

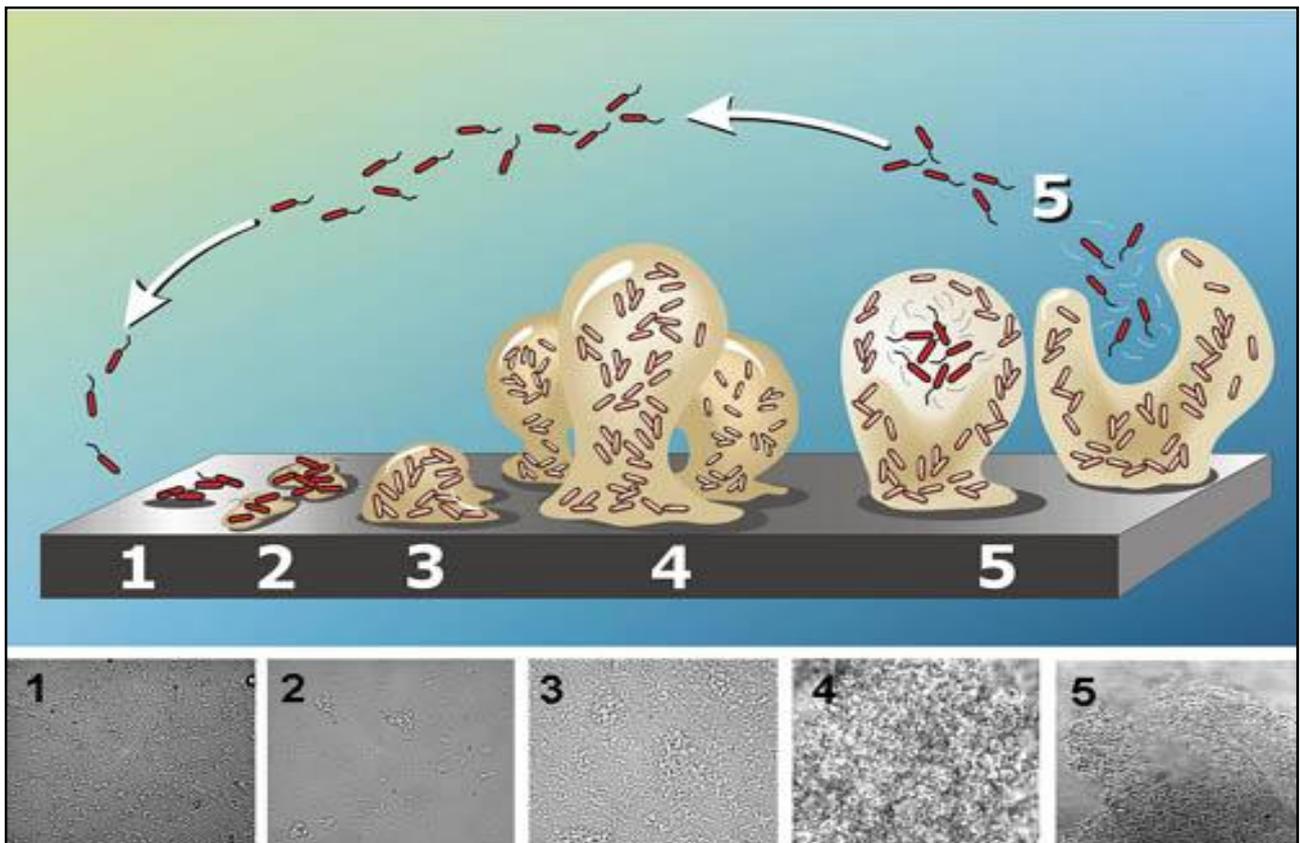
## Scotmas Healthcare – Factsheet Water Systems Biofilms

Almost immediately after a clean tank or pipe surface comes into contact with water, an organic layer deposits on the water/solid interface. These organics form a *conditioning layer* that neutralises excessive surface charge and surface free energy that may prevent a bacterial cell from approaching near enough to initiate attachment - the adsorbed organic molecules also serve as a potential nutrient source to bacteria. The development of a mature biofilm may take several hours or several weeks, depending on the water delivery system. *Pseudomonas aeruginosa* is a common pioneer bacterium and is used in a lot of biofilm research. In one experiment, researchers found that *Pseudomonas* cells adhere to stainless steel, even to electro-polished surfaces, within 30 seconds of exposure.



Biofilm build-up on a water pipe taken from a system previously treated with chlorine. Within the biofilm, are potentially disease-causing bacteria

### Biofilm Formation

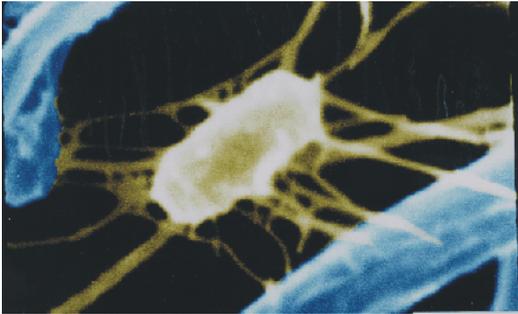


Each number corresponds with a stage in biofilm development. The photomicrographs are of an actual *Pseudomonas aeruginosa* biofilm.

### **1 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 detach. This initial attachment is based on electrostatic attraction and physical forces, not chemical attachments. Some of the reversibly adsorbed cells begin to make preparations for a lengthy stay by forming structures that may permanently adhere the cell to the surface; these cells become irreversibly adsorbed.

### **2 Glycocalyx Formation**



Biofilm bacteria excrete extra-cellular 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 most biocides. These extra-cellular polymers 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 polysaccharide 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.

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; soon a thriving colony of bacteria is established. A mature biofilm has more volume occupied by the loosely organised glycocalyx matrix (75-95%), than by bacterial cells (5-25%). Because the glycocalyx matrix holds a lot of water, a biofilm-covered surface is gelatinous and slippery.

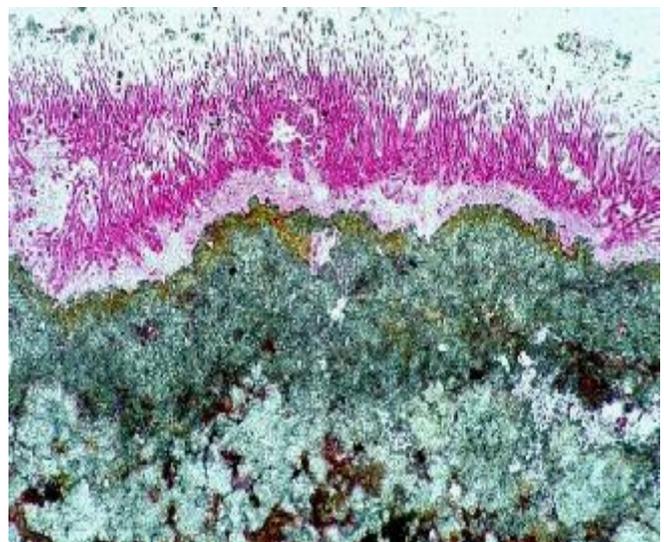
### **3 Secondary colonisers**

As well as trapping nutrient molecules, the glycocalyx net also snares other types of microbial cells through physical restraint and electrostatic interaction. These secondary colonisers metabolise wastes from the primary colonisers as well as produce their own waste, which other cells then use in turn. These other bacteria and fungi become associated with the surface following colonisation by the pioneering species over a matter of days.

### **4 A fully functioning biofilm**

The mature, fully functioning biofilm is like a living tissue on the pipe surface. It is a complex, metabolically co-operative community made up from different species each living in a customised micro-niche; biofilms are even considered to have primitive circulatory systems. The following paragraph imaginatively describes mature biofilms, extracted from an article called Slime City (Coghlan 1996):

*“Different species live cheek-by-jowl in slime cities, helping each other to exploit food supplies and to resist antibiotics through neighbourly interactions - toxic waste, produced by one species, may be hungrily devoured by its neighbour. 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 micro-zones provide the power to shunt the substances around the biofilm.”*

### **5 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 (commonly *Pseudomonas aeruginosa*) to colonise downstream sections of pipework. 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. 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 an initial organic coating for uncolonised pipe sections down stream, or as nutrient substances for other cell types.

Bacteria and other micro-organisms develop co-operative colonies or “consortia” within the biofilm. An anaerobic biofilm may develop underneath the aerobic layer. The biofilm thickness will reach equilibrium as flowing water detaches cells extending out into turbulent flow.

### **Why is Biofilm a Problem?**

The main problem with biofilms is that they provide a safe environment for a number of pathogenic micro-organisms to grow and multiply. Not only does the biofilm provide a source of nutrients for these micro-organisms, it also acts to protect them from some of the biocides, especially chlorine, which are commonly used to treat water.

The build up of general bacteria and biofilms in water systems is also a major source of taste and odour problems. Water stored for even a short period can develop a musty odour and taste. Biofilms can also cause turbidity and discoloration of the water. Where biofilms are present on heat exchange surfaces, efficiency of the heating system is greatly reduced, leading to an increase in energy costs.

#### **Pathogens found in Biofilm**

*E coli*  
*Legionella pneumophila*  
*Pseudomonas*  
*Arthrobacter*  
*Acinetobacter*  
*Sarcina*  
*Micrococcus*  
*Proteus*  
*Klebsiella*  
*Enterobacter*

***Fortunately, there is an answer to the problems created by biofilm. That answer is chlorine dioxide, which will penetrate and remove the biofilm and destroy the bacteria on contact.***