GENERAL CLEANING RECOMMENDATION FOR REMOVAL OF MICROBIAL RESIDUE

In nature and in process systems, microorganisms rarely exist as single cells or pure cultures. Microorganisms usually exist as a monoculture or a mixed culture of different microorganisms. Microorganisms within a biofilm, such as *Pseudomonas* or *Burkholderia* species, are commonly encased in a slimy matrix referred to as an Extracellular Polymeric Substance (EPS) that is essential for the microorganisms' survival (Hall-Stoodley, 2002). The EPS is a complex mixture of polysaccharides, nucleic acids and proteins. Its composition can vary greatly due to the different microorganisms present and different growth conditions (Manuzon, 2009). The presence of EPS is very important as it increases the microorganisms' resistance to environmental stresses, antimicrobial agents and cleaning agents. Therefore, the removal of the EPS prior to sanitization or disinfection is critical.

Microbial contamination of process equipment can affect biopharmaceutical, pharmaceutical (solid and liquid dose), medical device, dietary supplement, active pharmaceutical ingredient, cosmetic and personal care product manufacturing, as well as numerous other regulated and non-regulated industries. Microbial contamination, if not eradicated from the system or at least minimized prior to the next manufacturing batch of product, can impact the production batch as well as subsequent batches, or products.

For example, an oral solid dose manufacturer performed a continuous coating process of a single product over six days using a high viscosity coating. During manufacturing, the coating for bioburden was routinely sampled. Throughout the six day coating process, the manufacturer remained below their microbial alert and action levels. However, when the manufacturer changed to a new low viscosity coating, the in-process microbial counts exceeded the alert levels with *Pseudomonas aeruginosa* by day three of the six-day coating process. When the manufacturer returned to the high viscosity coating, there were no issues in running the process. In this case, *Pseudomonas aeruginosa* was part of the native flora in the coater. In the low viscosity coating, the microbe was able to proliferate resulting in out-of-specification results, shorter production runs, discarded coating solution, wasted coated product and lost revenue. The corrective action was a thorough cleaning of the coating equipment with CIP 100® Alkaline Process and Research Cleaner and an increase in the concentration of the Process QDS® Process Disinfectant/ Sanitizer. The increase in the Process QDS disinfectant was equal to broad spectrum disinfectant concentration effective against Gram negative bacteria, such as *Pseudomonas aeruginosa*.

The importance of removing the microbial residue as well as microbial efficacy, is demonstrated in Figure 1. Figure 1 displays three images:

1. A light microscopy image of fluorescently labeled *Pseudomonas aeruginosa* cultured in a glass tubular cell under continuous flow conditions (left).
2. The cultured *Pseudomonas aeruginosa* exposed to a ready-to-use sporicidal agent, Spor-Klenz® Ready-to-Use Cold Sterilant (center).
3. A formulated alkaline cleaner agent and detergent, ProKlenz® ONE High Performance Alkaline Cleaner at 5% v/v for 30 minutes at 60°C (140°F) (right).

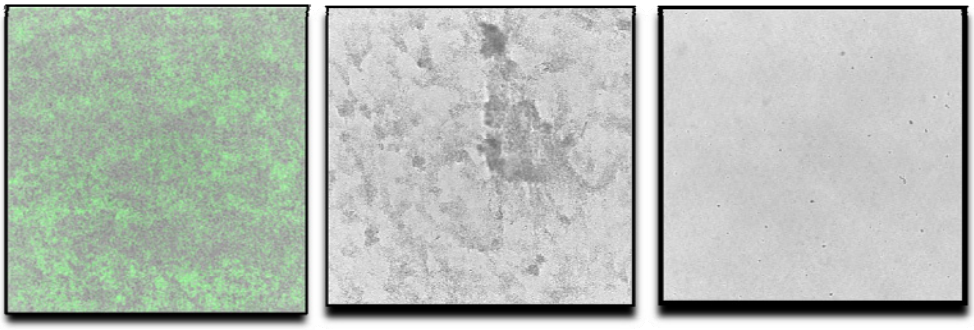

Figure 1: Photographs of *Pseudomonas aeruginosa* (control, Spor-Klenz Ready-to-Use Cold Sterilant treated and ProKlenz ONE High Performance Alkaline Cleaner treated) were provided by the Center for Biofilm Engineering (CBE) in Bozeman, MT

Viability as measured by fluorescence in both conditions was lost in approximately three minutes for the sporicidal agent and six minutes for the alkaline cleaner. However, the center image treated with sporicide, formulated to kill microbes, was not visibly clean. The soil associated with the microbial attachment was still evident. The sample that was cleaned with formulated alkaline cleaner experienced successful removal of the microbial residue.

The general recommendation that has been effective in removing and/or controlling microbial contamination is a 3-part process.

- The first part is to determine the cleanability of the system. The cleanability of the system addresses the following engineering concerns; spray coverage, orientation and dimensions of dead legs, selection and maintenance of valves, tubing and gaskets, flow velocity in the system during cleaning, drainability, material of construction and slopes (Verghese, 2010; Rivera, 2011).

- The second part is to determine the cleaning parameters to remove the process soil, microbial soil and any water scale or rouge on the surface. Cleaning parameters would include cleaning agent, concentration, time, temperature and water quality for the cleaning method used (Verghese, 1998). The use of a formulated alkaline detergent, such as CIP 150® Alkaline Cleaner, ProKlenz ONE High Performance Cleaner, ProKlenz 120 High Performance Cleaner or CIP 100 Alkaline Cleaner alone or in combination with ProKlenz Booster High Performance Detergent Additive at 5% v/v for 30 minutes at 60°C (140°F) should be effective at removing microbial soil. If there is water scale or rouge present, then it is recommended, following the alkali pre-cleaning, to perform a derouging step with CIP 200® Acid-Based Cleaner or ProKlenz® TWO High Performance Acid Cleaner. Refer to Technical Tip 410-200-3017 or 410-200-3073, General Procedure for Derouging Stainless Steel Surfaces. The report produced from the PACE® evaluation service can be utilized to customize the process soil removal, derouging and/or water scale removal through laboratory testing if time permits.

- The third element involves a disinfection/sterilization of the equipment and associated piping using a product with microbial efficacy claims against the target microbe or microbes associated with the microbial contamination. The concentration, temperature, time and water quality will be specified on the product label and followed. Common disinfectants/sterilants used for disinfection of process equipment include Spor-Klenz Ready-to-Use Cold Sterilant, Spor-Klenz Concentrate Cold Sterilant or Process QDS Process Disinfectant/ Sanitizer.

This three-part approach focuses on the importance of good engineering design, a robust cleaning procedure designed to remove the soil from the surface and proper selection of a disinfectant/sterilant effective against the microbial contamination.

References:

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