IMD-W™ Online Water Bioburden Analyzer Biofilm Detection Capability

The ability to quickly detect and monitor biofilm growth within pharmaceutical water systems is of paramount importance. Once formed, biofilms are notoriously difficult to remove, and when incompletely removed, can result in rapid regrowth and proliferation. Although point-of-use samples can provide an indication of biofilm presence, it may take many of these intermittent samples before an increasing trend in colony forming units is seen and biofilm is indicated. Now, with online water bioburden analyzers (OWBAs), water loop bioburden levels can be monitored continuously and changes or upward trends in bioburden detected more quickly. Two studies are reviewed in this white paper supporting the use of the IMD-W™ OWBA as a predictive monitoring tool in the detection of biofilms.

Biofilm Study 1: - gLab, Fujisawa Technology Center, Azbil Corporation

Experimental Design

A study was performed at Azbil's gLab facility with the goal of evaluating IMD-W biofilm detection capability. A *Methylobacterium extorquens* (NBRC #15911) biofilm was grown for approximately 60 days, with no media or nutrients added to the purified water system, and a stainless steel reactor was used as a test bed for biofilm growth and release. The biofilm reactor was connected to a 0.05 µm ultrapure water supply that continuously supplied water to the system at 90mL/min.

An IMD-W system and membrane filtration unit were connected in parallel to the output of the biofilm reactor, shown in Figure 1.

Figure 1: *Stainless steel biofilm reactor and biofilm coupon.*

Biofilm was confirmed using scanning electron microscopy (SEM), Figure 2, and fluorescence microscopy with lectin staining to confirm extracellular polysaccharide substance (not shown).

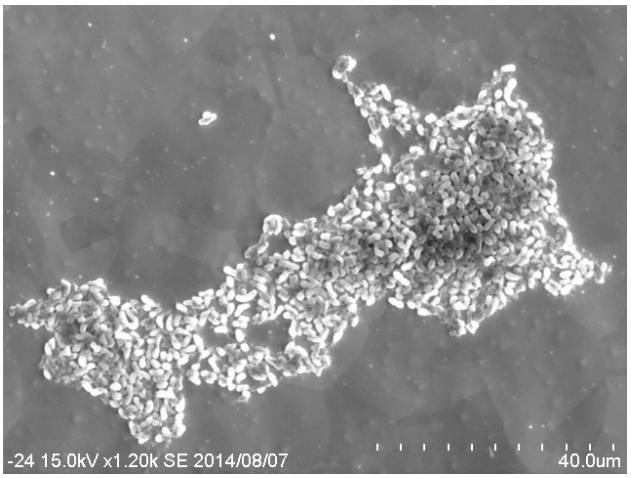

Figure 2: *SEM image of M. extorquens biofilm.*

The IMD-W system sampled continuously providing biologic count/mL results, and membrane filtration samples were collected concurrently providing CFU/mL data. The 0.45 µm membrane filters were transferred to R2A culture media and incubated for greater than five days at 30-35° C.

Results

Upon biofilm release, the IMD-W system immediately detected an increase in biologic counts as shown in Figure 3. An increase was also seen with the culture-based method, but to a lesser degree, with the increase being approximately 100x lower in magnitude than the IMD-W response and resulting in 0 CFU/mL. In the setup, initial background data also was obtained with the IMD-W system and membrane filtration unit to determine a baseline level of IMD-W biologic counts and CFU, respectively, for comparison.

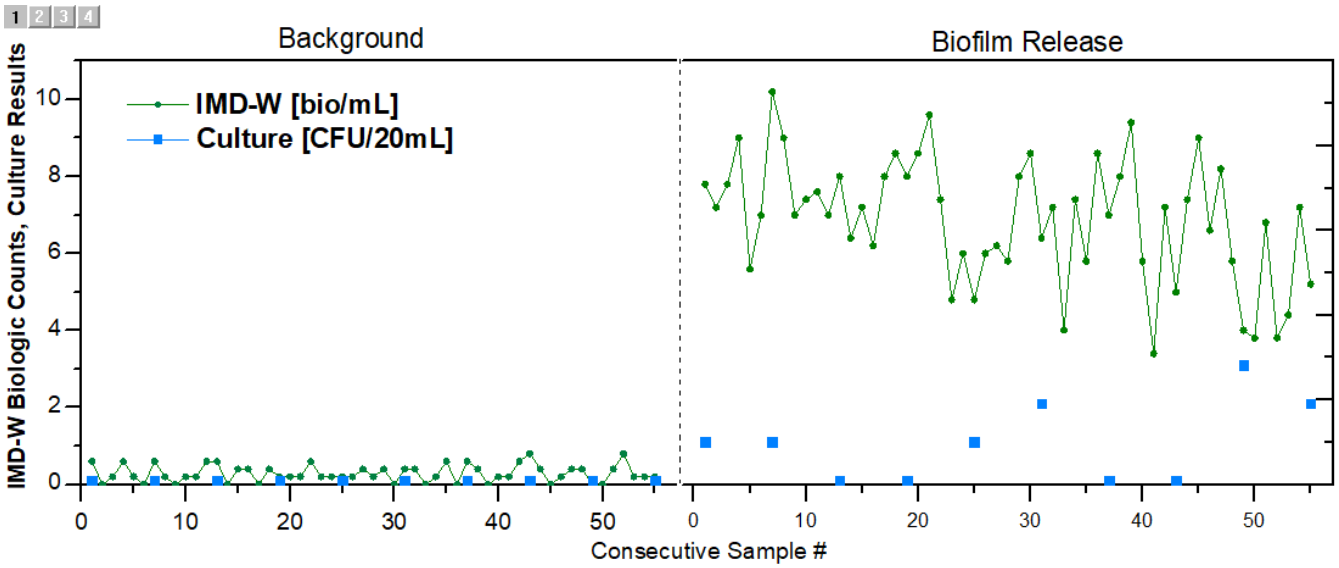

Figure 3: *Comparison of IMD-W biologic counts and culture results before and after biofilm release.*

In Figure 3, note that the IMD-W biologic counts are reported per mL whereas the culture results are reported per 20 mL so that the increase in CFU can be seen. Furthermore, IMD-W biologic count data points occur every minute and are connected by a line because the data is continuous, whereas the traditional method provides only discrete measurements.

Biofilm Study 2 – Center for Biofilm Engineering, Montana State University

Experimental Design

A second investigation into the IMD-W system's ability to detect detached and existing biofilm from an upstream water supply was undertaken at the Center for Biofilm Engineering at Montana State University. A continuous stirred tank CDC Biofilm Reactor (BioSurface Technologies) was used to grow a *Pseudomonas aeruginosa* (ATCC 15442) biofilm according to the ASTM E2562 standard method over 24 hours in the batch phase and 24 hours in the continuous phase with low levels (10mg/L) of tryptic soy broth media. A constant influent flowrate supplied growth media to the system, while an overflow spout maintained the reactor liquid volume at a constant level. The reactor contained eight removable rods that each contained three removable biofilm growth coupons for imaging and growth-based analysis. After 24 hours, effluent samples were taken with the IMD-W system and biofilm samples were taken from the removable coupons. Effluent heterotrophic plate counts (HPC), biofilm cell density, and IMD-W biologic counts were obtained over three consecutive days.

CDC Biofilm Reactor
(Image Courtesy Center for Biofilm Engineering)

Effluent heterotrophic plate counts (HPC) represent planktonic viable cell counts in units of CFU/mL, *biofilm cell density* represents biofilm counts present on a coupon in units of CFU/cm^2, and *IMD-W biologic counts* are based on microbial intrinsic fluorescence instead of growth.

For HPC, 10 mL of effluent was collected from the effluent tubing, and for biofilm counts, each coupon was dropped into a separate vial containing 10 mL of standard method dilution water (SMDW). These samples were vortexed and sonicated (3:2) for 30 seconds for each run, then diluted and drop plated on R2A agar plates, in triplicate. Plates were then incubated for 24 hours at 35°C, followed by enumeration of CFU.

IMD-W biologic counts were obtained through sampling of the effluent from the CDC reactor. A 1:100,000, or 10^{-5}, dilution of effluent in 0.1 µm-filtered RO water was made such that the maximum particle detection limit on the IMD-W system was not exceeded. From this sample, the average number of IMD-W biologic counts per mL was obtained.

Results

HPC, biofilm cell density and IMD-W biologic count data were obtained over three consecutive days, as shown in Figure 4. All three trended upward, showing increased counts for each successive day of CDC reactor operation. Across the three days of sampling, the IMD-W system reported lower biologic counts than HPC; however, this may be due to the disaggregation step used to reduce clustered cells into single cells during HPC enumeration. Detached biofilms typically contain both single cells and cell clusters, both of which would be counted by the IMD-W system as a single biologic count. Overall, the IMD-W system trends well with HPC and biofilm cell density results, indicating that it can be used to detect biofilm in a water system.

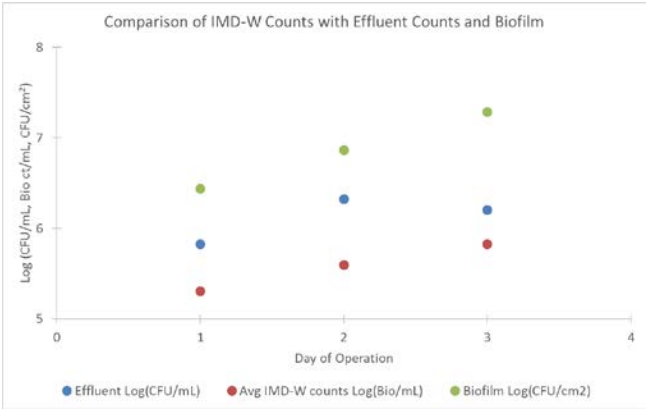

Figure 4: *Comparison of HPC, biofilm counts and IMD-W biologic counts over three consecutive days.*

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Conclusions

Two biofilm studies were performed with the IMD-W OWBA. Both studies showed that the IMD-W system has the sensitivity to detect biofilm in a water supply. With this adequate biofilm detection sensitivity and continuous, real-time detection capability, the IMD-W system can be used as a trending tool and early warning indicator of biofilm's presence in a pharmaceutical water loop.

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