

# Biofilm

By Alistair Cameron, Microbiologist & Peter Ford, Scotmas Limited

THE INSTANT A CLEAN PIPE IS FILLED WITH WATER, A BIOFILM BEGINS TO FORM...

## 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 (Mittleman 1985).

These organics are said to form a *conditioning layer* which neutralises excessive surface charge and surface free energy which may prevent a bacterium cell from approaching near enough to initiate attachment. In addition, the adsorbed organic molecules often serve as a nutrient source to bacteria.

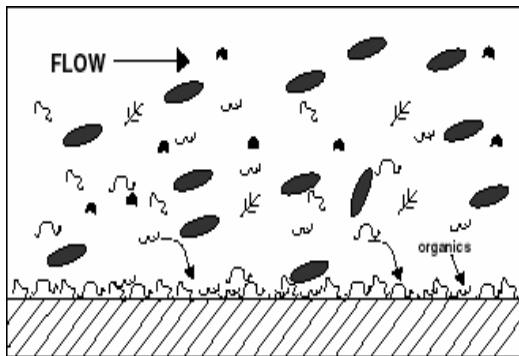


Figure 1. (left) Adsorption of organic molecules on the clean surface forms a conditioning film (Characklis 1990).

## ***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 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.

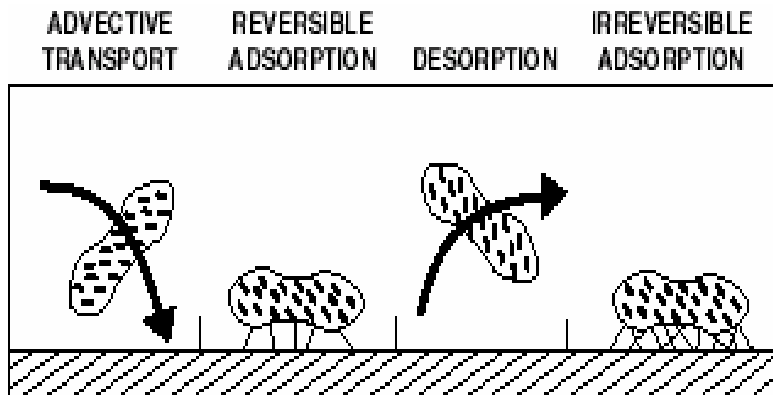


Figure 2. (left) Transport of bacteria cells to the conditioned surface, adsorption, desorption, irreversible adsorption (Characklis 1990).

## ***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 most biocides.

According to Mittleman (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 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.”

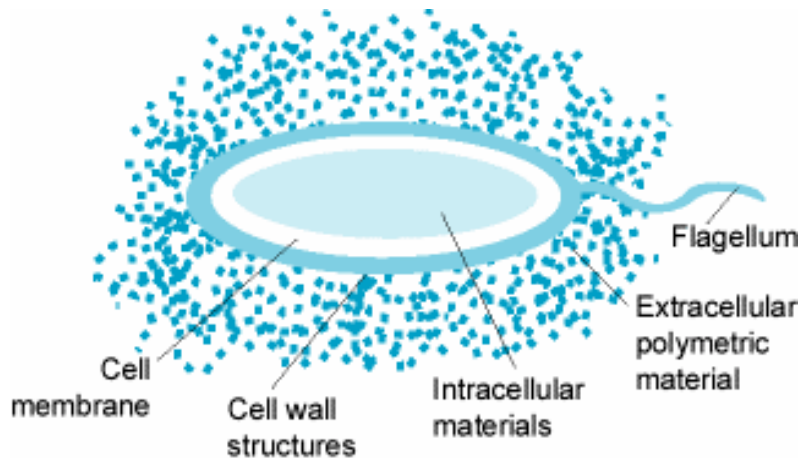
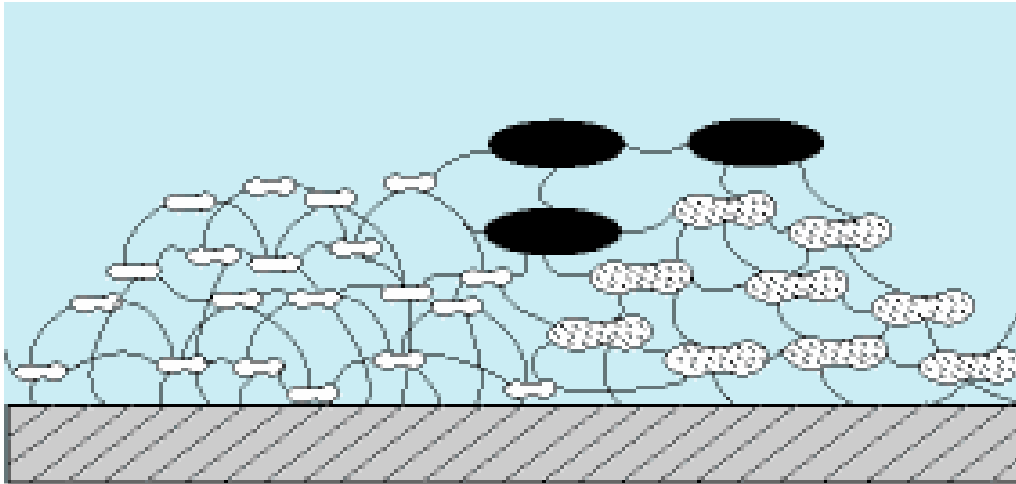


Figure 3. (left) Wild bacteria are “hairy” cells with extracellular polymers which stick to surfaces (Mittleman 1985).

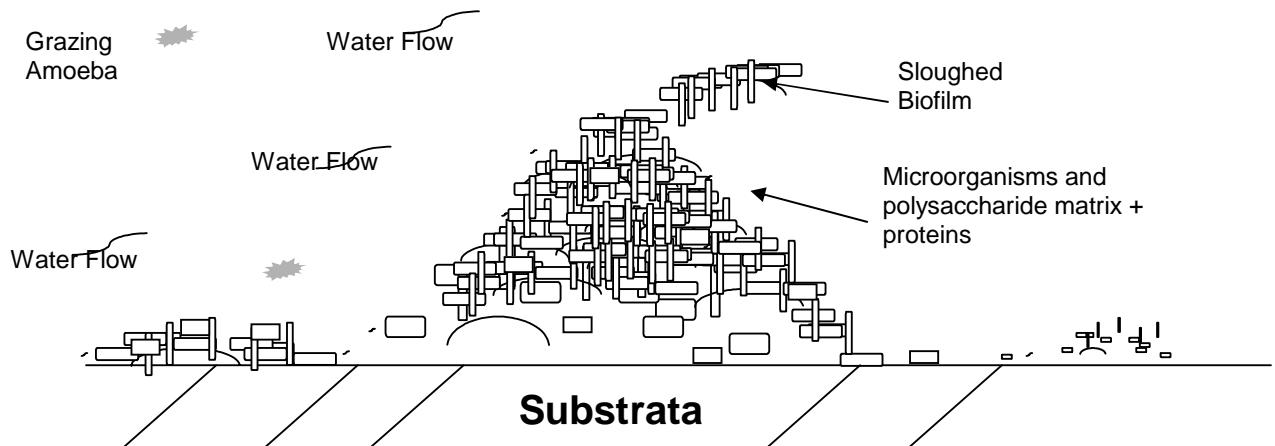
As nutrients accumulate, the pioneer cells proceed to reproduce. The daughter cells then produce their own glycocalyx, greatly increasing the volume of ion exchange surface. Pretty soon a thriving colony of bacteria is established. (Mayette 1992). In a mature biofilm, more of the volume is occupied by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

**Figure 4. (below) Biofilm is made up of microbes strung together in a “spider’s web” of extracellular polymers (Mittleman 1985).**



## ***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. According to Borenstein (1994), these “other bacteria and fungi become associated with the surface following colonisation by the pioneering species over a matter of days.”



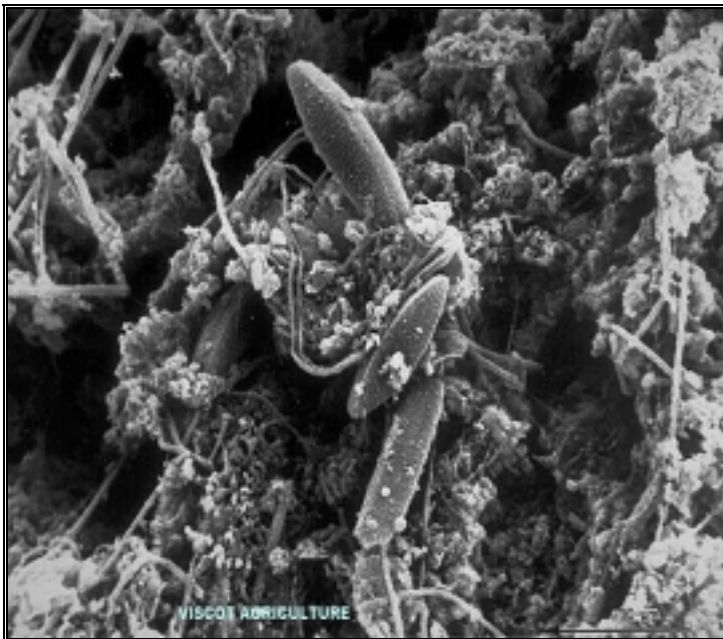
## FULLY FUNCTIONING BIOFILM

- ***A co-operative “consortia” of species***

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. Mature biofilms are imaginatively described as follows in 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 might 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.”*



The photograph to the left is the result of electron-microscopic magnification and shows up graphically the complex nature of a well established, mature biofilm.

## ***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. 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 an initial organic coating for uncolonised pipe sections down stream or as nutrient substances for other cell types.”

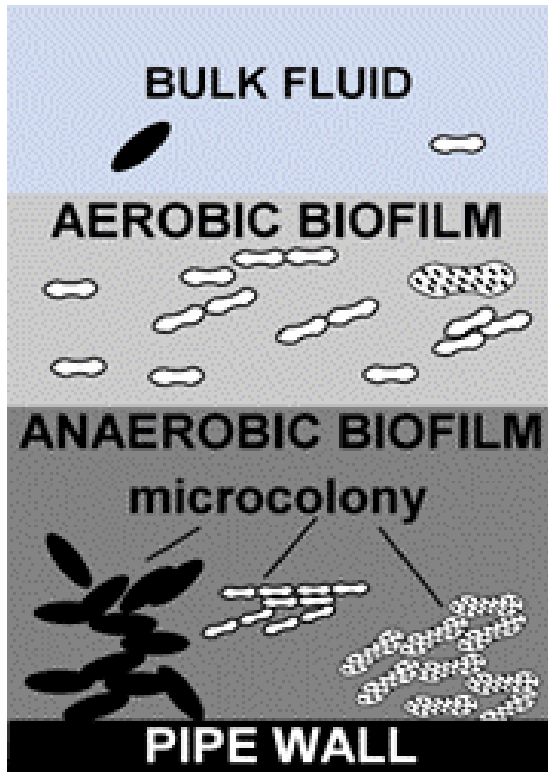


Figure 5. (left) Bacteria and other micro-organisms develop co-operative colonies or “consortia” within the biofilm. An anaerobic biofilm may develop underneath the aerobic layer. The biofilm thickness will reach an equilibrium as flowing water detaches cells extending out into turbulent flow. (Borenstein 1994).

## ***How fast does biofilm develop?***

According to Mittleman (1985), 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 (Vanhaecke 1990) researchers found that *Pseudomonas* cells adhere to stainless steel, even to electropolished surfaces, within **30 seconds of exposure**.