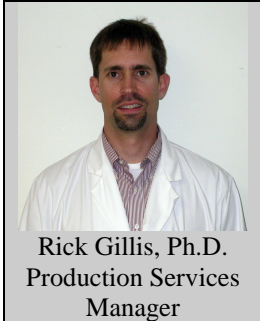


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Steam-in-Place (SIP), the Problems and a New Solution

Validation engineers face many challenges when validating steam-in-place or sterilization-in-place (SIP) systems. Of great concern is the loss of biological indicators within the expanse of process piping during validation. This may be the reason many validation protocols do not recommend biological indicators and only specify thermal monitoring. This approach, albeit good in theory, is misleading and can have dangerous consequences.

SIP is a process commonly used to sanitize or sterilize process piping. SIP is performed to remove or kill attached bacteria within the piping systems. Bacteria are extremely prolific and adapt to virtually all environmental conditions on the planet. Some estimate that bacteria have existed on this planet for billions of years. The propensity of bacteria to live in myriad environments is testimony to their opportunistic nature. One fairly recent discovery is that bacteria have the ability to live in a communal nature attached to a surface, termed a biofilm. Biofilms were first documented in alpine streams. To the surprise of the researchers, bacterial communities were found to be existing and even thriving in these environments with very low nutrients. As more research evolved in this area, biofilms were discovered in virtually every hydrated environment.

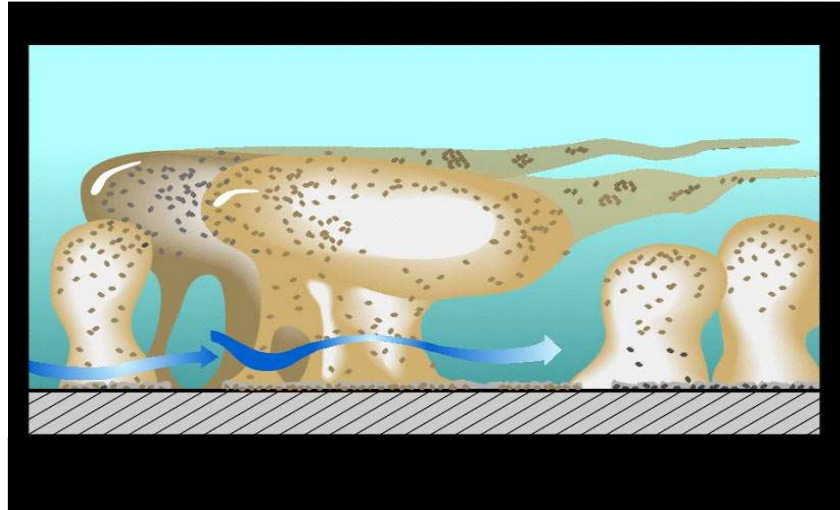


Figure 1. Schematic of a mature biofilm.
(Reprinted from Center for Biofilm Engineering Image Archive)

Bacteria prefer biofilm communities, which afford protection and resistance conditions that are not available to their free-floating counterparts. Biofilms can be widely characterized as a community of microorganisms encased in a slime matrix attached to a surface (Figure 1). These microscopic communities have been found to exist in nearly all aqueous environments where bacteria thrive, from deep-sea hydrothermal vents, distilled water systems, heart valves, and human teeth. There are many advantages for bacteria to live in a biofilm. An attached existence in a flowing environment allows energy conservation for nutrient acquisition. Bacteria do not have to move as the “nutrient conveyor belt” goes by; they can pick and choose from the available “smorgasbord”. Protection is provided by “safety in numbers” from predation and attack by amoebae and macrophage. The character of the slime matrix shields the bacteria from harsh or caustic environments. This is often the root cause of boil orders for public drinking water systems. Biofilm bacteria can live on the distribution piping and may not be effectively killed by the continuous chlorination. In this case, there are a few “poor souls” on the outside of the biofilm that sacrifice themselves for the preservation of the community. The dead carcasses then form a barrier to the chlorine, which shelters the bacteria living inside. These biofilms may get quite large in size until they eventually fall off in chunks, an event termed sloughing. Often, these biofilms contain *E. coli* bacteria, which, when found in high concentrations during routine water monitoring, can initiate a boil order (Figure 2).

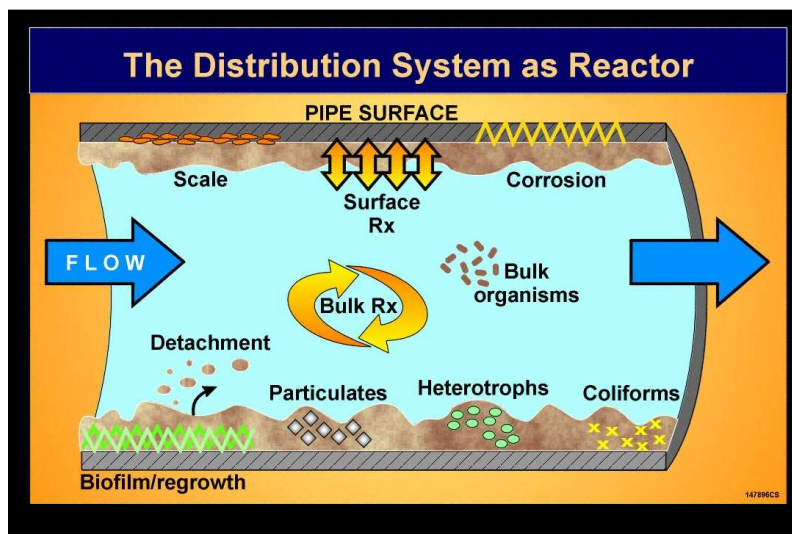


Figure 2. Diagram of a mature biofilm in a process piping system.
(Reprinted from Center for Biofilm Engineering Image Archive)

Biofilms can also be problematic in any fluid distribution system. The milk industry is very sensitive to the difficulties in removing biofilms from their systems, which are easily formed during distribution of this nutrient-rich product. Stainless steel piping has been used in this industry for years. Efforts are made to make the inside surfaces of the piping as smooth as possible to disallow the bacteria a good “foothold”. As one might imagine, the rougher the surface is, the easier it is for the bacteria to find refuge from the flowing environment. Many bacteria are less than 1/100th of a millimeter in diameter, so even the most seemingly obscure scratches in the surface are usually sufficient to harbor bacteria (Figure 3).

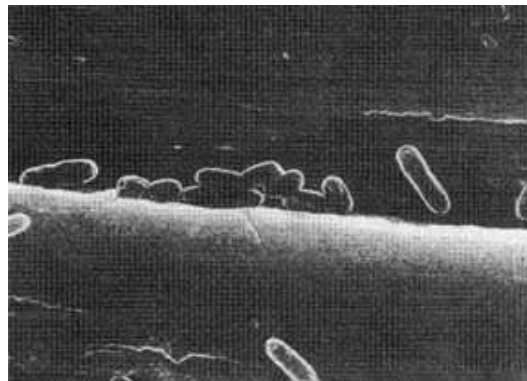


Figure 3. Bacteria in a stainless steel “scratch”. From: Gillis and Gillis, *Ultrapure Water*, 1996

It is easy to imagine how bacteria would have little problem living in a nutrient-rich product like milk. The more perplexing thought is that bacteria are equally able to exist in ultrapure water systems and WFI water, where the nutrients are extremely low. Although the bacteria that colonize WFI systems (e.g. *Pseudomonas* spp.) are usually different than those that may colonize milk systems (*Listeria* spp), the problems are equally disruptive. Although *Pseudomonas* species do not typically cause disease, they

are a type of bacteria that have a cell membrane that contains a substance called lipopolysaccharide (LPS) or endotoxin. This endotoxic compound is also referred to as a pyrogen. Pyrogens can cause severe fever or death. Thus, great lengths are taken to minimize any possibility that biofilms can form in the process piping. Removal of biofilms in these systems can be very difficult. Figure 4 describes a simplified scenario of a biofilm forming in a piping system dead leg. Dead legs are defined as lengths of intersection piping that are six pipe diameters or greater in length. Even at 6X the internal pipe diameter, these reservoirs are typically very difficult to sanitize or sterilize

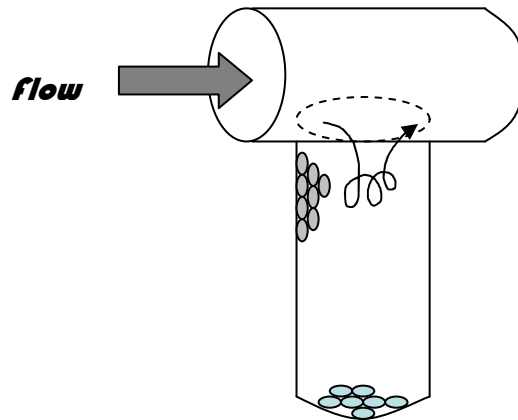


Figure 4. Diagram of biofilm formation in a deadleg.

due to the reduced flow. The farther away from the main process flow, the less turbulence and the more conducive the environment is to permitting a biofilm condition. SIP is a common method used to sterilize process piping systems. This method involves turning the entire piping distribution system into a large autoclave. Vent valves are installed on dead legs to minimize the amount of air that could be trapped during the SIP cycle, thereby increasing sterilization efficacy. Furthermore, extensive thermal monitoring is done during SIP to assure that the entire system reaches adequate sterilization temperatures for appropriate times.

Sterilization is a process that renders a process or material free from living microorganisms. It takes a biological challenge to demonstrate that the system has been sterilized. Biological indicators are typically used. The convenient placement of biological indicators during SIP operation, particularly during validation has been a problem in the past. Custom devices had to be developed in the past to accommodate BIs. A problem often encountered was the loss of the BI during the validation cycle. For this reason, many engineers doing SIP validation did not employ biological indicators. It is a fact that the biological indicator is the only true measure of sterility as there is no other way to effectively monitor steam penetration throughout the system. Furthermore, steam kills much more effectively than hot air. By only employing thermal monitoring, an assumption is made that a region has reached proper temperature and sterilization has occurred. If only thermal monitoring were done and no BIs were used, one cannot assume that lethal steam reached a specific area. The biological indicator is the only

system available that integrates all critical process parameters to accurately measure sterilization effectiveness. The saturated steam has 10 times the lethality that hot air has. Biofilms are problematic in any process fluid distribution system. At the heart of this problem is that bacteria prefer to be stuck to surfaces. This is a fact that will never change. An effective SIP process will provide greater assurance that the bacteria are killed and that the biofilm potential is minimized. A properly validated SIP cycle, done in conjunction with the EZTest[®] self-contained BI and the SmartGasket[®], is one way to ensure that the potential for biofilm formation is minimized.



Figure 5. The Smart Gasket[®] with associated fittings.

SGM Biotech, Inc. offers a solution of concomitantly holding and retaining an EZTest[®] self-contained BI in the piping system with a design to assist in biological indicator placement called the SmartGasket[®]. Two different designs are available, in-line placement, and top-loading. The SporeTrap Gasket is also available for spore strips. Multiple sizes are also available to accommodate sanitary fitting sizes from $\frac{3}{4}$ inch to 4 inch, depending upon the application. These gaskets simply replace your normal sanitary gaskets for the validation process. We invite you to visit our website (www.sgmbiotech.com) for more information.

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