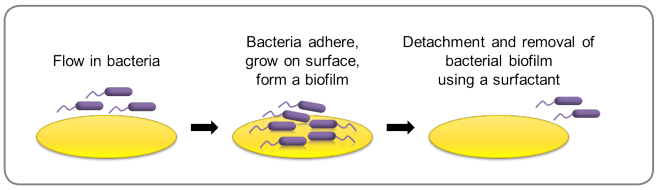


Figure 1. Bacterial detection scheme using a quartz crystal microbalance to monitor the growth and removal of bacteria from the gold-coated sensor surface. Using a flow cell, bacteria enter the system and attach to the gold-coated surface. Bacteria are left to grow in either stagnant conditions or under continuous flow with growth media added. After several hours of bacterial growth and biofilm formation, a surfactant is introduced to detach and remove bacteria from the surface.

## Method

Bacterial species Pseudomonas aeruginosa (strain PA01) was cultured overnight in lysogeny broth (LB) growth media at 37 °C. Cells were centrifuged to remove the supernatant and reconstituted in fresh LB to obtain a cellular density of approximately 4 million cells/µL. A gold-coated quartz sensor was rinsed with ethanol and deionized water and allowed to dry before its insertion into the qCell T. To prime the quartz sensor prior to bacterial addition, LB growth media was loaded into the qCell T at a flow rate of 150 µL/min. After total sensor surface coverage was established, the bacteria were loaded into the system at a flow rate of 80 µL/min for 30 minutes, after which flow was stopped.

Figure 2 shows the changes in frequency (Δf) and damping (ΔD) during bacterial cell deposition. The observed frequency and damping shifts in opposite directions, with different amplitudes, is indicative of a viscoelastic layer (i.e., bacterial biofilm) on the sensor surface. From this data, it is possible to determine the mass and thickness of the bacterial biofilm on the surface.

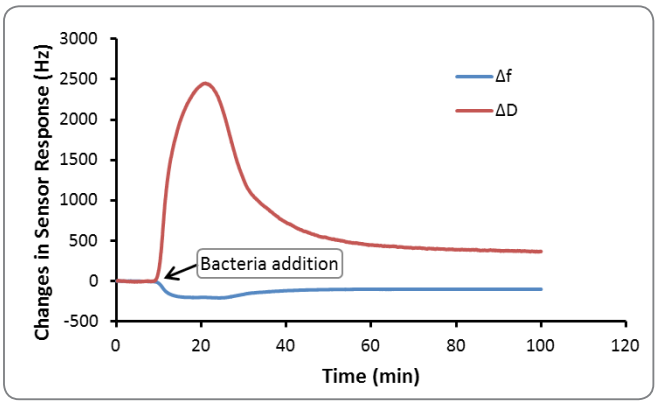


Figure 2. Frequency (blue) and damping (red) traces of bacterial cell deposition. P. aeruginosa bacterial cells are added once a stable baseline is reached for the growth media. The cells take approximately 1 hour to adhere to the gold surface and reach equilibrium.

Once the bacterial cells have stably attached to the surface, they can be perturbed using chemicals. Figure 3 shows how the qCell T can be used to investigate biofilm removal. As previously discussed, P. aeruginosa was deposited and grown on the surface of the sensor. After 5 hours of stagnant growth, fresh LB growth media was provided at a flow rate of 10 µL/min. After several hours of bacterial growth and biofilm formation, a solution of 1% SDS was loaded into the system at a flow rate of 150 µL/min. The removal of the bacteria from the surface was determined by the sensor's response as it returned to the original baseline and confirmed by visual inspection using the device's optical window. In addition, the effluent from this experiment can be also collected to perform cell counts and used to check the viability of the bacteria.

## Conclusion

Bacterial deposition and removal was successfully monitored in real-time by the qCell T QCM. The observed frequency and damping shifts in opposite directions but with different amplitudes suggest that a viscoelastic layer (i.e., bacterial biofilm) is formed on the sensor surface. More importantly,

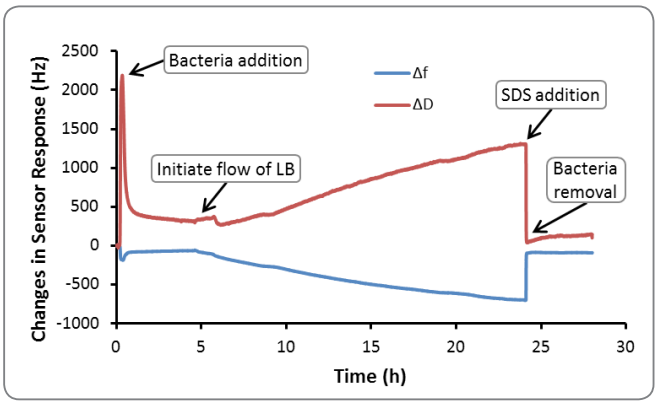


Figure 3. Frequency (blue) and damping (red) traces of bacterial cell removal. P. aeruginosa bacterial cells are deposited on the sensor and allowed to grow for 5 h under stagnant conditions. Fresh LB growth media is initiated at a flow rate of 10 µL/min. After 24 hours of bacterial growth, a 1% SDS solution is added to remove cells from the sensor surface. The return of the sensor response to the original baseline indicates cell removal and can be used to quantify the surfactant's effectiveness for bacterial removal.

the qCell T can be used to evaluate the effectiveness of compounds for removing biofilms.

## References

[1] Costerton, J.W., P.S. Stewart, and E.P. Greenberg, Bacterial biofilms: A common cause of persistent infections. Science, 1999, 284(5418), 1318-1322.
[2] Singh, P.K., et al., Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature, 2000, 407(6805), 762-764.
[3] Boucher, H.W., et al., Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clinical Infectiou Diseases, 2009, 48(1), 1-12.

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3T GmbH & Co.KG
Gartenstraße 100
78532 Tuttlingen
Germany

Tel.: +49 (0)7461-165 03-0
Fax: +49 (0)7461-165 03-29
E-Mail: info@3t-analytik.de
www.3t-analytik.com