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Innovative Solutions for Microbial Control

Understanding the Difference Between Biofilm Kill vs Biofilm Removal EPA Labeling Claims

The EPA maintains two separate claims on biofilm: kill of biofilm bacteria on hard surfaces and removal of biofilm from surfaces. This paper explores the key differences between the two and how it can affect food safety and sanitation programs, as well as chemical mechanisms for controlling biofilm.

Biofilm Kill vs Biofilm Removal

Biofilm occupies an interesting space in the U.S. Regulatory environment. The EPA has long recognized the protective habitat created by biofilms is a public health concern and should be treated and regulated as a pest. Pesticide regulations are designed to fit control/kill claims to specific organisms or classes of organisms. Biofilms act as protection for a variety of pathogens and organisms, making regulation difficult. Because of this, the EPA has two different classes of claims related to biofilm: removal of the biofilm from a hard surface and kill of bacteria within the biofilm. Understanding the key physical and regulatory differences between these claims is essential for control of biofilm in food production environments. Studies have shown that "biofilms are the root cause of more than 60 percent of foodborne disease outbreaks." (Han, 2017, p.1).

Biofilm Biological + Physical Traits

A basic understanding of the biological and physical traits of biofilm helps highlight the differences in the regulatory claims. Biofilm is constructed of an extracellular polymeric substance (EPS), a matrix of polysaccharides, lipids and proteins (López, 2010). It is secreted by a wide range of bacteria, including foodborne pathogens like *Escherichia coli* and *Listeria monocytogenes* as well as other pathogens like *Staphylococcus aureus*. The main function of this structure is to protect and house the microbial colony. However, the EPS provides more than just a home for bacteria, it also allows for important mechanisms such as quorum sensing, that have an impact on public health in food production. Quorum sensing is a cell-to-cell signaling mechanism that allows bacteria to communicate by releasing chemical signals to help the colony survive adverse threats. It has been shown that this unified sensing allows biofilm to behave like a multicellular organism and spread beneficial mutations that contribute to antibiotic tolerance and antimicrobial resistance, which is especially problematic for the control of pathogenic bacteria (Sakuragi, 2007). In addition to aiding organism-level resistance to antimicrobials, the biofilm itself provides resistance mechanisms for certain antimicrobials.

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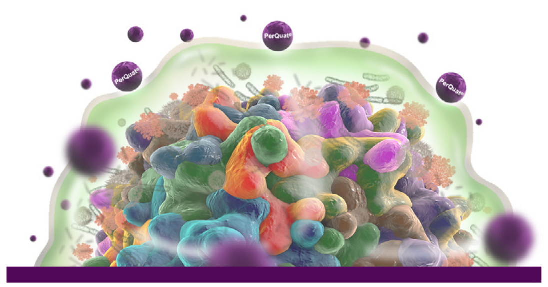
Biofilm Resistance to Chemical Agents

The mechanisms for the biofilm's resistance to common control chemistries is unique to the type of chemistry used but can share a common theme: they are most often related to difficulties in penetration of the EPS. Certain classes, like quaternary ammonium compounds (QACs) are unable to penetrate deep within biofilm layers, due to molecular interactions between the hydrophobic, cationic long chain QAC molecule and the EPS (Bridier, 2011). This causes QAC's to become bound up near the surface, leaving the deepest organisms untouched.



QAC MOLECULES STICKING TO SURFACE

Demonstration of hydrophobicity and charge interactions between QACs and biofilm leading to insufficient penetration and limited interaction between the antimicrobial and deep organisms.

Sodium hypochlorite (bleach) is another commonly used cleaning and sanitizing chemical agent, and like QACs it is not effective beyond the biofilm surface in most use cases. Hypochlorite ions are very reactive and can rapidly oxidize the outer layers of the EPS structure. If enough ions are present, it will fully oxidize and remove the full structure. However, because there is no true penetration, it requires a high dose and is an inefficient approach. In addition, some studies have shown that sub-lethal levels of chlorine can cause stress reactions within the biofilm that make the biofilm and the organisms it houses more resistant to chlorine attack (Dhakal, 2016).

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BIOFILM AFTER BLEACH TREATMENT

Simple oxidizing chemistries like hypochlorite or other halogens will react with the other layers of biofilm, causing stress reactions that can further protect biofilm from chemical attack.

Peroxide-acid based chemical agents like peracetic acid and other peracid blends have gained popularity as final sanitizers within the last 20 years in the food processing space. These compounds show the ability to penetrate the biofilm matrix, allowing for contact with internal organisms and antimicrobial effects within the biofilm. However, these peracid compounds have been shown to leave both the EPS structure intact as well as adhered cells behind after treatment, leaving a relatively intact biofilm to aide in repopulation (Pan, 2016). Internal testing has shown bacteria can repopulate an intact EPS structure within 48 hours. If the structure is completely removed, it will take closer to one week (168 hours) for the biofilm matrix to begin reforming.

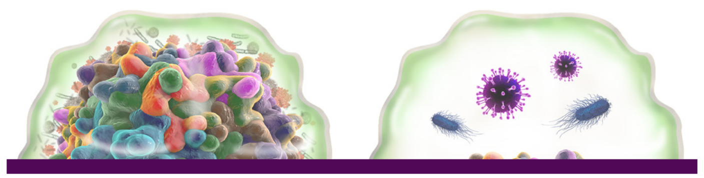
Because of biofilm resiliency and the difficulty associated with full control of biofilm, combination chemistries like Sterilex's PerQuat® technology prove to be much more effective on penetration, kill and removal of biofilm. Sterilex's PerQuat was the first chemistry on the market approved by the EPA to remove public health biofilms and it has an approved biofilm kill claim, making it the most complete biofilm solution available.

EPA Biofilm Kill vs Biofilm Removal Claims

It is easier to understand the difference between the EPA's biofilm kill and biofilm removal claims once there is a better understanding of biofilm chemical control mechanisms. In 2017, the EPA approved the biofilm bacteria kill claim which only refers to the control of bacteria within a biofilm. Also, this claim only applies to the specific organisms identified in the data submitted, which may not cover all bacteria in the multi-species biofilms encountered in food processing environments. Additionally, this efficacy data used to obtain the claim is not generated from multi-species biofilms, which does not reflect reality in food processing environments. It has been shown that the multi-species biofilms like those encountered in food processing environments have more complex and stronger resistance mechanisms than mono-species biofilms (Flemming, 2016). Most importantly, this claim does not cover the physical removal of the EPS structure from surfaces. It only applies to the reduction of specific bacterial populations within biofilm structures. In contrast, the biofilm removal claim applies to removal of the biofilm structure from hard surfaces. Each of these claims are effective on biofilm bacteria, but the different mechanisms for attacking biofilm (structure vs. bacteria) create real world differences in foodborne pathogen mitigation plans.

Biofilm Repopulation

Leaving the biofilm structure on a surface creates an ideal environment for recolonization and repopulation. If resistant microbial cells repopulate, the new population will be much harder to control without fully removing the biofilm matrix. This can also create a "system seeding" issue. Seeding dispersal, one of the life cycle stages of biofilm, occurs when a portion of the biofilm breaks off to attach to a new surface (López, 2010). This creates the potential for facility or system-wide inoculation of antimicrobial resistant bacterial populations. Because of their lack of removal capability, chemical agents that only kill bacteria within a biofilm are not a good solution for systems that already have biofilm. These chemicals will not kill every biofilm forming bacteria in the system and will allow repopulation of the established EPS within 24–48 hours (Müsken, 2018).



BIOFILM KILL

Biofilm kill claims only refer to reductions of specific biofilm causing bacteria within the biofilm EPS matrix, not the removal of the matrix itself. There will also be some viable population of bacteria left behind with the EPS matrix.

Biofilm removal claims are not granted by simply showing efficacy against specific bacterial populations, but rather by submitting data that demonstrates the biofilm's EPS matrix has been removed. It is possible to have both a biofilm removal claim and a bacteria in biofilm kill claim simultaneously kill claim. The biofilm kill claim and biofilm removal claim differ in some key aspects. From a physical standpoint, the ability to remove biofilm requires the ability to penetrate the biofilm down to the bare hard surface. Because of this, products that have removal claims may also demonstrate kill efficacy within those biofilms as well as removal ability. In addition to preventing public health issues, removing the biofilm can solve some of the ancillary problems seen with biofilm formation such as heat transfer rate reduction and microbiologically influenced corrosion on surfaces.

BIOFILM KILL + REMOVAL

The EPA biofilm removal claim requires demonstrating full removal of the biofilm EPS matrix from the surface.

Conclusion

Utilizing registered pest control agents is a key part of any food safety plan to mitigate foodborne pathogen transmission risk. Understanding the physical and legal ways to eradicate and control pests helps identify the best action plan. Biofilm is a registered pest, and unique in the way it is regulated and controlled. Special care should be taken when evaluating biofilm claims when constructing or evaluating a food safety plan. Compared to chemistries designed to only kill biofilm bacteria, chemical agents with both biofilm removal and biofilm bacteria kill claims provide the best protection on surfaces.

“ **Special care should be taken when evaluating biofilm claims when constructing or evaluating a food safety plan.**

References

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