Biofilm in the Greenhouse

White Paper

Biofilm exists in any plumbing system. In all cases, biofilm fouls plumbing, pumps and filters. In greenhouses, biofilm not only causes trouble with mechanical systems, it harbors pathogens dangerous to our crops.

Understanding biofilm is essential to growing healthy plants.

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Biofilm in your greenhouse.

If your greenhouse or nursery has irrigation pipes, filters or tanks you have biofilm in them and it is probably causing problems. Biofilm is ubiquitous. It lives in virtually every water system and it is a significant contributor to plant diseases. It grows in acid or base pH environments; warm water or cold; nutrient-rich water or clear; municipal water, well water or pond water; disinfected water or not. Researchers have observed the initial stages of biofilm formation in as little as 5 seconds after flowing freshly chlorinated municipal water through a new, clean, highly polished stainless steel pipe. It's a real deal.

The intent of this paper is to provide general information on how biofilm is established, how it functions, why it is of particular interest to growers, and some guidelines on how to reduce or eliminate it.

What Is Biofilm?

Biofilm is everywhere.

Anywhere there is water, there is biofilm. Biofilm can be aerobic or anaerobic. It is far more than just algal slime or scum buildup from water impurities.

> **Technically:** A layered aggregate or micro-colonies of predominantly bacterial cells (both primary and opportunistic), living in a protective hydrated glycocalyx matrix of Extra-Cellular Polymeric Substances (EPS) of DNA, nucleic acids and polysaccharides

> **Less technically:** Biofilm is the scum or slime on surfaces continuously exposed to water. That "icky stuff" that forms on our teeth overnight? Its mostly biofilm. Biofilm has been known about since at least 1684 when Anton van Leeuwenhoek, known as the "father of microbiology" observed a man's tooth in his microscope and commented that: "The number of these animicules (microorganisms) in the scurf of a man's teeth are so many that I believe they exceed the men in a kingdom."

A key to understanding how biofilm works and how it can be destroyed or treated is to understand how it develops.

Biofilm formation occurs in 5 stages:

The 5 Stages of a Biofilm

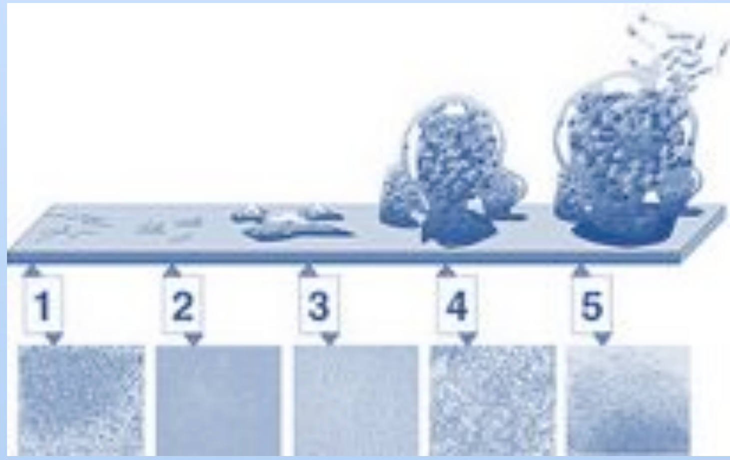


Image from FRT (Free Radicals Technology)

Stage 1: Surface Conditioning: A very fine organic monolayer is deposited on surfaces exposed to water, conditioning it to allow attachment of bacteria. This begins within moments of exposure to water, even sanitary municipal water. It is reversible if flushed or killed immediately and not re-exposed.

Stage 2: Pioneering: Free-floating pioneer bacteria arrive and attach themselves to the conditioned surface. They then switch on a mechanism to secrete EPS (Extracellular Polymeric Substance) that affixes them to the surface and holds the cells together in protective matrix. This is irreversible. This matrix protects them against detergents, antibiotics, most chemical disinfectants, even abrasion.

Stage 3: Colonization: Once the protective matrix is formed the cells divide and recruit other cells through a unique form of chemical communication that identifies it as a hospitable environment.

Stage 4: Maturing: Formation of a fully functioning biofilm, with a three-dimensional structure containing cells packed in clusters with channels between them to transport water, chemical communication, nutrients and waste removal. Any microbe, pathogenic or not, may become enmeshed in the matrix.

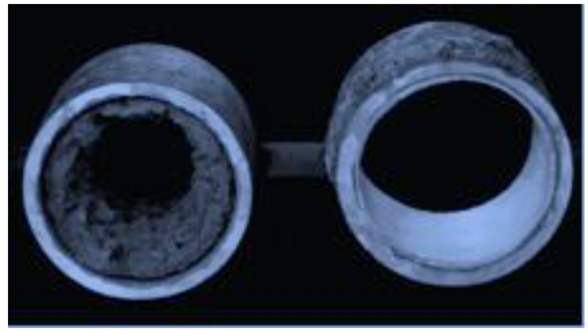
Stage 5: Dispersion: Detachment and Dispersion of cells and re-initiation of new biofilm formations begins. Pieces spread downstream and colonize new surfaces, and repair damage to the existing matrix. These microbes decrease disinfectant levels by increasing disinfectant demand, posing a direct risk to crops

We've always known that pipes accumulate a scum or slime layer over time, and that sometimes it becomes thick enough to cause clogging or disease outbreaks. It was often assumed that this was really only a long-term problem, that as long as it doesn't clog the pipes too much it isn't a significant concern. Microbes in a biofilm can indeed contribute to pipe corrosion. Iron and sulfur bacteria embedded in a biofilm will cause advanced corrosion. Sulfur-reducing microbes can generate hydrogen sulfide gas causing a rotten egg odor and decreasing pH. But these are not the most typical problems caused by biofilms.

We often think of biofilm as the thick visible layer of green and or brown scum inside a pipe or on a tank wall. This is obvious biofilm.

But often not recognized as biofilm is the slippery mostly invisible material that can only be felt inside a pipe, tank wall or other wetted surface…that too is biofilm, the most common type. Even before the slippery scum can be detected it is there and building.

Contrary to popular belief, light is not necessary for a biofilm to develop. It can grow in completely opaque pipes, buried far below the ground. Painting pipes or using schedule 80 PVC will not eliminate this problem.



In the above photo both pipes contain a biofilm. The pipe on the left is a very mature biofilm with combined layer of corrosion deposits on top of a biofilm layer. The pipe on the right is recently cleaned but none-the-less contains a more typical invisible layer or matrix of biofilm.

Why is Biofilm a Problem?

On the human side, we've all read the stories about disease outbreaks on **large cruise ships**.

Biofilm in the water pipes and exposed surfaces is the primary culprit! Biofilms are implicated in most outbreaks of Legionnaire's disease resulting from Legionella bacteria that detaches from biofilms in water and air handling systems in cruise ships, hotels and hospitals. Biofilm is also implicated in many other human diseases from Appendicitis to Pneumonia, gastrointestinal infections and many allergies. The National Institutes of Health (NIH) estimates that more than 65% of all human microbial infections are caused by biofilms.

Municipal water authorities are also learning about how to deal more effectively with biofilm based on recent research results. Following is a transcript of a brief NPR report about the City of Milwaukee Wisconsin learning about the effects of biofilm in their public water system. Note in the report that the EPA is primarily interested in how they deal with their distribution system…the piping, because the EPA recognizes that without solving that the problem isn't solved.

A NPR Report on **Milwaukee** city water stated: "The EPA is actually starting a whole new containment monitoring requirement that 's going to go into effect soon, and they've got 30 chemicals on that list; and it just came out this week and we are already testing for 22 of those 30. And we test for them in the source water, the plant finished water and the distribution system. All that the EPA wants us to do is the distribution system," Lewis says. Technology has influenced the utility's ability to get ahead of the requirement curve.

"The biggest, biggest change here in Milwaukee was the change from chlorine as the major disinfectant to ozone as the major disinfectant," Lewis says. What prompted that change was a public health crisis – the 1993 outbreak of Cryptosporidium. It sickened hundreds of thousands, killed some people and forced residents to boil their water. "Chlorine does not kill Cryptosporidium as we all learned the hard way but ozone does. And although ozone was put in as a disinfectant it is so powerful of these emerging contaminants that we're coming to understand are out in the lake are just blown to smithereens by ozone when they come into the plant," Lewis says.

Even the **beer industry**, brewing and serving, has found that biofilm can even make your favorite beer taste funny if not dealt with.

If biofilm is so prevalent and such a problem then why haven't we been talking about it for longer than just the past 10 years or so?

Although biofilms have been known about for hundreds of years, most of the body of research that has discovered and exposed the true nature and processes of biofilm has been established only in the past 20 years, with most of that research maturing and being reported just over the past 10 years.

In 1990 the National Science Foundation in the United States founded the Engineering Research Center at Montana State University under the direction of biofilm research pioneer William Charaklis. It is now known as the Center for Biofilm Engineering (CBE) at MSU. Other research groups now also exist in the US, England, and Germany. The research results coming out of CBE, and from other researchers, have uncovered a vast level of new knowledge about biofilm over the past 10 to 15 years; how it is formed, how it functions, its potential benefits, the problems it causes, and what works and doesn't work to eliminate it.

Institutions such as Scripps Research Institute in La Jolla CA, and CBE @ MSU are discovering the significant roll played by biofilms in the causes of human disease as well as plants and

animals. This includes biofilms formed inside the systems of the human body, biofilms formed in the drinking water systems we use, and even diseases passed on from the bites of pests that have bacteria harboring biofilms in their digestive tracts. For example the bacterium **Yersinia pestis**, the cause of human bubonic plague, is lodged in a persistent biofilm in the intestines of fleas, which is then transferred to humans through a flea bite.

Among the mountain of things learned about biofilm from 20 years of intense research, the discovery or recognition of three simple facts rises to the top when it comes to understanding how we have to deal with biofilm.

1) **Unless otherwise expressly destroyed, Biofilm exists on virtually every non-sterile surface exposed to water, whether living or non-living.**

2) **Biofilms establish or re-establish themselves extremely quickly. Destroying biofilm must be a continuous process.**

3) **Biofilm is very tough to eliminate, much tougher than was previously believed. It is alive, it has a protective mechanism and very few chemicals or treatments are able to destroy it.**

Many of the discoveries about biofilm explain problems experienced by growers, and other industries, for years. Some of these have been assumed to be caused by other factors, some were just mysteries that we learned to live with. Along with providing answers to some age-old water quality problems, the research has also shed new light on how to reduce and eliminate biofilm, much of which was not even suspected.

Research has proven that some existing methods of treatment simply do not work or are far less effective than previously believed. Conversely, other treatments have been discovered to be very effective or more effective than previously believed

Why Is Biofilm Important to Growers?

Greenhouse and nursery operations are particularly susceptible to biofilm problems. Biofilm establishes itself and thrives most easily in a nutrient rich environment, with warm temperatures that are more conducive to bacterial growth, and with a large surface-area to water-volume ratio. This of course describes the typical greenhouse or nursery operation's irrigation system.

Storage ponds and/or tanks, filters, thousands of feet of small diameter (1-6") pipes, filled with fertilizer injected water to assure an ample food supply to the biofilm, running exposed through heated environment, and/or exposed to sunlight all day, and irrigation emitters surrounded by and continuously exposed to plants and other sources of pathogens. ***It is biofilm heaven***. If you have a greenhouse with pipes, filters, any kind of water storage, without a doubt you have biofilm and it is most likely stressing your plants and your facility.

Why Biofilm Loves Horticulture

- High use of untreated surface or well water
- Nutrient injection into water stream
- High pipe surface area (lots of small pipes)
- High temperature environment
- Light permeable piping system
- Exposed/unprotected outlets (hose ends, nozzles)
- Small orifices to plug (drippers, mist nozzles, etc.)

Most growers have observed symptoms associated with biofilm for years, but the connection is often not made to biofilm as the culprit.

The more obvious problems, of course, include **clogging of drip tubes**, filters, valves and emitters. These lead to extra labor costs, crop shrink, and general headaches.

More difficult to connect symptoms are also very common. Frequent examples include occasional or chronic infections from water borne pathogens such pythium, phytopthera; chronic algae problems that get tougher and tougher to combat; and the reducing efficacy of some types of water treatment chemicals and the corresponding need to increase chemical concentrations to maintain appropriate disinfection capacity.

These and other problems are often directly associated with the adverse effects of biofilm in the water systems. This also contributes to extra labor, crop shrink, as well as more costly problems of poor plant shelf life and reduced performance for the consumer.

Biofilm clogged pressure compensating emitters

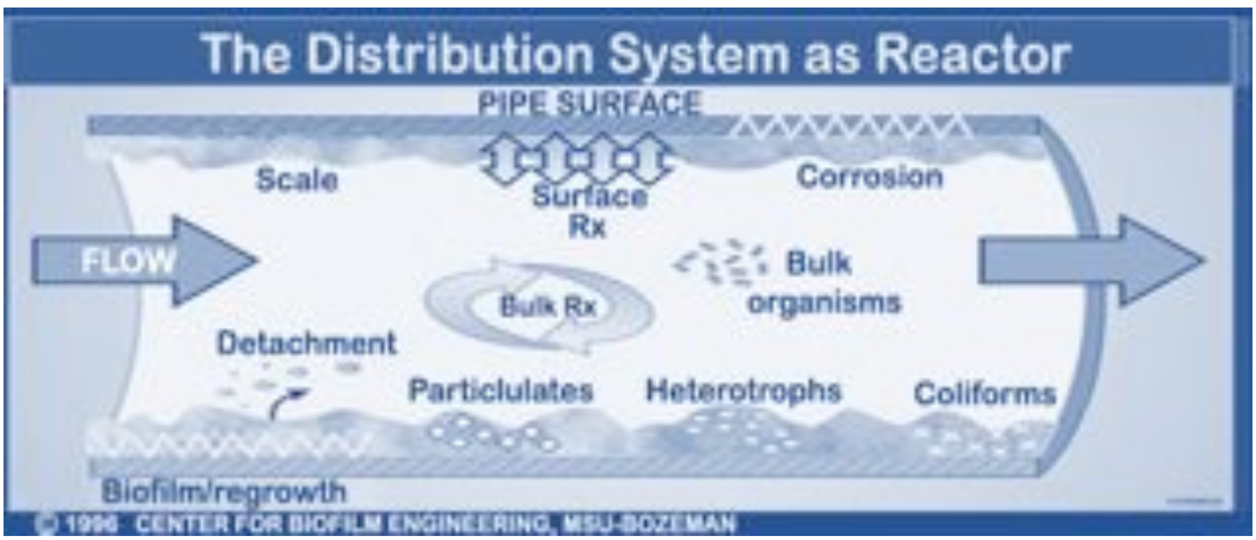
Biofilms are alive and play host to all types of living organisms. Bacteria, Viruses, Protozoa, Invertebrates, Algae and algal toxins, Fungi, various Pseudomonas species, E coli, staphylococci and Microbial toxins are among the most common invading pathogens that can be found in biofilms.

The biofilm in your pipes will generally include whatever microorganism has been, or is currently, in your facility, plus whatever rode in on the stream from your municipal water supply, well, or surface water supply (stream, river, pond, or lake).

If you've suffered a pythium outbreak in the past, it is very likely that same pythium strain resides in the biofilms in your irrigation system. Biofilm doesn't just grow downstream from the water source, it can also begin at the outlet of a hose or irrigation emitter and then travel upstream.

Consequently, where hoses and irrigation emitters come into contact with plants, sidewalks, pathways, or human hands, any microorganisms at these sites can and do transfer onto the surfaces to begin the formation of a biofilm. This also explains why some typically human carried pathogens end up in irrigation systems not otherwise exposed.

Biofilm is increasingly being recognized as a significant plant disease source. Biofilms act as a slow-release mechanism for persistent contamination of the water and pathogen release to the plants. Biofilms cause significant damage every year by providing a usually unidentified, unknown, sometimes even mysterious source of pathogens that damage crops and hurt the reputation of growers due to lower crop quality and sometimes food borne illnesses from vegetable producers.



Biofilms also present an oxygen demand, reducing important and often already depleted levels of dissolved oxygen to the plants.

Some biofilms may be beneficial to plants, but most contribute to or cause a number of common plant diseases. Many infections of pythium, phytopthera and other water borne or water transferable pathogens are the direct result of biofilm. Biofilms also attach to fruits and vegetables and consequently transfer bacteria harmful to humans, making erradication even more critical for vegetable production greenhouses and packing operations.

Can Biofilm Be Controlled?

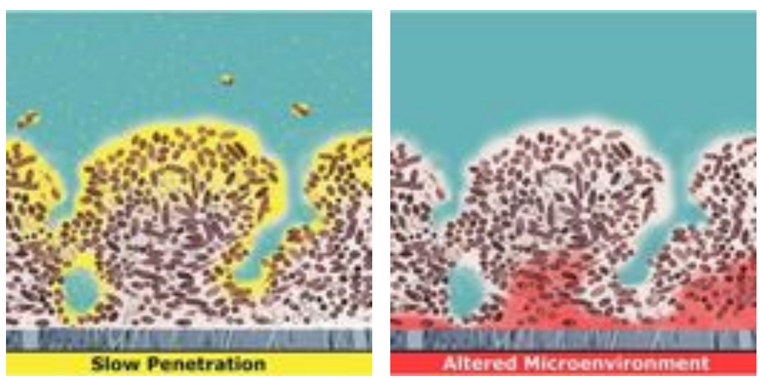
Once formed, a biofilm is very difficult to remove and keep away.

Efforts to combat biofilm have met with varying degrees of success. These include adding more effective filtration to deal with the debris that sloughs off of a mature biofilm; periodic high velocity line flushing to scour off the biofilm; chemical shock treatments to kill the biofilm when it gets too thick; and disinfection systems to kill the microbes that make a biofilm. A lot of money has been spent over the years to deal with biofilm; unfortunately much of it spent with inadequate results simply because the problem wasn't fully understood. While this is true of the horticulture industry; it extends to virtually every industry from greenhouses to municipal water authorities to major hospitals.

Many methods previously thought to destroy or eliminate or reduce biofilm have been discovered to be ineffective or only partially effective.

In some situations, partial effectiveness may be worse than no treatment at all. Even a minor layer or section of biofilm remaining after attempted removal will quickly reestablish. Researchers have observed that an "injured" biofilm will often reestablish faster and further than the original biofilm. The protective mechanism of the biofilm can allow microbes to be only injured rather than killed or destroyed by disinfectants and other partially successful attempts at treatment. These injured microbes are then able to recover and re-colonize and many will have established a higher level of resistance to the chemical that was used.

Many chemicals, most notably chlorine, interact with the biofilm in a way that slows the penetration of disinfectant through the top layer of the biofilm, consuming it, weakening it. Another protective mechanism of the biofilm is the altered microenvironment in the base matrix of the biofilm. This is a generally anaerobic environment and the very low oxygen levels in the matrix itself prevent oxidizers from functioning.



The Biofilms Hypertextbook Alfred B Cunningham, John E Lennox and Rockford J Ross, Eds 2001-2008

Water treatment without dealing with biofilm is addressing less than half the problem and will result in continued disease problems from water borne pathogens.

What works for effective biofilm treatment, particularly in irrigation systems? It is important first to clearly define success in biofilm treatment. Successful biofilm elimination will accomplish the following 7 objectives:

1. **Substantially reduces, kills or destroys the free-floating cells/microorganisms that colonize a biofilm and that break off of an established biofilm.**

2. **Reduces or eliminates the buildup (stages 3 & 4) of a mature biofilm.**

3. **Destroys the Glycocalyx matrix that forms the life-supporting base and protects the biofilm.**

4. **Accomplishes the destruction of the matrix and kill the free-floating microorganisms at concentrations/levels that are not toxic to and do not damage plants.**

5. **The biofilm treatment is able to continuously maintain biofilm free pipes, tanks and irrigation delivery systems (emitters, etc.) without potentially harmful intermittent biofilm formations**

6. **The treatment does not contribute to, or minimizes, the potential for clogging of downstream irrigation delivery system(s).**

7. **The treatment maintains or improves the overall quality of the water in the process of eliminating biofilm. This includes maintaining correct pH levels, Dissolved Oxygen levels, and levels of salts.**

The Most Effective Biofilm Treatment Methods:

1. **Continuous Ozone treatment**: Municipal water systems are adding ozone to water systems to destroy biofilms because the chlorine in most municipal water systems has been found ineffective even at very high concentrations. Ozone meets all 7 criteria of effective biofilm treatment for horticulture. It kills free floating cells and destroys the biofilm matrix **on contact** at very low concentrations, and leaves no negative residual that can be harmful to plants. It does not effect pH or increase salts and it charges the water with dissolved oxygen in the process.

2. **Continuous Chlorine Dioxide Injection**: Chlorine dioxide is able to penetrate the mature biofilm and will, over time, kill the biofilm matrix. However, Cl02 is somewhat selective in what it kills and the concentration required is very close to the levels that are toxic to many plants so it must be monitored closely.

3. **Mixed Oxidative Solutions**: These are blends of oxidizers that will effectively kill biofilms including the matrix. They are also effective at concentrations that are very close to the toxicity susceptibility levels of some plants and must be carefully monitored.

4. **Shock treatments with some forms of acid**: If other methods mentioned above are not immediately available or affordable, this will work in a pinch as a periodic treatment to reduce and/or temporarily remove biofilm. However, this fails the test of continuous treatment and plant safety, and often clogs filters, valves and irrigation emitters.

What does not work to destroy biofilm in irrigation systems?

- **Heat Pasteurization:** This is capable of killing the organisms that contribute to biofilms but does nothing at all to contact and reduce or destroy a biofilm. After the water is cleaned in the heat exchanger it is delivered back to biofilm laden pipe system re-infesting the cleaned water with no means of attacking the biofilm.

- **UV Light (low or high pressure):** Identical problems associated with heat pasteurization. Does nothing to contact and reduce or destroy biofilm.

- **Chlorine:** While chlorine can be effective at reducing many free-floating microbes, it is ineffective at destroying a biofilm even at very high concentrations and extended contact times. A process called reaction-diffusion interaction slows the penetration of the chlorine through the biofilm layers slowing the effectiveness of the chlorine until the neutralizing capacity of the biofilm itself is able to overpower the chlorine. Experiments have shown that even after an hour and a half of exposure to chlorine the biofilm was not penetrated or destroyed. In irrigation systems with fertilizer injected into the water chlorine is effectively rendered inert as a disinfectant within a few seconds upon exposure to nitrates.

  Research demonstrated that biofilm bacteria may be 150-3,000 times more resistance to free chlorine and 2-100 times more resistant to monochloramine than free floating bacteria.

- **Most biocides** fail to kill the base microbes in a biofilm matrix simply because they are consumed by and therefore fail to penetrate past the surfaces layers of the biofilm.

  "Biofilm bacteria display a resistance to biocides that may be considered stunning." - LeChevallier 1988

- **Straight Hydrogen Peroxide** has limited efficacy in biofilm destruction because the biofilm contains enzymes that split hydrogen peroxide into water and oxygen and while it remains mobile in the biofilm it is consumed in the surface layer faster than it diffuses, thereby rendering it ineffective unless used at very high dilution rates. Even in biofilms that are more susceptible, Hydrogen Peroxide is often too slow and too selective to be fully effective in an irrigation system. Hydrogen Peroxide compounds can be effective as a shock treatment. Some Hydrogen Peroxide products that are combined with peroxyacetic acid or other oxidizers are effective

- **High Velocity Line Flushing/Scouring**: Bacteria cemented to surfaces under the laminar layer of matrix resist high shearing forces from flushing forcefully with water, even water with an abrasive compound. This cannot be relied on to destroy a biofilm. It will knock down the maturing buildup (stages 4 & 5) and slough that downstream but will generally not destroy the matrix itself. Often flushing will worsen the problems caused by biofilm because the sloughed material clogs filters and drippers, and the biofilm will always quickly rebuild itself even thicker and more robust than before.

- **Copper Ionization**: This is effective against some thin celled pathogens but is minimally effective on destroying the biofilm Glycocalyx/matrix, particularly in the relatively short contact times allowed in most irrigation pipe systems.

In conclusion, biofilm in the irrigation distribution of greenhouses and nurseries is costing producers millions per year in added labor, product shrink, reduced sell-through at retail, and added chemical costs for the resulting disease treatment. The available solutions are very limited, but readily available. Dramm stands ready to assist you to evaluate your irrigation distribution system and develop a game plan to correct problem areas and to eliminate the biofilm problem that surely lurks in your system

1. **You have biofilm in your pipes unless you have already taken steps to eliminate it.**
2. **The biofilm in your pipes is providing a constant slow release of pathogens to your plants.**
3. **The pathogens coming from a biofilm can be sufficient to overcome the plants resistance either alone or when added to already existing pathogen levels.**
4. **Primary treatment of the water coming into the irrigation system is inadequate. Secondary action from a residual must be established to deal with the biofilm.**
5. **Continuous treatment is required to maintain an acceptably biofilm free environment.**