ClorDiSys

"The Chlorine Dioxide People"

Application Note #42

Chlorine Dioxide and Biofilms

A biofilm is defined as a "microbially-derived sessile community, which is cells that are irreversibly attached to a surface or to each other." More simply put, microorganisms attach themselves to surfaces and develop these very robust biofilms. Biofilms can be found in natural environments, on surfaces around the home, and even more alarmingly, they can be found in food processing facilities.

Cells in a biofilm have the unfortunate ability to survive both cleaning and sanitization. This resistance to sanitizers increases with the maturity of the biofilm. In the last decade, a number of studies have been conducted to determine a variety of sanitizers' efficacy against biofilms. In 2010, the Department of Food Science at Purdue University compared the effect of chlorine dioxide gas, aqueous chlorine dioxide, and aqueous sodium hypochlorite treatments on the inactivation of *Listeria monocytogenes* containing biofilms.

Listeria monocytogenes is a food-borne pathogen with the highest mortality rate. It has the ability to adhere to and grow on a variety of surfaces found in food processing plants. The Purdue study proved that the biofilm developed from the five-strain mixture of Listeria was more resistant to the sodium hypochlorite treatment than either liquid or gaseous chlorine dioxide (CD). More specifically, "aqueous CD resulted in a significantly greater log reduction of biofilm cells for shorter treatment times (2, 4 and 6 min) as compared to CD gas treatment. This may be a result of slow diffusion of CD gas into glass sample tubes and/or the time required for the gas to dissolve in water present on the surface of the biofilm. The difference in log reductions between treatments was smaller and not significant for the longer treatment times" (R. Vaid et al., 2010). However, they did cite further research needed to be done to establish levels of CD and treatment times required for complete inactivation of biofilm cells for the purpose of equipment sterilization and inactivation of biofilms from food processing equipment surfaces.

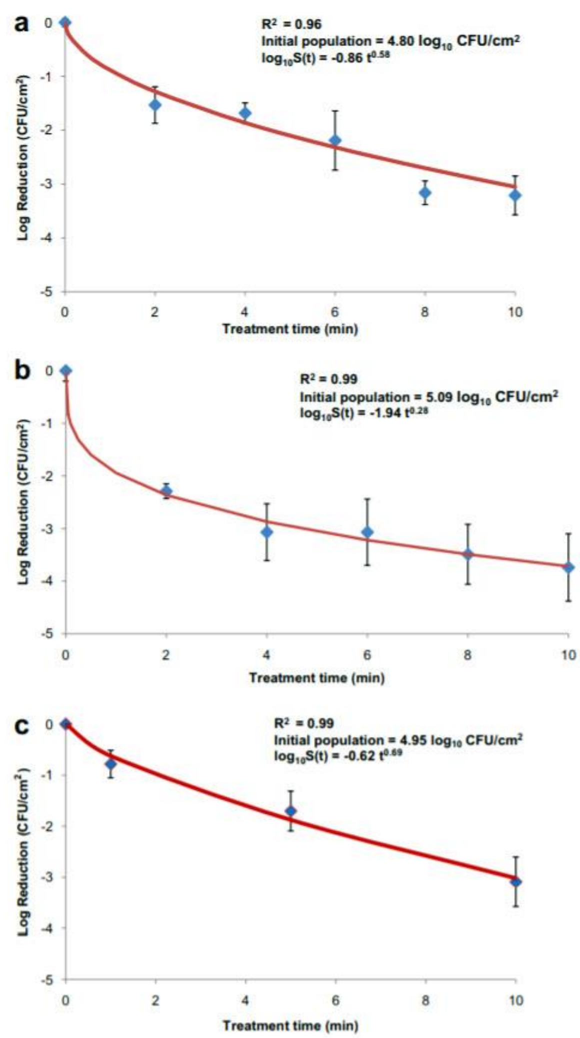

Figure 2. Effect of (a) chlorine dioxide gas (0.3 mg/l @ 75% RH), (b) aqueous chlorine dioxide treatment (7 mg/l) (middle) and (c) sodium hypochlorite (50 mg/l) on four day old five strain Listeria monocytogenes biofilm (points on the graph indicate the mean log reduction ± std. dev. (log_{10} CFU/cm^2), n = 3 at each time point).

Reprinted from *Comparison of inactivation of Listeria monocytogenes within a biofilm matrix*, by R. Vaid et al., 2010, Food Microbiology, 27, p.982.

Thus, chlorine dioxide's efficacy against biofilms in both the gaseous or aqueous state was taken a step further by the Republic of Korea's Department of Biotechnology and University of Georgia's Center for Food Safety in 2014. This team evaluated chlorine dioxide's ability to kill *Bacillus cereus* spores in biofilm formed on stainless-steel coupons (SSCs). *Bacillus cereus* is a spore-forming bacterium that can cause foodborne diseases. The study pointed out that while aqueous CD has "the advantage of being easy to produce and handle compared to gaseous ClO2," its residual moisture may promote the growth of molds after treatment of food-contact surfaces. It was determined that the antimicrobial activity of chlorine dioxide gas was higher than that of the aqueous. More specifically, results reported "when treated with gaseous ClO2, vegetative cells and spores were inactivated within 6 h. The number of B. cereus spores attached to SSCs was not significantly different from the number of B. cereus spores in biofilms. However, when treated with gaseous ClO2, 6 h was required to inactivate spores in biofilms, compared to 1 h for spores simply attached to SSCs. Regardless of whether spores were in biofilms or attached on the surface of SSCs, gaseous ClO2 was an effective sanitizer" (H. Nam et al., 2014). Researchers recommended the use of chlorine dioxide gas to control hazardous microorganisms in biofilm formed on food-contact surfaces as part of the "clean-in-place" (CIP) system since processing lines provide a closed system during cleaning and sanitization, but further evaluation of this application was suggested.

References

Nam, H., Seo, H., Bang, J., Kim, H., Beuchat, L., Ryu, J. (2014) Efficacy of gaseous chlorine dioxide in inactivating Bacillus cereus spores attached to and in a biofilm on stainless steel. *International Journal of Food Microbiology, 188,* 122-127.

Vaid, R., Linton, R., & Morgan, M. (2010) Comparison of inactivation of Listeria monocytogenes within a biofilm matrix using chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite treatments. *Food Microbiology, 27,* 979-984.