STERILEX®
Innovative Solutions for Microbial Control

How Biofilm Affects Drinking Water Quality

Water is a vital resource for both humans and animals. Drinking water quality and the cleanliness of the drinking water system (DWS) play an important role in the general health and performance of livestock, including broiler chickens. Clean water has little value if it is being delivered to birds via a DWS containing pathogens housed within a biofilm. Biofilms cost the U.S. billions of dollars every year in energy losses, equipment damage, product contamination and medical infections. Biofilms pose a tremendous risk to both human and animal health. All DWSs eventually develop some type of biofilm. In a 2019 study on the "Occurrence and Characterization of Biofilms in Drinking Water Systems of Broiler Houses," Maes, et al. found that "63 percent of the analyzed surfaces were identified as carrying biofilm. This is a much higher number compared to surfaces in the food industry where the presence of biofilm (determined in the same way) was suspected in only 17 percent of the cases" (p. 11).

Biofilm

Biofilm is a grouping of microorganisms that stick together, embedded in a self-produced matrix of extracellular polymeric substance (EPS) composed of proteins, polysaccharides and other materials. The EPS is composed of long, sugary molecular strands that not only attach biofilm to a surface, but also trap nutrients, provide structural support and protect microbes from antimicrobial treatment and disinfectants.

A contaminated water supply and DWS is one of the fastest ways to introduce disease pathogens to a flock. Waterlines inside broiler houses create an ideal environment for biofilms to form with a combination of warm temperatures, low flow rates, and sufficient nutrients. Researchers are becoming increasingly aware of the role biofilm plays in animal health. "Studies into the role of water in *Campylobacter* infection in chickens have identified the importance of factors such as biofilm in protecting the organisms" (Sparks, 2009, p. 459).

In addition to forming biofilms, pathogens can also attach to pre-existing biofilms and survive for days, weeks or even longer. "Biofilms in drinking water systems can serve as an environmental reservoir for pathogenic microorganisms and represent a potential source of water contamination, resulting in a potential health risk for humans if left unnoticed" (Wingender & Flemming, 2001, p. 417). A study illustrating the biofilm building capacity of *Salmonella enterica* strains from the poultry farm environment concluded that the biofilm building capacity is strongly dependent on incubation temperature and might be a function of adaptation to the host environment. Therefore, the control of biofilm as a reservoir for pathogens such as *Salmonella* in the farm environment is crucial to the overall improvement of food safety (Schonewille, et al., 2012).

> Maes, et al. found that 63% of the analyzed surfaces were identified as carrying biofilm. This is a much higher number compared to surfaces in the food industry where the presence of biofilm[...] was suspected in only 17% of the cases.

Water Quality

Biofilm associated with drinking water can directly impact water quality and drinkability by causing aesthetic and organoleptic problems such as color, odor and taste degradation due to chemical compounds released. More importantly, such biofilm poses a threat to human and animal health by hosting pathogenic or toxin-producing bacteria, viruses, protozoa, algae, fungi and invertebrates. (Farkas, Ciataras, Bocos, 2012) Additionally, "low concentrations of chlorine residuals, stagnant water, plastic pipes and particle accumulation in distribution systems may increase the generation of taste and odor compounds by favoring biofilm formation and microbial activity" (Farkas, Ciataras, Bocos, 2012, p. 151).

Water testing on farms can be an important tool in managing bird health and performance. Wingender and Flemming (2011) found that "95 percent of the bacterial numbers in a DWS are located at the surfaces while only 5 percent are found in the water phase and detected by sampling as commonly used for quality control" (p. 418). Due to the concentration of bacteria located at the surfaces, water testing results may only be a muted indicator of a much larger problem. Swabbing the DWS may allow for additional discovery. Water testing helps ensure water is within an acceptable pH range, contains an acceptable amount of minerals and nutrients and is not contaminated. Water tests should pull samples from both the source and the end of the DWS to provide the most complete information.

> "...despite regular disinfection, most sampled surfaces on the inside of the DWS of the broiler houses showed high microbiological counts.
>
> *– V. Maes, et al.*

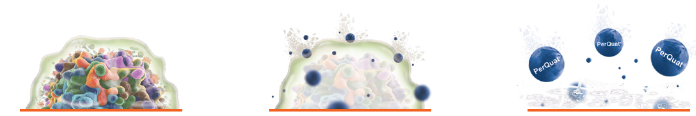
Sanitation and Disinfection

Medications, such as antibiotics, and additives, such as organic acids and vitamins, are often administered through the DWS and "can serve as a nutrient source for microorganisms and benefit biofilm formation" (Mae, et al., 2019, p. 2). Cleaning techniques, such as flushing, and the use of on-bird sanitizers may reduce bacterial growth and can temporarily prevent water quality issues, but these practices alone are not capable of eliminating biofilm. "Disinfecting water and water supplies and controlling microbiological issues related to water is considered an important measure to minimize waterborne diseases in broiler production" (Maharjan, et al., 2016, p. 269).

Oxidizing chemistries, such as chlorine bleach and PAA are often used to disinfect the water and DWS between flocks. These chemistries may kill free-swimming and surface level biofilm-related microorganisms, but leave the EPS structure intact, particularly the inner layers. Biofilms protect pathogens from disinfection and allow organisms injured by environmental stress and disinfectants to recover and grow. Maes, et al. (2019) identified that "despite regular disinfection, most sampled surfaces on the inside of the DWS of the broiler houses showed high microbiological counts" (p. 10). Bacteria can repopulate in an intact EPS structure within 48 hours.

Sterilex's patented PerQuat® technology was the first chemistry to receive EPA-registered anti-biofilm claims for industrial and public health use sites. It has the unique ability to collapse the protective matrix, penetrate biofilm, kill pathogenic microorganisms, dissolve and chemically scrub the biofilm structure from a surface. The active ingredients in Sterilex's FortiSolve™ are based on quaternary ammonium compounds and hydrogen peroxide. The primary role of the quaternary ammonium compounds is to serve as a phase transfer agent, combining with the peroxide ion to form a "PerQuat" molecule. This combination provides a powerful level of performance through multiple physical and chemical mechanisms of action, including hydrolysis and oxidation.

The Power of PerQuat Technology



Polysaccharide matrix — PerQuat molecule penetrating matrix — PerQuat molecule breaking up biofilm using oxidation + hydrolysis

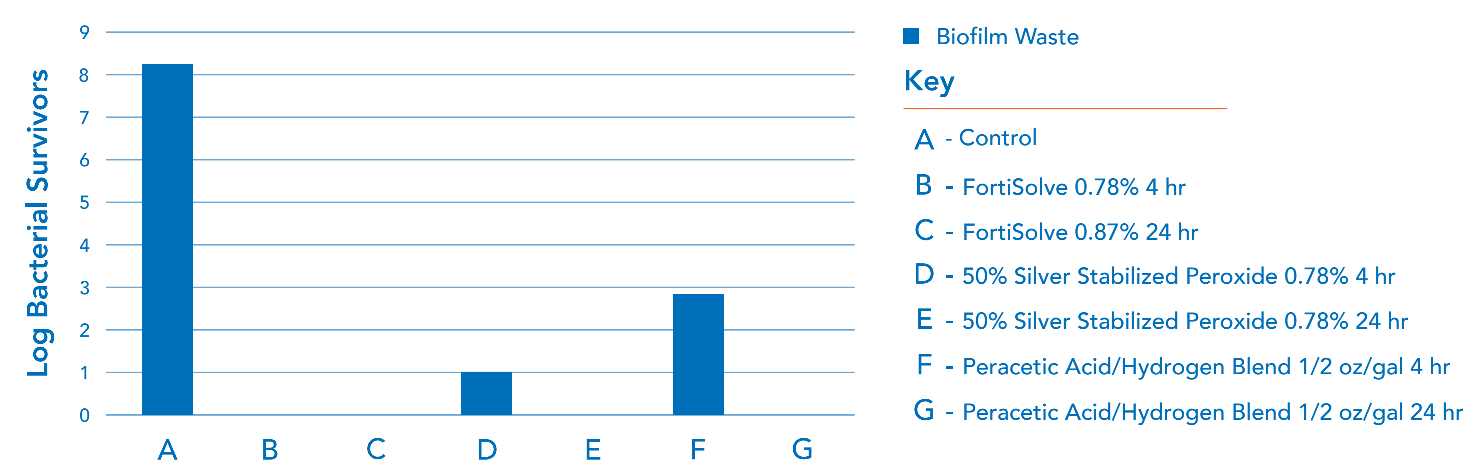
At the 2008 Virginia Poultry Health and Management Seminar, Dr. Susan Watkins identified that Sterilex products used at 2.5% had the greatest reduction in aerobic plate counts (APC) with more than a 4-log reduction when compared to bleach, citric acid and 50% hydrogen peroxide products.

Bacterial Test Results (log) Figure 1

TYPICAL CHEMISTRIES	RATIO	START	4 HRS	24 HRS
PerQuat	2.50%	5.60	1.29	<1.00
Peroxide (35%)	3.00%	6.74	5.45	1.97
Bleach	0.78%	6.98	5.03	5.14
Citric Acid	0.78%	7.56	7.52	7.33
Silver Stabilized Peroxide	0.78%	5.86	4.23	3.24
Silver Stabilized Peroxide	3.00%	5.87	3.00	<1.00

S. Watkins, "Good Water, Good Birds," presented at the Virginia Poultry Health and Management Seminar, Roanoke, VA, April 8-9, 2008.

Survivors (log) Figure 2



> "The Sterilex 2.5%... had the greatest reduction in APC with more than a 4 log reduction.
>
> *– S. Watkins, University of Arkansas*

In March 2020, Sterilex conducted an in-house waterline survey using biofilm waste. The in-house biofilm waste was created via method: EPA MLB SOP MB-19: Growing a biofilm using the CDC Biofilm Reactor. *Salmonella enterica* ATCC 10708, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 11229 and *Saccharomyces cerevisae* ATCC 834 were placed into the CDC Biofilm Reactor and taken through the batch phase and the continuous stirred tank reactor (CSTR) phase as described in the method. After the CSTR phase was complete, the waste was collected and used for testing. CFU/mL of bacterial load was calculated for the generated waste. The study showed (Figure 2) that FortiSolve when used at a concentration of 0.05 ounces/gallon eliminated biofilm waste bacteria in four hours whereas the same concentration of either a 50% silver stabilized peroxide or a peracetic acid/hydrogen peroxide blend showed bacterial survivors in the same timeframe.

Conclusion

If human and animal hosts are susceptible and exposed to contaminated water, a health risk is present (Wingender & Flemming, 2011). Biofilms are considered a public health threat because of their outstanding resistance to antibacterial treatments and disinfection. Controlling biofilms is an important and challenging step in delivering high-quality water to livestock. Research has shown that more than half of analyzed surfaces in broiler water systems carry biofilm, while less than 20% of surfaces in the food industry show a presence of biofilm. DWS should be cleaned and sanitized between every flock using an EPA-registered disinfectant labeled to both remove biofilm and kill biofilm bacteria. "Clean and safe water is a basic requirement for optimizing production" (Maharjan, et al., 2016, p. 266).

"Biofilms are considered a public health threat because of their outstanding resistance to antibacterial treatments and disinfection.

References

Farkas, A., Ciatara, D., Bocos, B. (2012). Biofilms Impact on Drinking Water Quality. *Ecological Water Quality—Water Treatment and Reuse*. 141-160. https://www.researchgate.net/publication/300805037_Biofilms_Impact_on_Drinking_Water_Quality

Sparks, N.H.C, (2008). The Role of the water supply system in the infection and control of *Campylobacter* in chicken. *World's Poultry Science Journal*, *65(3*), 459-474. https://doi.org/10.1017/S0043933909000324

Schonewille, E., Nesse, L., Hauck, R., Windhorst, D. Hafez, H., Vetby, L. (2012). Biofilm building capacity of *Salmonella enterica* strains from the poultry farm environment. *FEMS Immunology & Medical Microbiology*, *65(2)*, 360-365. https://doi.org/10.1111/j.1574-695X.2012.00966.x

Maes, S., Vackier, T., Huu, S., Heyndrickx, M., Steenackers, H., Sampers, I., Raes, K., Verplaeste, A., Reu, K. (2019). Occurrence and characterization of biofilms in drinking water systems of broiler houses. BMC Microbilogy, 19(77) 1-15. https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-019-1451-5

Maharjan, P., Clark, T., Kuenzel, C., Foy, M.K., Watkins, S. (2016). On farm monitoring of the impact of water system sanitation on microbial levels in broiler house water supplies. *Journal of Applied Poultry Research*, *25(2)*, 255-271. https://doi.org/10.3382/japr/pfw010

Wingender, J., Flemming, H. (2011). Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health*, *214(6)*, 417-423. https://doi.org/10.1016/j.ijheh.2011.05.009

Sterilex Animal Health products are available through MWI Animal Health. Please contact your MWI representative or Sterilex for more information.

111 Lake Front Drive, Hunt Valley, MD 21030 | 1.800.511.1659 | **www.sterilex.com** | marketing@sterilex.com