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The key to understanding and controlling bacterial growth in Automated Drinking Water Systems, Second Edition
by Paula H. Dreeszen - June 2003

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Definitions

adsorption - the physical process occurring when one substance adheres to the surface of another. Adsorption is a physical process which occurs without chemical reaction.

aerobic - require air or free oxygen for growth
anaerobic - grow without air or free oxygen
AWS - 'automated watering system'
boundary layer - the laminar or quiescent zone at the pipe wall where flow velocity falls to zero
chemotaxis - movement of organisms in response to a chemical gradient
flagella - plural of flagellum. Some bacteria have more than one flagellum.
flagellum - long filamentous appendage that propels bacteria; literal meaning is "whip"; see Figure 3
glycocalyx - extracellular polymeric substances (sticky polymers) that bacteria excrete to adhere to solid surfaces and to trap nutrients
hydrophilic - having the characteristic of absorbing water
hydrophobic - having the characteristic of repelling water (example: Teflon is a hydrophobic material.)
laminar flow - fluid movement in smooth, continuous, nonturbulent parallel layers which do not mix with each other
motile - movement independent of water
oligotrophs - bacteria and other organisms able to grow in nutrient-limited environments
planktonic - free-floating microorganisms whose movements are controlled by water movement (not attached to surfaces)
RA - 'roughness average' or the arithmetic average deviation from the center line of a surface
RDS - 'room distribution system', part of an automated watering system
RMS - the 'root mean square' of the deviations from the center line of a surface
sessile -attached to solid surfaces (opposite of planktonic)
surface charge - the balance between the numbers of positive and negative charges exposed at a solid surface
surface free energy - the degree of hydrophobicity or hydrophilicity of a surface

Introduction

What are biofilms?

"More properly known as ***biofilm****, slime cities thrive wherever there is water - in the kitchen, on contact lenses, in the gut linings of animals. When the urban sprawl is extensive, biofilms can be seen with the naked eye, coating the inside of water pipes or dangling slippery and green from plumbing."* (Coghlan 1996)

Simply put, biofilms are a collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface. You are already familiar with some biofilms: the plaque on your teeth, the slippery slime on river stones, and the gel-like film on the inside of a vase which held flowers for a week. Biofilm exists wherever surfaces contact water.

More than 99 percent of all bacteria live in biofilm communities. Some are beneficial. Sewage treatment plants, for instance, rely on biofilms to remove contaminants from water. But biofilms can also cause problems by corroding pipes, clogging water filters, causing rejection of medical implants, and harboring bacteria that contaminate drinking water.

Why learn about biofilms?

"Microbiologists have traditionally focused on free-floating bacteria growing in laboratory cultures; yet they have recently come to realize that in the natural world most bacteria aggregate as biofilms, a form in which they behave very differently. As a result, biofilms are now one of the hottest topics in microbiology." (Potera 1996)

As in any water system, 99 percent of the bacteria in an automated watering system is likely to be in biofilms attached to internal surfaces. Biofilms are the source of much of the free-floating bacteria in drinking water, some of which can cause infection and disease in laboratory animals.

One common biofilm bacteria, *Pseudomonas aeruginosa*, is a secondary pathogen which can infect animals with suppressed immune systems. Besides being a reservoir of bacteria which can affect animal health, biofilms can also cause corrosion in stainless steel piping systems. In order to design and operate automated watering systems that deliver the bacterial quality required by our customers, we should understand how biofilms develop, some of the problems they can cause, and how they can be controlled. Understanding bacteria in biofilms is one step in preparing for the future. We are currently meeting the most demanding microbiological water quality requirements of many of our customers by supplying chlorinated reverse osmosis water and by maintaining water quality through flushing and sanitization. (Refer to *Microbiological Survey of Automated Watering Systems*, D209, Dreeszen 1996.) But, what if chlorine use in animal drinking water is prohibited? Or, what if water quality requirements become even more stringent with the use of new specialized animals?

Of course, you might just want to learn about biofilms to marvel at the ability of bacteria to adapt to their environment and to evade our attempts to eliminate them. •

Steps in Biofilm Development

The instant a clean pipe is filled with water, a biofilm begins to form. The development of the biofilm occurs in the following steps.

Surface conditioning

The first substances associated with the surface are not bacteria but trace organics. Almost immediately after the clean pipe surface comes into contact with water, an organic layer deposits on the water/solid interface (Mittelman 1985). These organics are said to form a "conditioning layer" which neutralizes excessive *surface charge* and *surface free energy* which may prevent a bacteria cell from approaching near enough to initiate attachment. In addition, the adsorbed organic molecules often serve as a nutrient source for bacteria.

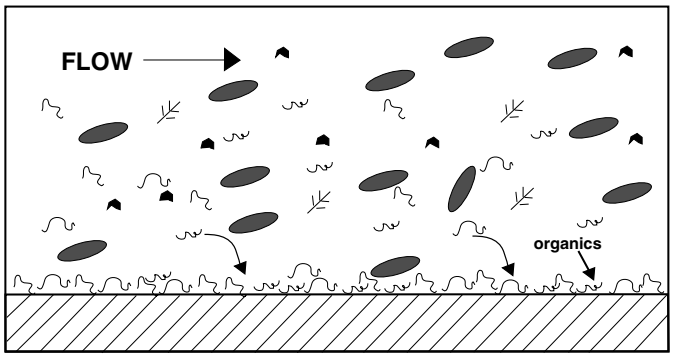


Fig. 1
Adsorption of organic molecules on a clean surface forms a conditioning film. (Characklis 1990)

Adhesion of 'pioneer' bacteria

In a pipe of flowing water, some of the *planktonic* (free-floating) bacteria will approach the pipe wall and become entrained within the *boundary layer*, the quiescent zone at the pipe wall where flow velocity falls to zero. Some of these cells will strike and adsorb to the surface for some finite time, and then desorb. This is called *reversible adsorption*. This initial attachment is based on electrostatic attraction and physical forces, not any chemical attachments. Some of the reversibly adsorbed cells begin to make preparations for a lengthy stay by forming structures which may permanently adhere the cell to the surface. These cells become *irreversibly adsorbed*.

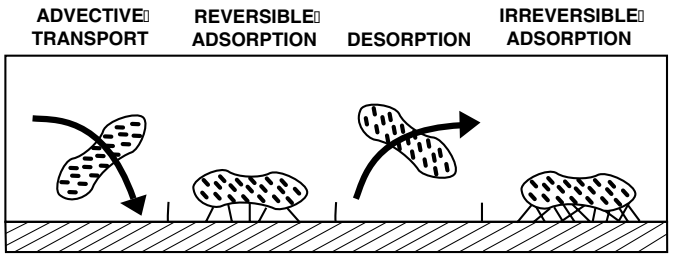


Fig. 2
Transport of bacteria cells to the conditioned surface, adsorption, desorption, and irreversible adsorption. (Characklis 1990)

Glycocalyx or 'slime' formation

Biofilm bacteria excrete *extracellular polymeric substances*, or sticky polymers, which hold the biofilm together and cement it to the pipe wall. In addition, these polymer strands trap scarce nutrients and protect bacteria from biocides. According to Mittelman (1985), *"Attachment is mediated by extracellular polymers that extend outward from the bacterial cell wall (much like the structure of a spider's web). This polymeric material, or glycocalyx, consists of charged and neutral polysaccharides groups that not only facilitate attachment but also act as an ion-exchange system for trapping and concentrating trace nutrients from the overlying water. The glycocalyx also acts as a protective coating for the attached cells which mitigates the effects of biocides and other toxic substances."*

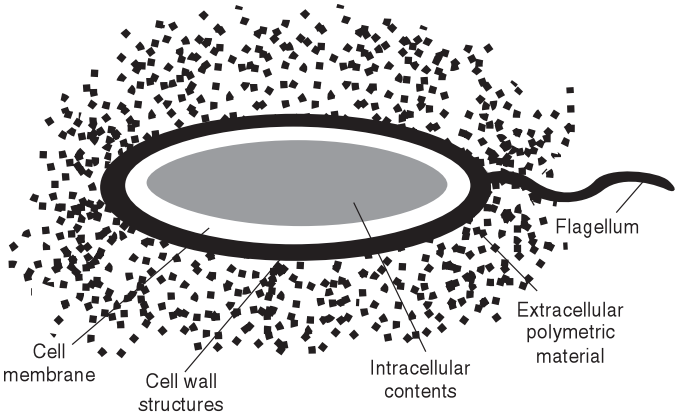


Fig. 3
Wild bacteria are "hairy" cells with extracellular polymers which stick to surfaces. (Mittelman 1985)

As nutrients accumulate, the pioneer cells proceed to reproduce. The daughter cells then produce their own glycocalyx, greatly increasing the volume of ion exchange surface. Pretty soon a thriving colony of bacteria is established. (Mayette 1992)

In a mature biofilm, more of the volume is occupied by the loosely organized glycocalyx matrix (75-95%) than by bacterial cells (5-25%) (Geesey 1994). Because the glycocalyx matrix holds a lot of water, a biofilm-covered surface is gelatinous and slippery. Biofilm is made up microbes and a "spiders web" of extracellular polymers.

4. Secondary colonizers

As well as trapping nutrient molecules, the glycocalyx net also snares other types of microbial cells through physical restraint and electrostatic interaction. These secondary colonizers metabolize wastes from the primary colonizers as well as produce their own waste which other cells then use in turn. According to Borenstein (1994), these *"other bacteria and fungi become associated with the surface following colonization by the pioneering species over a matter of days."*

5. Fully functioning biofilm

A cooperative "consortia" of species

The mature, fully functioning biofilm is like a living tissue on the pipe surface. It is a complex, metabolically cooperative community made up of different species each living in a customized microniche. Biofilms are even considered to have primitive circulatory systems. Mature biofilms are imaginatively described in the article "Slime City":

"Different species live cheek-by-jowl in slime cities, helping each other to exploit food supplies and to resist antibiotics through neighborly interactions. Toxic waste produced by one species might be hungrily devoured by its neighbor. And by pooling their biochemical resources to build a communal slime city, several species of bacteria, each armed with different enzymes, can break down food supplies that no single species could digest alone. The biofilms are permeated at all levels by a network of channels through which water, bacterial garbage, nutrients, enzymes, metabolites and oxygen travel to and fro. Gradients of chemicals and ions between microzones provide the power to shunt the substances around the biofilm." (Coghlan 1996)

Biofilms grow and spread

A biofilm can spread at its own rate by ordinary cell division and it will also periodically release new 'pioneer' cells to colonize downstream sections of piping. As the film grows to a thickness that allows it to extend through the boundary layer into zones of greater velocity and more turbulent flow, some cells will be sloughed off. According to Mayette (1992), "These later pioneer cells have a somewhat easier time of it than their upstream predecessors since the parent film will release wastes into the stream which may serve as either the initial organic coating for uncolonized pipe sections down stream or as nutrient substances for other cell types."

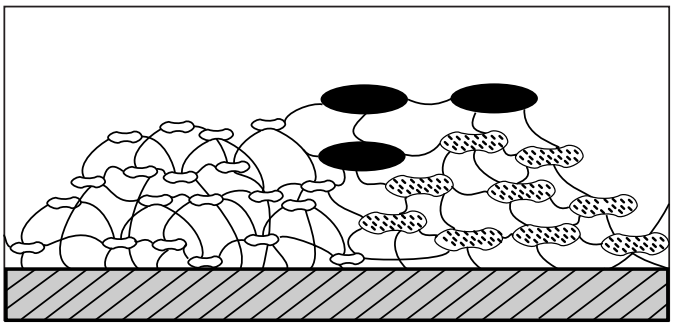


Fig. 4
Bacteria and other microorganisms develop cooperative colonies or "consortia" within the biofilm. An anaerobic biofilm may develop underneath the aerobic layer. The biofilm thickness will reach an equilibrium as flowing water detaches cells extending out into turbulent flow. (Borenstein 1994)

How fast does biofilm develop?

According to Mittelman (1985), the development of a mature biofilm may take several hours to several weeks, depending on the system. *Pseudomonas aeruginosa* is a common 'pioneer' bacteria and is used in a lot of biofilm research. In one experiment (Vanhaecke 1990, see test summary pg 5), researchers found that *Pseudomonas* cells adhere to stainless steel, even to electropolished surfaces, within 30 seconds of exposure. •

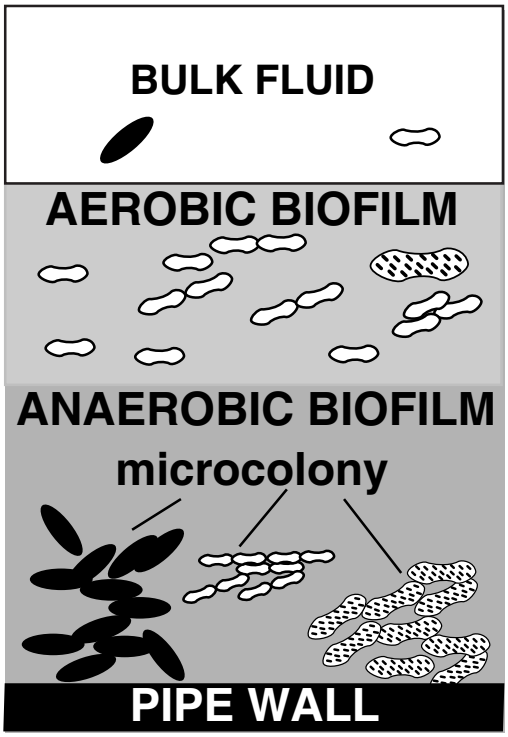


Fig. 5
Biofilm periodically releases a 'pioneer' cells

Benefits to Bacteria: food and protection

The association of bacteria with a surface and the development of a biofilm can be viewed as a survival mechanism. Bacteria benefit by acquiring nutrients and protection from biocides.

Food

Potable water, especially high-purity water systems, are nutrient-limited environments, but even nutrient concentrations too low to measure are sufficient to permit microbial growth and reproduction. Bacteria and other organisms capable of growth in nutrient-limited environments are called *oligotrophs*. Bacteria have evolved the means to find and attach to surfaces in order to increase the chances of encountering nutrients.

What advantages are offered by adhesion to surfaces and development of biofilm?

1. Trace organics will concentrate on surfaces.
2. Extracellular polymers will further concentrate trace nutrients from the bulk water.
3. Secondary colonizers utilize the waste products from their neighbors.
4. By pooling their biochemical resources, several species of bacteria, each armed with different enzymes, can break down food supplies that no single species could digest alone.

What means have bacteria developed to find and attach to surfaces?

1. Motility and *chemotaxis*

Motile bacteria can swim along a chemical concentration gradient towards a higher concentration of a nutrient. The movement of organisms in response to a chemical (nutrient) gradient is called *chemotaxis*. *Pseudomonas aeruginosa* is one of the motile bacteria which uses a *flagellum* to move toward higher nutrient concentrations at the pipe wall. In a study on the attachment of *Pseudomonas* to stainless steel surfaces (Stanley 1983), researchers put cells in a blender to remove the *flagella*. They found that the rate of cell attachment decreased at least 90% when flagella were removed.

2. *Hydrophobic* cell wall

Many organisms faced with the starvation conditions encountered in purified water systems respond by altering their cell wall structure to increase their affinity for surfaces. By altering the protein and lipid composition of the outer membrane, the charge and hydrophobicity can be changed. The cell wall becomes *hydrophobic*. "Such hydrophobic cells want nothing more than to find their way out of the water column. Once in the *boundary layer* (the dead zone at the piping wall where flow velocity falls to zero), they are attracted to the pipe surface" (Mayette 1992).

3. Extracellular polymer production

Once at the surface, bacteria cells anchor themselves to the surface with their sticky polymers. Simple shear (flushing) is no longer adequate to remove these cells. (See section on flow velocity, page 8.)

Protection from disinfectants

"Once the microorganisms have attached, they must be capable of withstanding normal disinfection processes. Biofilm bacteria display a resistance to biocides that may be considered stunning." (LeChevallier 1988).

This researcher demonstrated that biofilm associated bacteria may be 150-3000 times more resistant to free chlorine and 2-100 times more resistant to monochloramine than free-floating bacteria.

Another researcher's work (Anderson 1990, see test summary below) suggests that *Pseudomonas* has a clever way of eluding its attackers: It secretes a sticky slime that builds up on the pipe interior. A germicide flushed through the water distribution system kills free-floating microbes, but it can't touch bacteria embedded in the slimy biofilm.

"When bacteria are in a film, they are very resistant to biocides. "In fact, they often produce more exopolymers after biocide treatment to protect themselves" (Borenstein 1994).

Test Summary: *Pseudomonas* Disinfection and Regrowth in Pipes

Roger Anderson and his colleagues at the Atlanta-based Centers for Disease Control took plastic pipes and filled them with water contaminated with two strains of *Pseudomonas*. After allowing the bacteria to incubate for eight weeks, the scientists emptied out the infested water and doused the pipes with germ-killing chemicals, including chlorine, for seven days. They then refilled the pipes with sterile water and periodically sampled the "clean" water. The team reported that both strains survived in the chemically treated pipes and reestablished colonies there.

How does the biofilm provide protection from disinfectants?

1. Protective shield

In order to destroy the cell responsible for forming the biofilm, the disinfectant must first react with the surrounding polysaccharide network. The cells themselves are not actually more resistant, rather they have surrounded themselves with a protective shield. The disinfectant's oxidizing power is used up before it can reach the cell.

2. Diffusion limitations

When cells are attached to a pipe wall, delivery of the disinfectant is limited by the rate of diffusion of the compound across the boundary layer and through the film. It takes a higher concentration over a longer contact time for the disinfectant to reach the bacteria cells in a biofilm compared to free-floating organisms. •

New discoveries

Recent research from the Center for Biofilm Engineering has dispelled some earlier assumptions about bacteria and biofilms.

Center for Biofilm Engineering

The Center for Biofilm Engineering was established at Montana State University, Bozeman, MT, in 1990 by the National Science Foundation Engineering Research Centers program. Their mission is to advance the basic knowledge and education required to understand, control and exploit biofilm processes. **Visit their website at: www.erc.montana. edu**

Biofilm structure

In the past, microbiologists assumed that biofilms contained disorderly clumps of bacteria located in no particular structure or pattern. New techniques to magnify biofilms without destroying the gel-like structures have enabled researchers to discover the complex structure of biofilms as if viewing a city from a satellite. This structure is described in the recent article *"Slime City" (Coghlan 1996):*

"In most cases, the base of the biofilm is a bed of dense, opaque slime 5 to 10 micrometers (197-394 microinch) thick. It is a sticky mix of polysacharides, other polymeric substances and water, allproduced by the bacteria. Soaring 100 to 200 micrometers (3940-7840 microinch) upwards are colonies of bacteria shaped likemushrooms or cones. Above street level comes more slime, this time of a more watery makeup and variable consistency with a network of channels through which water, bacterial garbage, nutrients, enzymes, metabolites, and oxygen travel."

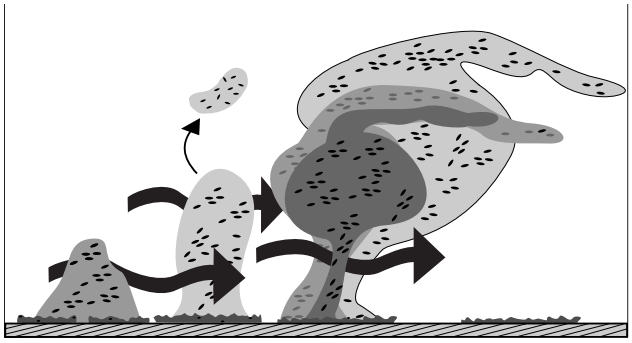


Fig. 6

Conceptual model of the architecture of a single-species biofilm based on direct observations using a confocal *microscope. Some microcolonies are simple conical structures, while others are mushroom shaped. Water currents (arrows) flow in channels between the colonies carrying nutrients and waste.* (Costerton 1995)

NOTE: In an automated drinking water system that is flushed regularly, the biofilm thickness should be less than 200 microns. See section on biofilm thickness and flow velocity, page 11.

Biochemistry of biofilm bacteria

Past researchers assumed that biofilm bacteria behaved much like solitary, free-floating microorganisms. Now, they are discovering that while it's true that biofilm bacteria have exactly the same genetic makeup as their free-roving cousins, their biochemistry is very different because they switch to using a different set of genes.

For example, the Center for Biofilm Engineering has studied how *Pseudomonas aeruginosa* forms biofilms. The instant the bacteria dock to glass, they switch on certain genes involved in the synthesis of *alginate* (an unusually sticky form of slime), switching them off again once the bacteria are engulfed in alginate.

Researchers now estimate that as many as 30 to 40 percent of the proteins present in bacterial cell walls differ between sessile and planktonic bacteria (called 'city dwellers' and 'free-rovers' by *Coghlan 1996)*. Some of the targets for antibiotics are not there anymore, so bacteria become difficult to kill. This is primarily a problem with biofilms inside humans and animals.

Chemical Signals

Researchers are studying the chemicals (called sigma factors) which signal bacteria to change their biochemistry to life in a biofilm *(Costerton 1995)*. If they can discover a *"reverse sigma factor"* which would change biofilm bacteria into planktonic free-floaters, it might be possible to dissolve biofilms by *"sending the equivalent of an evacuation signal." (Coghlan 1996)*

Implications for sanitization

Traditional disinfectant testing has been done with single-species free-floating laboratory cultures. The *CT* constant for a disinfectant is the product of (concentration) x (time) required to kill a particular bacteria. However, CT values shouldn't be extrapolated to bacteria in biofilms.

What does this mean for automated drinking water systems? For one thing, it explains how bacteria counts can be measured even when the water contains low levels of chlorine. Typical chlorine levels in tap water are between 0.5-2.0 ppm. This amount of chlorine has been shown to kill free-floating bacteria, but may not be enough to kill biofilm bacteria. Chunks of sloughed off biofilm can contain viable bacteria which show up in plate counts. This is a particular problem with *Pseudomonas* which is a great slime producer, and so is more chlorine resistant. One animal facility determined through their own testing that they need approximately 3 ppm chlorine in RO water to achieve low *Pseudomonas* counts. •

Factors that affect biofilm attachment and growth

Surface material

The material of the surface has little or no affect on biofilm development. Stainless steel is just as susceptible as plastic pipe. According to Mayette (1992), "piping material that microorganisms cannot adhere to has yet to be discovered. Studies have shown that microbes will adhere to stainless steel, Teflon, PVC and PVDF (Kynar) with nearly equal enthusiasm."

One article talks about incorporating an antimicrobial additive into plastic to delay or reduce the attachment of biofilm (Hamilton 1988). However, the chemical they used is not safe for drinking water supplies. Some ion exchange resins have been silver-coated to prevent microbial growth.

Test Summary:
Stainless Steel vs. PVC Surfaces

Researchers in Sweden (Pedersen 1990) compared biofilm development on stainless steel and PVC surfaces exposed to flowing municipal drinking water. After 167 days, they measured the number of micro-organisms growing on the surfaces. There was no difference in the amount of cells on the hydrophilic electropolished stainless steel and the hydrophobic PVC. However, they did find that a rougher 'matt' finish stainless steel had 1.4 times more micro-organisms than the electropolished steel. The two reasons proposed for the difference in surface roughness were

1. Rough surfaces have more surface area.
2. Rough surfaces provide more shielding from shear forces (flow).

However, silver-tolerant bacterial populations can develop (Flemming & Geesey 1990, pg.67). There are no practical examples of toxic surface coatings for drinking water.

Surface area

One major factor influencing biofilm development in purified-water systems is surface area. Industrial water systems, unlike most natural environments (lakes and rivers), offer a tremendous amount of surface area for attachment. RO membranes, DI resins, storage tanks, cartridge filters, and piping systems all provide surfaces suitable for bacterial attachment and growth (Mittelman 1985).

Surface smoothness

Although smoother surfaces delay the initial buildup of attached bacteria, smoothness does not appear to significantly affect the total amount of biofilm that will attach to a surface. According to Meltzer (1993), "no surfaces have been found that are exempt from biofouling. Surface structure does appear to influence the rate of fouling, but only initially over the first few hours of exposure. In general, smooth surfaces foul at a slower initial rate than do rough ones, but biofilm formation after a period of days is inevitable." This conclusion is based on research on *Pseudomonas* attachment to stainless steel (see test summary below).

Summary: Pseudomonas Attachment and Surface Roughness

Researchers in Belgium (Vanhaecke 1990) compared the rate of attachment of *Pseudomonas aeruginosa* to 304 and 316-L stainless steel with roughness values of 120-grit, 320-grit, 400-grit, and electropolished. They found that:

1. The maximum number of bacteria adhering per square centimeter was independent of the stainless steel type or of the surface roughness.
2. Measurable adhesion, even to the electropolished surfaces, occurred within 30 seconds.
3. Strains with *hydrophobic* cell walls attach at the same rate independent of the surface roughnesses tested. *Hydrophilic* strains attached faster to the roughest 120-grit surface and had minimal adhesion to the electropolished surfaces. (Remember, in pure water bacteria will alter their cell wall so it becomes hydrophobic.)

A study of the cleanability of stainless steel used for food contact surfaces (Milledge), concluded that *"if surface finish does have an effect on cleanability, it is probably small in relation to other factors during cleaning (temperature, detergent concentration, etc.)."* So far, no research was found that compares the ability to sanitize surfaces with different finishes in water systems.

There may be some advantages to smooth surfaces.

1. Smooth pipe should support less biofilm because it has **less total surface area** than rough pipe. This was seen in the testing by Pedersen (see test summary, page 10) but wasn't seen in testing by Vanhaecke (test summary above).
2. Smooth (especially electropolished) surfaces should have less biocorrosion. One cause of biocorrosion is currents that can flow between the peaks and valleys of a rough surface.

Flow velocity

High water flow rates may alter biofilm growth but will not prevent the attachment of bacteria to pipe surfaces. This conclusion is supported by Mittelman (Part 1 1985), Patterson (1991) and Meltzer (1994). High flow will not prevent bacteria attachment or remove existing biofilm for the following reasons:

1. Low flow in the boundary layer

Regardless of the water velocity, it flows slowest in the layers adjacent to pipe surfaces. Even when water flow in the center of the pipe is turbulent, the flow velocity falls to zero at the pipe wall. The distance out from the pipe wall in which the flow rate is not turbulent is called the *boundary layer* or *laminar sublayer*. The thickness of the laminar sublayer was calculated by Pittner (1988) for various flow velocities and for 6 pipe sizes (see following table). Pittner calculated that the shear forces within the laminar sublayer are much less than that required to dislodge a bacteria cell.

2. Strong adhesion by exopolymers

In water systems with continuous high-velocity flow, the bacteria that accumulate in biofilm tend to be filamentous varieties (like *Pseudomonas*) especially suited for attachment by filaments. The bacteria anchor themselves to the surface with their 'sticky' exopolymers.

Table 1: Laminar Sublayer Thickness (microns)
(Pittner 1988)

Pipe Size	Velocity (ft/sec)					
	0.2	1.0	2.0	5.0	8.0	12.0
E.I. RDS						
0.428"ID	*	*	125	55	37	26
1/2" Sch.80	*	*	136	60	40	28
1" Sch.80	*	265	146	65	43	30
2" Sch.80	537	291	158	69	46	32
3" Sch.80	563	305	165	74	48	33
4" Sch.80	582	312	170	75	50	34

**Flow may or may not be turbulent at these conditions*
Current E.I. RDS flush velocity is approximately 3-4 ft/sec.

Although high flow velocity will not prevent the attachment of bacteria to pipe surfaces, it does have the following effects on the biofilm structure.

1. Denser biofilm

According to *Mittelman (1985), "at higher flow rates, a denser, somewhat more tenacious biofilm is formed. As a result, these surfaces often appear to be free from foulants, since they are not slimy to the touch."*

2. Limited biofilm thickness

The maximum thickness of the biofilm can be considered to be the thickness of the laminar flow layer (see Table 1). In a constant flow system, *"an equilibrium thickness is reached which is dependent on water velocity and nutrients. Growth of the biofilm beyond the laminar layer will result in the release of planktonic 'pioneer' cells that will, conditions permitting, establish the biofilm in another section of pipe." (Patterson 1991)* In systems that have fluctuating water flow, such as automated watering systems with periodic flushing, bacteria will be sloughed off during the flush. This results in random 'particle showers' of bacteria which can explain day-to-day fluctuations seen in total bacteria count results.

Limited nutrients

Like other living creatures, bacteria require certain nutrients for growth and reproduction. Limiting these nutrients will limit bacteria growth, but *"nutrient levels in high-purity systems are unequivocally sufficient to permit microbial growth and reproduction to a troublesome extent" (Husted 1994).* Table 2 lists some sources of nutrients in purified water systems.

Table 2: Nutrients for bacterial growth found in pure water systems
(Mittelman 1985)

Nutrient	Sources
Organic Carbon	Humic and fulvic acids (source water) Pipe plasticizers and solvents Fiberglass-reinforced plastics (FRPs) Pump and gage lubricants Microbial byproducts Personnel Airborne dust
Nitrogen	Humic and fulvic acids (source water) Nitrates and nitrites (source water) Microbial byproducts Airborne dust
Phosphorus	Phosphates (source water) Microbial byproducts Airborne dust
Sulfur	Sulfates (source waters) Sulfuric acid (RO pretreatment) Membrane surfactants Airborne dust
Trace metals and salts	Source waters Process piping -reinforced plastics (FRPs) Stainless steel system components RO pretreatment chemicals Personnel Airborne dust

Substrate nutrition

Can bacteria get some of their nutrients from the pipe and fittings in a water system? Yes, according to Flemming & Geesey (p. 67, 1990). Most plastics are not biodegradable, but pipe cements and plastisizers that leach from epoxy resins, PVC pipe and polyamide pipe can be organic carbon sources for bacteria. Cellulose-based RO membranes can also be a nutrient source. That is why we must chlorinate RO feedwater. Also, bacteria can obtain trace metal nutrients from stainless steel and other metal components.

Purified water

Under perfect growth conditions, a bacterial cell divides into two daughter cells once every 20 minutes (Harfst 1992). This means that a single cell and its descendants will grow exponentially to more than 2 million cells in eight hours or to 4,000,000 pounds of bacteria in 24 hours! Of course, these growth rates are never actually realized (especially in clean drinking water) because they are limited by space and available nutrients.

Can bacteria be "starved to death" or at least inhibited in their growth by depriving them of organic nutrients and oxygen? Unfortunately, even minute amounts of organic matter will support many bacteria. This was explained in the following example by Pittner (1988):

"If only one part per billion of organic matter in a 1-milliliter water sample were converted to bacterial bodies (assuming the bacteria to be 20% organic matter and the specific gravity of bacteria to be about that of water), approximately 9,500 bacteria, each 1.0 micron in diameter, would be present in a 1-milliliter sample." or 1 ppb organic matter 9,500 bacteria/ml

Currently available technology cannot reduce nutrient levels completely, so total control of bacteria is not achievable by simply controlling nutrients. Similarly, "very small quantities of oxygen will adequately support luxurious bacterial growth even if the bacteria do not revert to anaerobic respiration, which most bacteria have the ability to do. For these reasons, a thriving bacterial population can exist even in high purity water systems." (Pittner 1988).

Nutrient-limiting environments can actually promote the attachment of bacteria to surfaces because that is where the trace organics accumulate and extracellular polymers in a biofilm capture trace nutrients.

Although we can't completely starve bacteria out, nutrient-poor reverse osmosis water will support less biofilm than regular tap water supplies. •

Biofilm Proportions and Watering Systems

So far, this paper has described how a biofilm layer develops on the inside of water pipes and how this layer will reach a certain equilibrium thickness depending on flow velocity and nutrient levels. And it has discussed how surface smoothness isn't a significant factor effecting biofilm attachment. Now, you may be wondering...

1. How does the size of a bacteria cell compare with the depth of grooves and bumps in the surface of typical stainless steel pipe? Do one or a million cells fit in these grooves?
2. If biofilm thickness is limited to say 100 or 50 microns by flushing, how many bacteria cells thick is this? Is it thick enough to expect a zone of anaerobic bacteria at the pipe wall or within the grooves of the pipe surface?
3. How does the thickness of the biofilm compare to the inside diameter of 1/2" stainless steel pipe? Can it be seen?

This section will show you graphically how the size and proportion of an individual bacteria cell compares to surface roughness, biofilm thickness, and pipe diameter.

Converting Units of Measure

1 inch = 1,000,000 or 1 x 10^6 micro-inch (μ in or μ ")
1 meter=1,000,000 or 1 x 10^6 micrometers (μ m)
1 meter=39.37 inches
1 micrometer (m)=1 micron=39.37 micro-inch (μ in)

Surface finish and cell size

For many years, the finish provided on stainless steel surfaces was a dairy standard defined by Number or *grit* such as #4 or 150 grit. **Grit finish** is used with mechanical polishing and refers to the number of grit lines per inch of abrasive; the higher the number, the smoother to finish. Although the dairy and pharmaceutical industries still use grit coding for finishes, they are moving toward a system where the surface roughness can be more accurately measured.

Surface roughness can be measured by a profilometer, a stylus device used to trace across the surface profile. The results are expressed either as **RA**, which is the arithmetic average deviation from the center line of the surface, or as **RMS**, which is the root mean square of the deviations

from the center line. RA or RMS values are given in either microns (same as micrometers or μ) or micro-inches (μ "). On Edstrom Industries' drawings, surface roughness is specified as RMS in micro-inches. RMS will be approx-imately 11 percent higher than the RA number for a given surface. [RA x 1.11 = RMS]

Pharmaceutical Water Stainless Steel Piping Finish

According to Meltzer (1993), "there is no universally accepted standard for surface finishing for stainless steel. Tubing in the US is usually of an interior 150 to 180-grit finish. Of four large pharmaceutical manufacturers in the Chicago area, two rely on 150-grit finishes, one on a 180-grit finish, and one on a 180-grit finish followed by electropolishing. Water-for-Injection is usually flowed through pipe finished to a 240 to 320-grit surface."

Smoothness of Plastic Pipe

According to Gillis (1996), extruded polypropylene and PVDF pipes are as smooth as electropolished stainless steel. There are no irregularities of significant magnitudes in the size range of a bacterial cell.

Finish on Edstrom Industries Fittings and Valves

For machined fittings and drinking valves, the default finish specified in the title block of our drawings is 64 microinch RMS. When a smoother finish is needed, for o-ring sealing surfaces for example, a smoother 32 or 16 microinch finish may be specified. If machined parts are electropolished, roughness should be reduced by 30-40%.

Finish on E.I. Stainless Steel RDS Tubing

The stainless steel tubing used in Edstrom room piping and manifolds has a welded seam, but it does not have a defined interior surface smoothness. It is a rolled finish which appears smooth but could have crevices formed by flattened metal during rolling. Assume it is no smoother than 180-grit finish.

Table 3. Surface Measurement Comparisons (Meltzer 1993)

RMS (microinch)	RMS (m)	RA (microinch)	RA (m)	Grit Size
80	2.03	71	1.90	80
58	1.47	52	1.32	120
47	1.2	42	1.06	150
34	0.6	30	0.76	180
17	0.43	15	0.38	240
14		12	0.30	320

These values are the average data of many tests. Because of the many variables that create this data, deviations of ± 5% would be considered well within good measurement parameters. From Bulletin on Material Welds and Finishers *by DCI, Inc.* (Meltzer 1993)

Profile height

On most surfaces the total profile height of the surface roughness, or the peak-to-valley height will be approximately four times the RA value. Knowing the measured roughness, an approximate profile of the surface can be drawn (see Figure 7).

Typical Size of Biofilm Bacteria Cells

A very common biofilm bacteria is *Pseudomonas aeruginosa*. Cells of *Pseudomonas* are rod shaped and approximately 0.3-0.8 microns wide by 1.0-1.2 microns long. This is equivalent to 12-31 microinches wide x 40-47 micro-inches long.

Comparing Surface Profile to the Size of Bacteria Cells

The roughness profile of various stainless steel finishes used in water systems is shown schematically in Figure 7. Notice that a 34" RMS or 180-grit finish (which is considered sanitary for dairy, food, and pharmaceutical uses) has scratches large enough to harbor bacteria. A 14" RMS (320-grit) finish, which is typical of Water-for-Injection applications, has scratches only as deep as approximately one bacteria cell. A 320-grit surface followed by electropolishing has only minor surface variations relative to cell size.

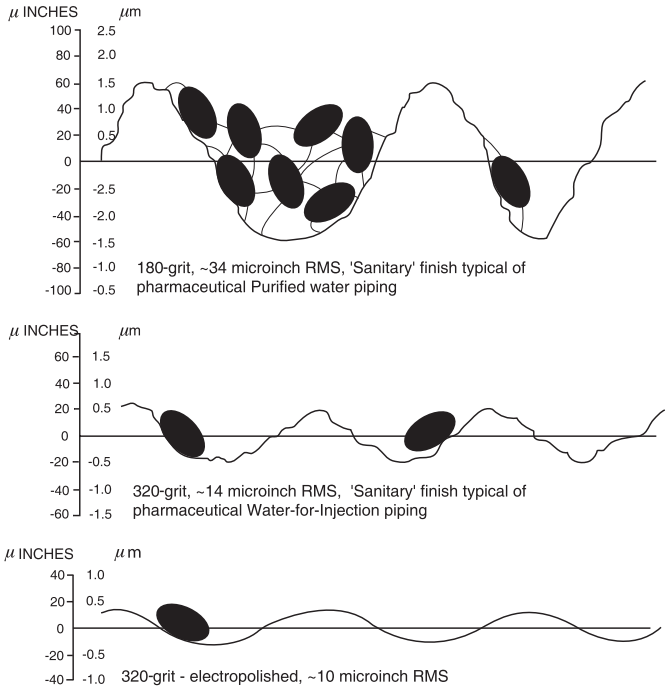


Figure 7.
Roughness profile of various stainless steel finishes

Figures 8 and 9 show actual photographs of *Pseudomonas* cells on a 180 grit stainless steel surface. The relative sizes of bacteria cells and surface scratches shown in these photos are similar to the 180-grit profile of Figure 7.

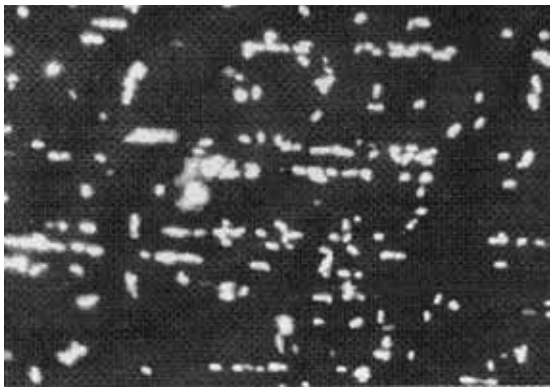


Fig. 8
Scanning electron micrograph at x 400 magnification of 180-grit mechanically polished 316L stainless steel surface after 180-minute incubation with Pseudomonas aeruginosa. *Notice that cells tended to congregate long polishing marks.* (Gillis 1996)

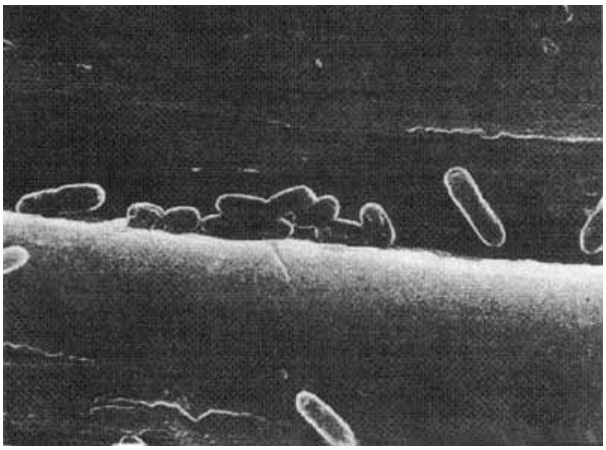


Fig. 9
Same 180-grit surface as Figure 8, but at x 5000 magnification. Notice scratches re large enough to harbor bacteria. (Gillis 1996)

Biofilm thickness and flow velocity

"Biofilms may consist of a monolayer of cells or can be as thick as 300-400 mm, as in algal mats." (Characklis 1990)
Flushing will limit biofilm thickness in automated watering systems. As discussed earlier (pages 11-13), shear forces caused by flushing will slough off biofilm which extends out into the turbulent flow in the center of a pipe. Therefore, the maximum thickness of the biofilm will be approximately the same as the laminar layer for a particular flow rate.

Table 1 (page 8) shows the approximate thickness of the laminar layer in microns for Edstrom Industries' 1/2" stainless steel RDS pipe for various flow velocities. In current automated watering systems, the RDS piping is flushed at approximately 1.5-1.75 gpm. This is an average flow velocity of 3-4 ft/sec. At 3-4 ft/sec, biofilm thickness should be limited to approximately 70-90 microns.

Figure 10 shows the maximum biofilm thickness for various flushing velocities in Edstrom Industries' stainless steel RDS piping. Remember that biofilm thickness is also limited by the available nutrients, so the equilibrium thickness may be less than if it was only limited by flushing velocity.

Compare biofilm thickness to an individual cell of *Pseudomonas* and notice that the biofilm could have many layers of bacteria. Also, notice that irregularities in surface finish are small compared to the maximum possible biofilm thickness. This explains what researchers have found (Vanhaecke 1990): the maximum number of bacteria adhering to a surface is independent of the surface roughness.

Anaerobic Surface Conditions

Aerobic bacteria near the outer surface of a biofilm consume oxygen. If the biofilm is thick enough, oxygen will be depleted at the pipe surface creating an *anaerobic* environment. Anaerobic surface conditions are undesirable because there can be more corrosion problems (see pages 13).

Could the biofilm in automated watering systems be thick enough to have anaerobic zones? One source (below) indicates that oxygen can be depleted within 30-40 microns of the water/biofilm interface. The depth of the oxygen gradient into the biofilm will vary depending on oxygen content in the bulk water, water temperature, and water flow, but this gives a rough idea of how far oxygen may diffuse.

"Aerobic P. aeruginosa biofilms grew to 30-40 μm in depth as monocultures, but increased in depth to 130 μm when the culture was amended with anaerobic bacteria. This indirect evidence suggests that depletion of oxygen - not of nutrients - limited the vertical development of the P. aeruginosa biofilm." (Costerton 1995)

If biofilm thickness in an automated watering system is only limited by flushing, it could be 50-125 microns thick and have some anaerobic zones. Of course, crevices such as o-ring pipe joints and threaded fittings can have much deeper biofilms and are most likely to have anaerobic zones.

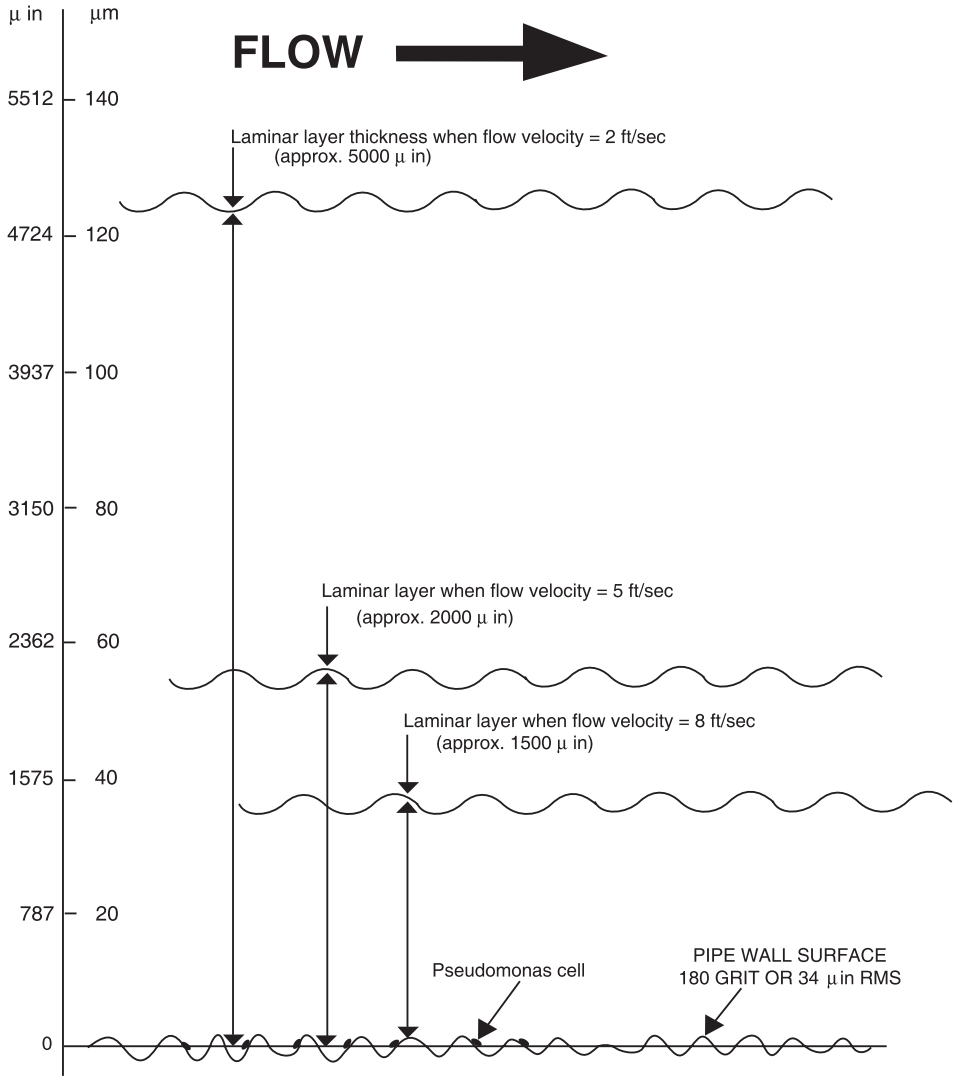
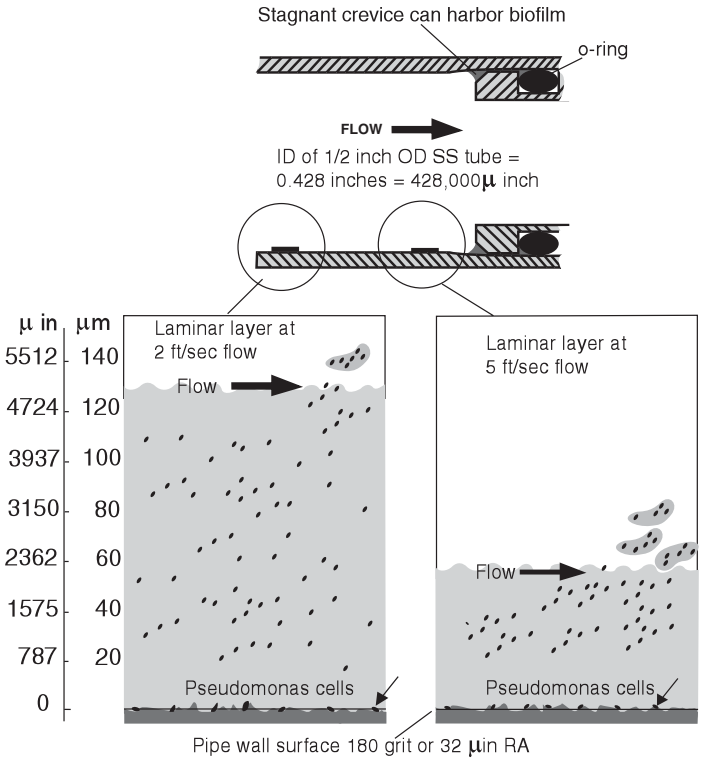


Fig. 10



Biofilm Thickness Compared to Pipe Diameter

The last proportional comparison is to look at the maximum biofilm thickness at the wall of RDS piping compared to the overall inside pipe diameter. This comparison is shown in Figure 11 which shows the inside diameter of Edstrom Industries' 1/2" OD stainless steel tubing (inside diameter = 0.428") and the cross section of an o-ring joint. The biofilm thickness is small compared to the overall pipe diameter and compared to the depth of biofilm which could develop in an o-ring joint crevice. •

Microbiologically Influenced Corrosion

The physical presence of microbial cells on a metal surface, as well as their metabolic activities, can cause *Microbiologically Influenced Corrosion* (MIC) or *biocorrosion*. The forms of corrosion caused by bacteria are not unique. Biocorrosion results in pitting, crevice corrosion, selective dealloying, stress corrosion cracking, and under-deposit corrosion. The following mechanisms are some of the causes of biocorrosion.

Oxygen depletion or differential aeration cells

Nonuniform (patchy) colonization by bacteria results in differential aeration cells. This schematic shows pit initiation due to oxygen depletion under a biofilm. (Borenstein 1994)

Stainless steels' protective film

Oxygen depletion at the surface of stainless steel can destroy the protective passive film. Remember that stainless steels rely on a stable oxide film to provide corrosion resistance. Corrosion occurs when the oxide film is damaged or oxygen is kept from the metal surface by microorganisms in a biofilm.

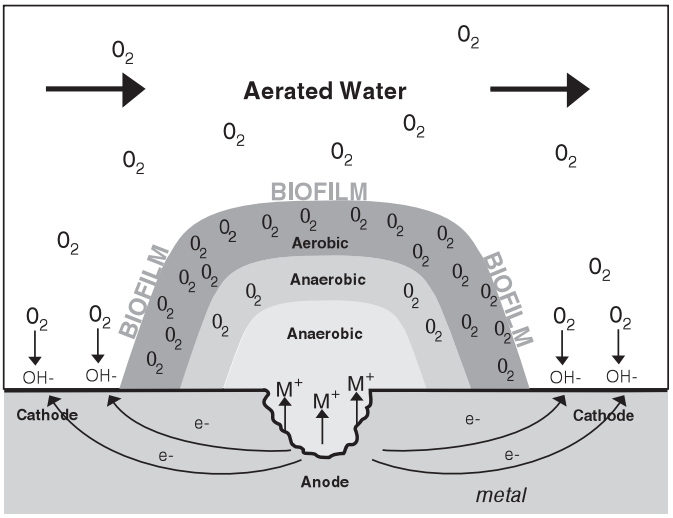
Sulfate-reducing bacteria

Oxygen depletion at the surface also provides a condition for anaerobic organisms like sulfate-reducing bacteria (SRB) to grow. This group of bacteria are one of the most frequent causes for biocorrosion. They reduce sulfate to hydrogen sulfide which reacts with metals to produce metal sulfides as corrosion products. Aerobic bacteria near the outer surface of the biofilm consume oxygen and create a suitable habitat for the sulfate reducing bacteria at the metal surface. SRBs can grow in water trapped in stagnant areas, such as dead legs of piping. Symptoms of SRB-influenced corrosion are hydrogen sulfide (rotten egg) odor, blackening of waters, and black deposits. The black deposit is primarily iron sulfide. (Borenstein 1994 and Geesey 1994)

"One way to limit SRB activity is to reduce the concentration of their essential nutrients: phosphorus, nitrogen, and sulfate. Thus, purified (RO or DI) waters would have less problem with SRBs. Also, any practices which minimize biofilm thickness (flushing, sanitizing, eliminating dead-end crevices) will minimize the anaerobic areas in biofilm which SRBs need" (Geesey 1994).

Byproducts of bacterial metabolism

Another corrosion mechanism is based on the by-products of bacterial metabolism.



Nonuniform (patchy) colonies of biofilm result in the formation of differential aeration cells where areas under respiring colonies are depleted of oxygen relative to surrounding non colonized areas. Having different oxygen concentrations at two locations on a metal causes a difference in electrical potential and consequently corrosion currents. Under aerobic conditions, the areas under the respiring colonies become anodic and the surrounding areas become cathodic.

Acid-producing bacteria

Bacteria can produce aggressive metabolites, such as organic or inorganic acids. For example, *Thiobacillus thiooxidans* produces sulfuric acid and *Clostridium aceticum* produces acetic acid. Acids produced by bacteria accelerate corrosion by dissolving oxides (the passive film) from the metal surface and accelerating the cathodic reaction rate (Borenstein 1994).

Hydrogen-producing bacteria

Many microorganisms produce hydrogen gas as a product of carbohydrate fermentation. Hydrogen gas can diffuse into metals and cause hydrogen embrittlement.

Iron bacteria

Iron-oxidizing bacteria, such as *Gallionella*, *Sphaerotilus*, *Leptothrix*, and *Crenothrix*, are aerobic and filamentous bacteria which oxidize iron from a soluble ferrous (Fe2+) form to an insoluble ferric (Fe3+) form. The dissolved ferrous iron could be from either the incoming water supply or the metal surface. The ferric iron these bacteria produce can attract chloride ions and produce ferric chloride deposits which can attack austenitic stainless steel. For iron bacteria on austenitic stainless steel, the deposits are typically brown or red-brown mounds.

Sanitization Methods for Biofilms

Biofilm can be removed and/or destroyed by chemical and physical treatments. Chemical biocides can be divided into two major groups: oxidizing and nonoxidizing. Physical treatments include mechanical scrubbing and hot water. An article by Mittelman (1986) has the most comprehensive information on treatment of biofouling in purified water systems.

Oxidizing biocides

Mittelman says the effectiveness of the oxidizing biocides in purified-water systems on an equal milligram-per-liter-dosage basis decreases in the following order: ozone > chlorine dioxide > chlorine > iodine > hydrogen peroxide

Chlorine

According to Mittelman (1986), "Chlorine is probably the most effective and least expensive of all oxidizing and nonoxidizing biocides." The activity of chlorine against attached biofilms is particularly high; not only are planktonic and biofilm bacteria killed, but chlorine also reacts with and destroys the polysaccharide web and its attachments to the surface. By destroying the extracellular polymers, chlorine breaks up the physical integrity of the biofilm.

Characklis (1990) recommends improving a chlorine treatment program by taking the following measures:

1. Increase the Chlorine Concentration at the Water-Biofilm Interface

As chlorine diffuses into a biofilm, it is used up in reactions with bacteria cells and extracellular materials. At low chlorine levels, biofilm bacteria can produce extracellular material faster than chlorine can diffuse through it so they are shielded in slime. By increasing the concentration, chlorine will diffuse farther into the biofilm. When it comes to disinfection of biofilms, high chlorine concentration for short durations is more effective than low concentration for long durations.

2. Increase the Fluid Shear Stress at the Water-Biofilm Interface

Simultaneous chlorine sanitization and flushing results in a higher uptake of chlorine by the biofilm and in greater biofilm detachment due to:

- Increased mass transfer of chlorine from the bulk water to the biofilm.
- Disruption of the biofilm during chlorination exposes new biofilm surfaces for chlorine attack.
- Decreased thickness of viscous or laminar sublayer.

3. Use pH Control

High pH favors hypochlorite-ion-promoted detachment of mature biofilms, and low pH enhances hypochlorous acid disinfection of thin films. Characklis proposed an interesting procedure would be to alternate between continuous chlorination at pH 6.5 and shock chlorination at pH 8. He doesn't imply that this has been tested.

Chlorine dioxide

Chlorine dioxide has biocidal activities similar to those of chlorine. Because it is unstable, it must be mixed and prepared on-site. Like chlorine, chlorine dioxide is corrosive to metals and must be handled with care.

Ozone

As an oxidizer, ozone is approximately twice as powerful as chlorine at the same concentrations. Like chlorine dioxide, ozone must be generated on-site because of its high reactivity and relative instability. Systems must be designed with appropriate ozone resistant materials.

"Ozone is usually dosed on a continuous basis at 1-2 mg/l. Success in employing higher dosages on a noncontinuous basis has been limited, possibly because of the limited solubility of ozone in purified water; it is difficult to produce high concentrations of ozone in solution" (Mittelman 1986). Although chlorine isn't as powerful as ozone when you compare 1-2 mg/l of each, chlorine can be used in higher sanitizing concentrations with equal disinfecting strength.

Hydrogen peroxide

"Hydrogen peroxide is frequently used as a biocide in microelectronic-grade purified-water systems because it produces no by-products; it rapidly degrades to water and oxygen. A 10% by volume solution in purified water appears effective in killing planktonic bacteria, but more studies are needed on the effectiveness against attached biofilm" (Mittelman 1986).

Non-oxidizing biocides

Quaternary Ammonium Compounds

In addition to their biocidal activity, quats are effective surfactants/detergents, which may be an important factor in their use for biofilm inactivation and removal from surfaces. Rinseability can be a problem as removal from a purified-water system often requires exhaustive rinsing.

Formaldehyde

Formaldehyde has been applied to pharmaceutical-grade systems. It is relatively noncorrosive to stainless steel. Its effectiveness against biofilm is questionable and it is a toxic carcinogen.

Anionic and Nonionic Surface-Active Agents

These surfactant or detergent compounds have limited biocidal activity against the bacteria in purified water systems. Applications may be found for these detergents in conjunction with other biocides to improve biofilm and other particulate removal.

Physical Treatments

Heat

Pharmaceutical Water-for-Injection systems use recirculating hot water loops (greater than 80°C) to kill bacteria. According to Mittelman (1986), when these systems are used on a continuous basis, planktonic bacteria are killed and biofilm development is *reduced*. Biofilms are even found in hot water (80°C). *Periodic* hot water sanitization can also be used to destroy bacteria in biofilm, but according to Collentro (1995) this requires a temperature of 95°C for a period in excess of 100 minutes. This would not be practical in an animal drinking water system!

Mechanical removal

From Mittelman: "Heavy biofilms cannot be removed from storage tank walls by the use of chemicals alone; mechanical scrubbing or scraping, high-pressure spraying, or a combination is also required. Mechanical removal of biofilm from distribution systems is impractical." For RO system maintenance, we don't routinely scrub storage tanks, but there is usually a continuous low chlorine level in the stored water, so heavy biofilms aren't allowed to develop.

Biocide resistance

Unlike antibiotics used to fight bacteria associated with human, animal, and plant diseases, bacteria do not develop the same type of resistance to industrial biocides. The difference between antibiotics and industrial biocides is that while an antibiotic may have a small number of target sites on or in the bacterial cell, all oxidizing biocides have a multitude of potential target sites. Chlorine, for example, is thought to have more than a hundred potential target sites on or in microorganisms. It is virtually impossible for microorganisms to develop a general resistance to such compounds (Mittelman 1986). However, bacteria *in a biofilm* can resist biocides because they are shielded in slime.

Biofilm recovery (Regrowth)

Bacteria associated with biofilms are much more difficult to kill and remove from surfaces than planktonic organisms. According to Characklis (1990), numerous investigators and plant operators have observed "a rapid resumption of biofouling immediately following chlorine treatment." Incomplete removal of the biofilm will allow it to quickly return to its equilibrium state, causing a rebound in total plate counts following sanitization.

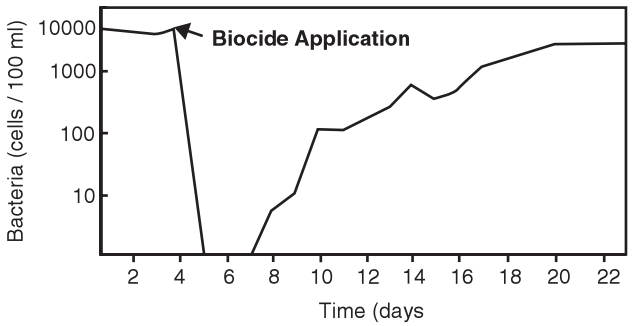


Figure 13 (Mittelman 1986), shows typical regrowth following sanitization. Initially, the bulk water bacteria count dropped to zero after sanitization, but this was followed by a gradual increase in numbers to levels at or below the pretreatment levels. In this example, regrowth started after 2 days and was back up to equilibrium levels after 20 days. This is similar to results seen in in-house sanitization testing at Edstrom Industries.

According to Characklis (1990), biofilm recovery may be due to one or all of the following.

1. The remaining biofilm contains enough viable organisms that there is no lag phase in regrowth. Thus, biofilm recovery after shock chlorination is faster than initial accumulation on a clean pipe.
2. The residual biofilm on the surface makes it rougher than clean pipe. The roughness of the deposit may provide a stickier surface which adsorbs more microbial cells and other compounds from the water.
3. The chlorine preferentially removes extracellular polymers and not biofilm cells, thus leaving biofilm cells more exposed to the nutrients when chlorination ceases.
4. Surviving organisms rapidly create more slime (extracellular polymers) as a protective response to irritation by chlorine.
5. There is selection for organisms less susceptible to the sanitizing chemical. This is usually the organisms that produce excessive amounts of slime like *Pseudomonas*.

Case Study: Sanitization Selecting for *Pseudomonas*

When several mice on long term studies died in 1996, this laboratory animal facility suspected that the cause was *Pseudomonas aeruginosa* which was found in rack manifold piping. They had never identified *Pseudomonas* in the water until after they chlorine sanitized their recirculating automated watering system (AWS) for the first time since the system was installed in 1982.

System Description: RO with no chlorine pretreatment followed by a carbon filter and deionization tanks before filling 3 storage tanks. Carbon and DI filters most likely add a lot of bacteria to the RO water. No chlorine is

allowed in the animal drinking water due to the nutritional studies being conducted. One tank is non-recirculating and holds more than 2 weeks water supply for non-chlorinated rack manifold flushing. The other 2 tanks are on recirculating loops supplying the AWS. There is no RDS flushing and low turn-over of tank water.

Bacteria Testing: Facility does regular testing of drinking water for bacteria. They regularly got total counts of 10,000-50,000 cfu/ml from the RO storage tanks and never had any animal health problems. When counts exceeded 100,000 cfu/ml, they decided to do the first system sanitization.

Sanitization: 20 ppm chlorine for 4 hour soak with all animal racks removed. The bacteria counts were very low for a couple weeks afterwards, but then increased (typical of regrowth following sanitization).

Speculation: Chlorine sanitization selectively promoted more chlorine resistant organisms like *Pseudomonas*. It was probably present all along, embedded in the mature biofilm attached to pipe and tank walls. A one-time sanitization with a low concentration like 20 ppm is not going to kill 100% of *Pseudomonas*. •

Detection and Enumeration of Bacteria

Routine monitoring of bacterial levels is an essential part of monitoring the quality of laboratory animal drinking water. The classic way to enumerate bacteria in water is to do a *plate count* which is to spread a known volume of sample on the surface of a laboratory medium and count the number of visible colonies that develop after a period of time. However, it should be recognized that plate counts may underestimate the total number of bacteria present in a watering system.

Most bacteria are in biofilms

Water samples only collect planktonic or free-floating bacteria. Free-floating bacteria in animal drinking water are either sloughed off of the biofilm or pass through from the incoming water supply. If a plate count test is low, one shouldn't assume that bacteria are not present in the watering system. More than 99% of the bacteria in water systems are in biofilms attached to pipe surfaces. If the integrity of a mature biofilm hasn't been disrupted by recent flushing or sanitization, it may not slough off many cells into the drinking water, but it is still there. As Smith (1987) puts it,

"When you take a water sample you are sampling only the "strays" and not the main "herd" of bacteria in the system."

Plate counts don't detect *all* viable bacteria

Plate counts are based on the ability of bacteria in a sample to grow on a defined nutrient medium. When bacteria grow on a nutrient, they form distinct colonies. Theoretically, a colony is derived from a single bacteria cell. Some underestimation of bacteria is caused by clumps of bacteria that form only one colony.

Another reason viable counts can be too low in nutrient-poor purified water is that the bacteria are in a starved state and cannot grow on rich nutrient media. Rich laboratory media are toxic to bacteria adapted to living in high-purity water systems. To get higher bacterial recoveries from purified waters, special media (R2A agar), decreased incubation temperatures, and increased incubation times are sometimes used.

Understanding particle showers

Sometimes the results of bacterial plate count testing seem very erratic. Samples taken from one point in the system may vary from less than 10 cfu/ml to TNTC (too-numerous-to-count). Or maybe the counts are usually low, but occasionally a high count appears. Some of this variability can be explained by understanding that biofilms periodically "shed", causing bacterial counts to skyrocket. According to Patterson (1991), "Sudden failure of the integrity of the biofilm at specific locations will result in bacteria and particle showers that occur randomly in time." •

Conclusions

"The situation, then, is that neither smoothness of surface, materials of construction, nor flow velocities long delay the advent of biofilm formation." (Meltzer 1994)

Bacteria constitute a very successful life form. In their evolution, they have developed successful strategies for survival which include attachment to surfaces and development of protective biofilms where they behave very differently than free-floating bacteria. Their successful strategies make it difficult for us to control biofilm growth in automated watering systems.

How biofilm bacteria evade our attempts to eliminate them:

- ✓ We **purify** water to remove nutrients and ask "How could anything live in it?
- ✓ They use their polymer web to concentrate nutrients. They live on nutrient levels we can't even measure.
- ✓ We **flush** water lines trying to scour them off the pipes.
- ✓ They cement themselves to surfaces with their sticky polymers under the laminar layer where shear forces are too weak to remove them.
- ✓ We **smooth** the inside surfaces of fittings so they can't take shelter in crevices and crannies.
- ✓ It doesn't matter. They will attach themselves speedily and inevitably anyway.
- ✓ We **sanitize** piping with chlorine.

They shield themselves in slime.

So what is the solution?

Purify anyway!
It will limit nutrients somewhat, especially nutrients for microbes like sulfate-reducing bacteria which cause corrosion problems. Nutrient-poor RO water will support less bacteria than tap water. This means a thinner biofilm. Besides, the animals will be getting better quality drinking water.

Flush anyway!
Periodic flushing will minimize the thickness of the biofilm. Thinner biofilm have less anaerobic zones and sanitizing chemicals will have a shorter distance to diffuse through to reach the pipe surface.

Minimize crevices anyway!

Maybe surface finish doesn't matter much as far as total biofilm accumulation, but eliminating large crevices (like o-ring joints) will eliminate deep pockets of biofilm which are harder to sanitize and are more corrosive. Also, electropolishing will aid in resisting corrosion.

Sanitize anyway!

If biofilm recovers in say 3 days after sanitization, knock it back down by sanitizing every 1-2 days. This could be done by automating chlorine or ozone sanitization.

There is no one easy answer. Unless a continuous chlorine level is allowed in a water supply, it will take a combination strategy. But, if we use every weapon we've got, it should result in a bacterial water quality which will satisfy the needs of our customers and the research animals. ●

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