Overcoming Biofilm Detection and Mitigation Challenges

to Improve Process Control in a Pharmaceutical Water-for-Injection System

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Abstract

The development and existence of bioburden in pharmaceutical water systems is often misunderstood. Microorganisms are always present and have a keen ability to adapt to their environment. This is especially true in a Water-for-Injection (WFI) system, where microbial attachment and biofilm growth will occur regardless of flow rate, material of construction, turbulent flow and low nutrient conditions. While industry makes every effort to control and eliminate bioburden, traditional sanitization methods are not one-hundred-percent effective at accomplishing this objective. Because of the limitations and time to result delay of conventional plate counting, we may be at a disadvantage for assessing bioburden, causing us to use water at risk. This poster explores real-life examples of biofilm in pharmaceutical water systems, and how real-time microbial detection could be used as a tool for improved risk management and process control.

Learning Objectives

- Explore phenomenon of microbial attachment and biofilm growth
- Discuss limitation of plate counts in detecting slow-growing or injured organisms
- Share examples of biofilm in water system components
- Build a case for real-time microbial detection as an early warning biofilm indicator

Biofilms

Microorganisms are ubiquitous in nature and possess amazing capabilities to adapt to extreme environmental conditions. In this poster, we will discuss microorganisms typically found in pharmaceutical water systems. In water, bacteria are initially planktonic, or free-floating, however, for survival, bacteria aggregate and grow in self-sustained communities called biofilms.

In a biofilm, bacteria use a phenomenon called quorum sensing to communicate and adapt to environmental stress by regulating gene expression for survival mechanisms. One such protective measure that impacts pharmaceutical water systems is the secretion of extracellular polymeric substances (EPS). This sticky slime layer enables the biofilm to adhere to surfaces and protects it from harm.

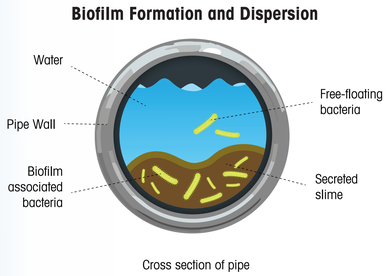

Figure 1: Biofilm in a Pipe Cross-section

Biofilms can be comprised of one species or multiple species. In a pharmaceutical water system, the most identified organisms are Gram-negative rod-shaped bacteria. The presence of Gram-negative bacteria is concerning because endotoxins, which are pyrogenic and harmful if injected into a patient, are composed of lipopolysaccharides (LPS), a key component of the Gram-negative cell membrane.

Prevalent species of concern include *Pseudomonas aeruginosa, Ralstonia picketti, and Burkholderia cepacia,* which are considered oligotrophic, meaning they can grow and survive in low nutrient environments. According to a National Institutes of Health (NIH) study, bacteria such as *Ralstonia picketii* will adopt starvation strategies that impact cell size and shape after 24 hours in high purity water. This can cause an issue in water systems because *R. picketti* can change its shape and squeeze through 0.2 µm filters.

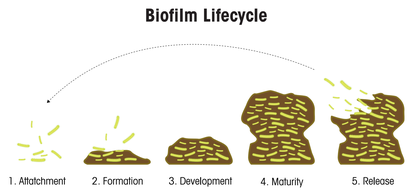

Figure 2: Biofilm lifecycle

Pharmaceutical Water Systems

Because microbial populations will always exist to some degree in high purity water, biofilm prevention and process control is paramount. Microbial control in pharmaceutical water systems requires robust hygienic design and engineering. Engineers must carefully consider construction materials, pipe geometry and layout, elimination of dead legs, and point-of-use distribution. Each water distribution loop is unique, varying by design, dynamics, and facility requirements. Production of high-purity water is regulated by international Pharmacopeia standards, including USP <1231>.

Key Risk Factors

- Seasonal feed water quality variations
- Component failures causing leaks and blockage
- Storage tank level fluctuations
- Valve operations affecting flow dynamics

Economic Impact

Biofilm contamination presents a major financial and operational challenge in the pharmaceutical industry. Every hour a water system is shut down, a facility endures production and revenue losses. Estimated remediation costs include:

Contamination event: $100,000 - $1,000,000

Unplanned sanitization: $5,000 to $40,000

Quality investigation: $5,000 to $20,000

Water system upgrade: $100,000 to $1,000,000+

Once established, biofilms are extremely difficult to completely eradicate because the members of the bacterial community work together to protect against disinfection chemicals and thermal sanitization. For example, in a hot WFI system, if the top layer of a biofilm community is exposed to high temperature waters, the lower layers can be protected by the metabolite layer and will continue to multiply. Organisms in a Hot WFI system may be in a stressed state, be spore formers, or Viable but Not Culturable (VBNC).

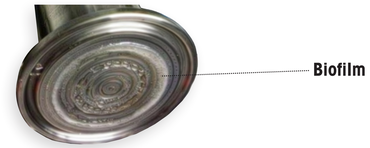

Figure 3: Biofilm on a pressure sensor

Limitations of Compendial Bioburden Testing Methods

One of the challenges of monitoring a high purity water system is that no one really knows the microbial concentration that is "expected" in such a situation. Planktonic cells will interact differently based on temperature and flow of the water through the piping.

- Culture methods are inefficient, time-consuming and susceptible to human error.
- Time to result is up to a week or longer, and it is well-established that as little as 0.1% of the actual microbial cells present in a sample can form colonies on standard bacteria growth media (USP <1223>).
- Traditional methods test the water at a single sampling location, but they don't test the water system and the impact that failed components can have on microbial activity.
- These factors lead to difficulty in identifying the presence of biofilms and draw out the root cause determination for months or longer.

Continuous Real-Time Monitoring

Water systems are ever changing, and plate counts alone cannot capture the impact of biofilm and VBNC organisms. With the advancement of real-time bioburden monitoring tools, we can now detect changes in biofilm as they happen with respect to hydraulic activities and maintenance. With real-time measurement, we are now able to:

- Enhance visibiliity into the water system
- Enable real-time process control for rapid response to microbial excursions
- Eliminate uncertainty of releasing at-risk water

Conclusion

Because an established biofilm can never truly be eradicated, we need automated detection tools in addition to conventional microbiological test methods to assess risk to the water system in real time. Real-time measurement offers a unique opportunity to monitor microbial trends within an active water system. More importantly, it serves as an early warning system that enables us to see actual microbial trends so that we can assess when they change or take a direction of concern. The industry has been monitoring trends in real-time for years with compendial measurements like TOC and conductivity, and real-time microbial detection adds another level of pharmaceutical water quality compliance.

References

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