Real-time Detection of Burkholderia cepacia and Biofilm in Pharmaceutical Water Systems

The ability to continuously monitor and quickly detect stressed objectionable organisms, like *Burkholderia cepacia,* or biofilm growth, within a pharmaceutical water system is of paramount importance. Certain organisms such as *B. cepacia* can be very difficult to detect with the culture media typically used to assess point-of-use samples. Identifying biofilm growth can be equally tedious, requiring numerous intermittent samples before CFU trends become apparent. Once discovered, both can be difficult to remove and, when incompletely removed, can persist and proliferate. With the use of bio-fluorescent particle counters (BFPC) like the BioVigilant IMD-W™ system, water loop bioburden levels can be monitored continuously and changes and trends in bioburden detected more quickly. Data is shared on the IMD-W system's counting sensitivity to *B. cepacia* and use of this online water bioburden analyzer (OWBA) as a predictive monitoring tool in the detection of biofilms and stressed organisms.

Burkholderia cepacia Detection

Thirteen industry-relevant organisms were utilized to challenge the IMD-W system and assess its detection performance as compared to the traditional culture-based method. [1] This testing included *Burkholderia cepacia* (NBRC 14595). *B. cepacia* was cultured aerobically for two days at 32°C. The bacteria were streaked onto TSA plates and incubated at approximately 32°C for 40-48 hours to achieve the stationary phase. *B. cepacia* then was harvested in sterile, distilled water and washed through centrifugation at 2,100*g* for three minutes. The supernatant was removed and the pellet was re-suspended in filtered distilled water. Optical density measurements at 600nm (OD_{600}) were utilized to estimate concentration, followed by dilution with filtered distilled water to reach the target microbial concentration. This dilution was utilized in the system testing and plated on TSA to perform a final titer check. Five target concentrations were tested, including 0.1 CFU/mL, 1 CFU/mL, 10 CFU/mL, 100 CFU/mL, and 1000 CFU/mL. Six replicates were performed at each concentration tested.

Figure 1 shows test results from two IMD-W systems (particle and biologic counts per mL), a Rion KS-42B reference liquid particle counter (counts per mL), and a Pall MicroFunnel with 0.45um filter used for water sampling and then cultured on TSA media (CFU per mL).

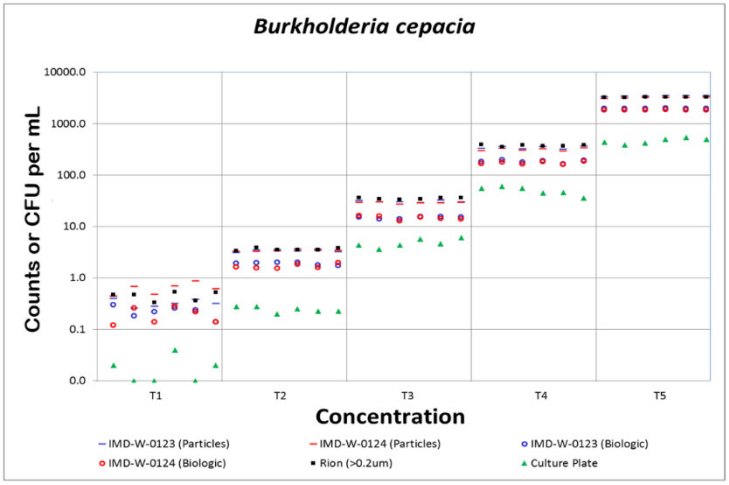

Figure 1- *IMD-W biologic and total particle counts for B. cepacia. Results for six replicates at five concentrations as compared to the traditional culture-based method and a reference Rion liquid particle counter.*

These systems were run in parallel on a test water loop to obtain side-by-side test data.[1] The IMD-W systems showed higher *B. cepacia* recoveries as compared to the traditional culture results for all five W system's, equivalent to, or better than, detection sensitivity as compared to the traditional culture-based method with the *B. cepacia* tested. This sensitivity, coupled with the IMD-W system's continuous monitoring and result reporting capability, make it well-suited for the more timely detection of such objectionable organisms.

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Methylobacterium extorquens Biofilm and Stressed Organism Detection

An investigation also was undertaken to evaluate the IMD-W system's biofilm detection capability. A Methylobacterium extorquens (NBRC #15911) biofilm was grown on stainless steel coupons over the course of approximately 60 days in a stainless steel reactor.[2] After 60 days, one coupon was used to confirm the presence of a biofilm using scanning electron microscopy (SEM) and fluorescence microscopy with lectin staining to confirm extracellular polysaccharide substance.[2] A second coupon was removed and placed into a secondary biofilm reactor that was connected to a 0.05 µm ultrapure water supply that continuously supplied water to the system at 90mL/min. No media or nutrients were added to this reactor. An IMD-W system and membrane filtration unit were connected in parallel to the reactor output.

The IMD-W system sampled continuously, providing biologic count/mL data, while episodic membrane filtration samples were collected concurrently to provide CFU/mL data. The 0.45 um membrane filters were transferred to R2A culture media and incubated for more than five days at 30-35°C. Upon biofilm release, the IMD-W system saw an immediate increase in and continued presence of biologic counts, as shown in the biofilm release portion of Figure 2.

An increase also was seen with the culture-based method, but to a much lower and less consistent degree. Note that in Figure 2 IMD-W biologic counts are reported per mL while the culture-based method CFU results are reported per 20mL to give higher resolution on the increase in counts observed. Note that four of the ten culture results were 0 CFU/20mL. The background data in Figure 3 was obtained using the same procedure, but with the insertion of a blank stainless steel coupon into the secondary reactor.

In addition to the biofilm testing, concentrated suspensions of *M. extorquens* were stored in purified water at 4°C and 25°C for 60 days. Periodic testing to assess the impact of starvation and stress on *M. extorquens* recoveries then was performed. Figure 3 shows the results of testing with the IMD-W system and traditional culture-based method through use of R2A media, as used in the biofilm testing. After 60 days, IMD-W biologic count recoveries were three logs greater than the culture-based CFU recoveries for the *M. extorquens* sample stored at 25°C. The culture-based recovery dropped sharply across the 60-day test whereas the IMD-W biologic counts detected remained quite stable. The difference in *M. extorquens* recovery between the IMD-W system and the culture-based method was less pronounced with 4°C storage, but at this temperature, the IMD-W system still saw higher retrievals after 60 days than the traditional method. In this testing, *M. extorquens* culturability was impacted to greater degrees by starvation and stress than microbial fluorescence.

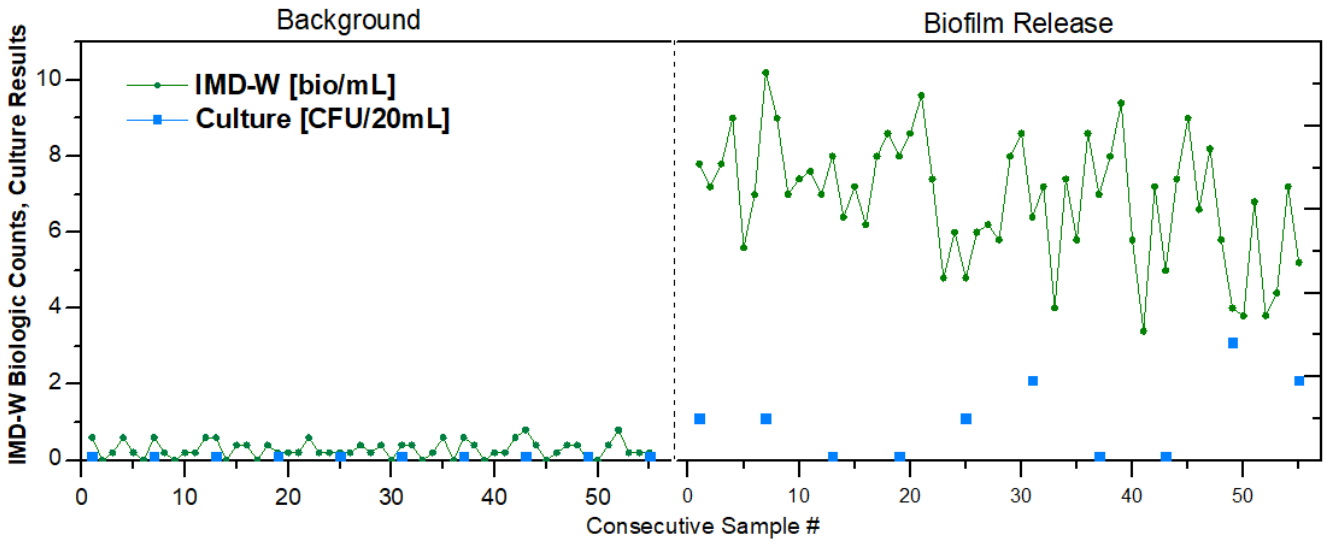

Figure 2- *Comparison of IMD-W biologic counts and culture results before and after an M. extorquens biofilm release.*

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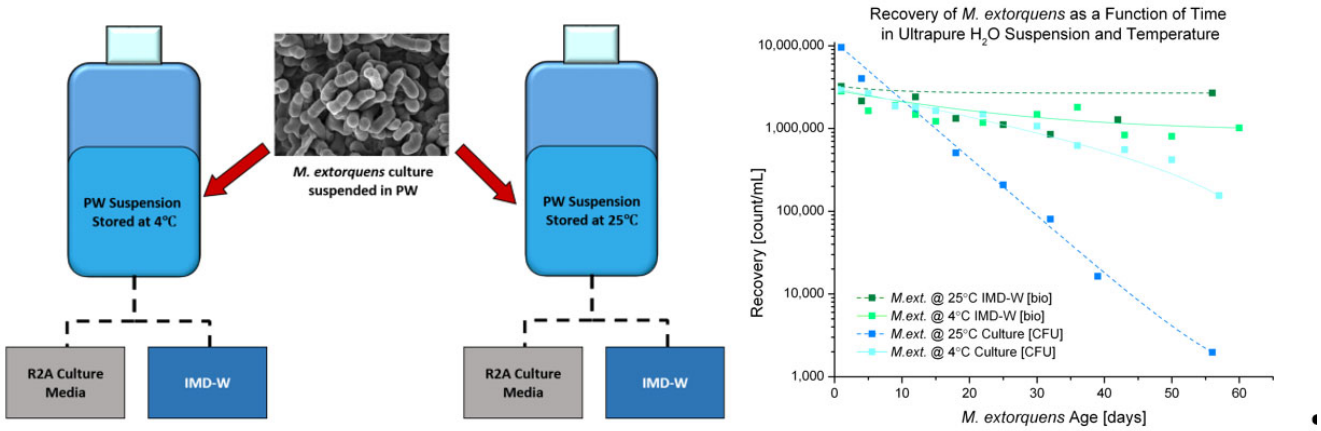

Figure 3- *IMD-W biologic count and culture-based CFU recovery of M. extorquens stored at 4°C and 25°C for 60 days. Periodic sampling was performed to assess the impact of starvation and stress on the two detection methods.*

Conclusions

The IMD-W system has been shown to have an equivalent to, or better than, ability to detect objectionable organisms like *B. cepacia,* as compared to the traditional culture-based method, and sufficient sensitivity to detect biofilm. The IMD-W system also showed greater *M. extorquens* recoveries, and results that were less impacted by starvation and stress than the traditional growth-based method. With an equivalent and often higher detection sensitivity, combined with a continuous, real-time detection capability, the IMD-W system can be used as a trending tool and early warning indicator for the presence of objectionable and often difficult-to-culture organisms as well as biofilm in a pharmaceutical water loop.

References

1. IMD-W Microbial Challenge Test Results Fact Sheet, LI025 September 24, 2019.
2. IMD-W Online Water Bioburden Analyzer Biofilm Detection Capability White Paper, LI030 May 3, 2018.

2005 W. Ruthrauff Road, Suite 151 ▪ Tucson AZ 85705 ▪ Telephone (520) 292-2342 ▪ Fax (520) 292-2365

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