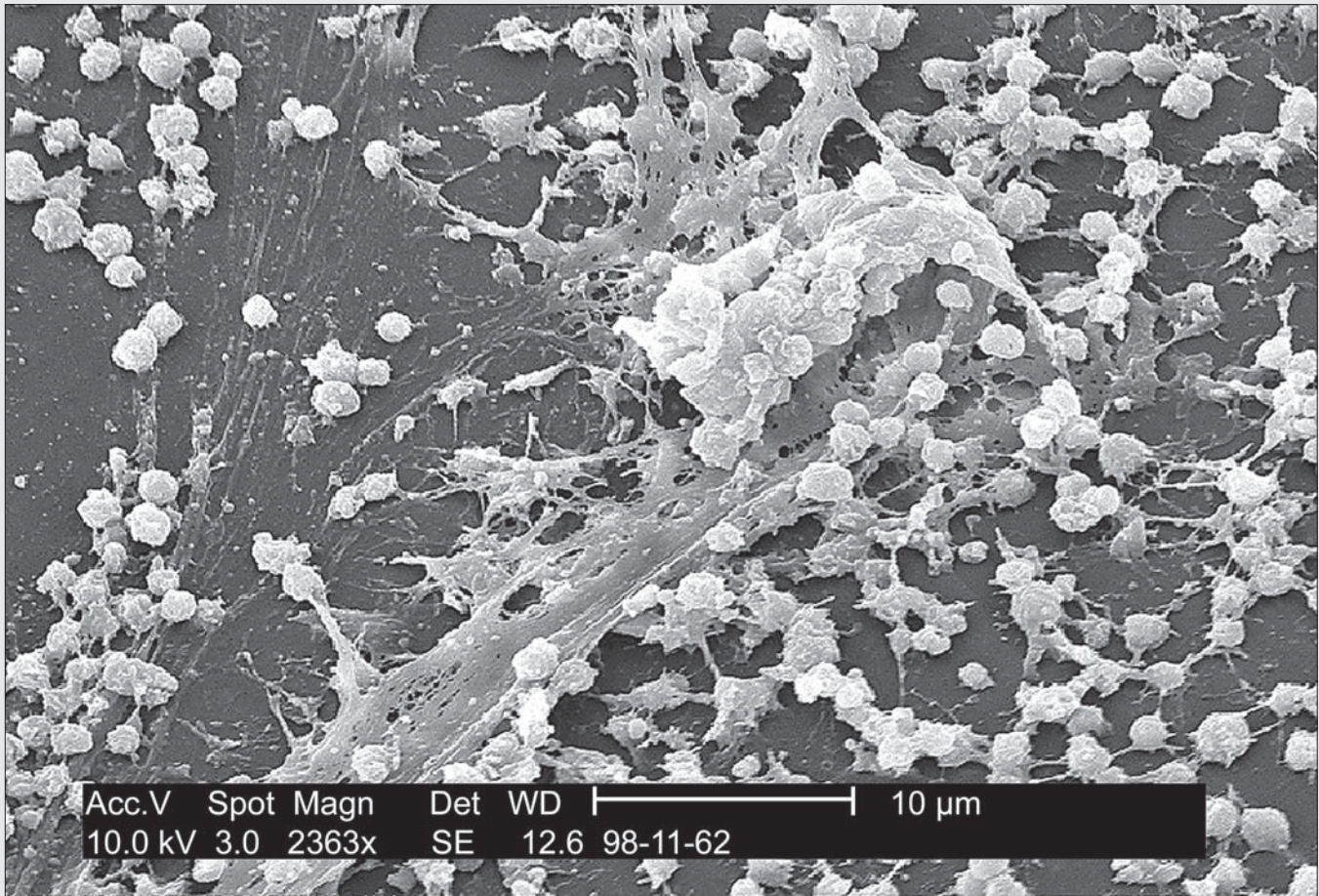


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The above photo depicts an E.coli (ATCC 11775) biofilm grown on PC (polycarbonate) coupons using a CDC biofilm reactor. Microorganisms often colonize, and adhere strongly to living and non-living surfaces forming biofilms, and at times, demonstrate an increased resistance to antimicrobials. Biofilms on indwelling medical devices pose a serious threat to public health.

BIOFILMS: Friend or Foe?

By NICOLE KENNY, B.Sc, Assoc.Chem., Director of Professional & Technical Services, Virox Technologies Inc



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Scanning electron micrograph of a *Staphylococcus* biofilm on the inner surface of a needleless connector. A distinguishing characteristic of biofilms is the presence of extracellular polymeric substances, primarily polysaccharides, surrounding and encasing the cells. Here, there polysaccharides have been visualized by scanning electron microscopy.

Picture yourself a contestant on Jeopardy. Alex Tricab has just asked you to choose the category. You're lagging behind the leader by \$400. All but one of the \$500 questions have been taken, and the last category has had extremely difficult and often perplexing questions. With as much confidence as you can muster you blurt out, "Alex, I'll take *Natural Phenomena* for \$500."

You take a deep breath as Alex reads the question: "What does the plaque on your teeth, the slippery slime on river stones, the gel-like film on the inside of a flower vase, the unsightly stains in toilet bowls, the gunk that clogs your drains, otitis media (ear infections) and bacterial endocarditis (infection of the inner surface of the heart) have in common?"

Your brain goes blank, your heart stops, your mouth goes dry and you think,

"Why didn't I listen to my mother and take more science courses?" But in that split second you also remember a documentary you watched on CNN about whirlpool tubs and you know the answer.

"Alex, what are BIOFILMS?"

THE ISSUE

Biofilms are nothing new to our world. As described in the Jeopardy question, they can be evident in any environment that has a flow of water and a surface to which to stick. In fact, since 1684 scientists have been striving to determine how to manage biofilms. More than three centuries ago, Antonie van Leeuwenhoek studied dental plaque (which he referred to as scurf), and made the following conclusions: "From whence I conclude, that the vinegar with which I washed my teeth, killed only those animals which were on the outside of the scurf, but did not pass thro the whole substance of it."¹

Biofilms can be dangerous or beneficial depending on where they are found and of which organisms they are comprised. In industry, biofilms are responsible for billions of dollars in lost productivity due to equipment damage, notoriously famous for causing pipes to plug or corrode. However, in biotechnology, biofilms are used for treating environmental wastes such as sewage, contaminated ground water or soil. They are also used to produce a variety of biochemicals that can be used for manufacturing medicines or food additives. Even Mother Nature makes use of biofilms. Some biofilms attach to the plant roots of crops, and help cycle nutrients to and from the plant, which results in improved agricultural productivity. As a society, however, we most commonly associate biofilms with their related infections. Examples of these are otitis media and bacterial endocarditis,

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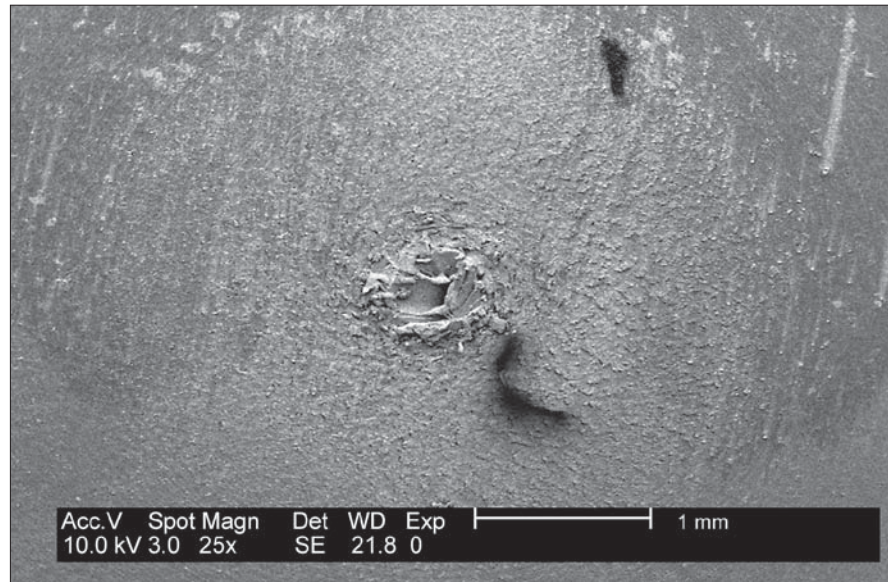
TO PLACE

which are caused by bacteria entering a fluid-filled part of the body. Most notable, however, are the healthcare related infections where biofilms can develop on medical device surfaces such as catheters, medical implants or wound dressings.²

By the way, biofilms happily colonize on many household surfaces such as toilets, sinks, countertops, cutting boards and coffee pots. Poor disinfection practices and ineffective cleaning products may increase the incidence of illness associated with the pathogenic organisms commonly found around the home.

THE COMPOSITION

The literal meaning of Biofilm is “life-slime.” The scientific definition of Biofilm is “the film or thin layer composed of cells of microorganisms such as bacteria, fungi, yeasts, protozoa and other microorganisms that are attached to a surface.”³ When the bacteria or fungi adhere to surfaces, they begin to excrete a slimy, glue-like substance



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The above low-mag SEM shows surface irregularities in biofilm culture material (polycarbonate) growing P. mirabilis bacteria. Biofilms, or the colonization of bacteria on living and non-living surfaces, are cultured in the laboratory to investigate the link between biofilm formation, infection and the development of antimicrobial resistance.

(technically called extracellular polysaccharide) that helps them stick to all kinds of surfaces such as metals, plastics,

rocks, implanted medical devices and even tissue. This slime layer also provides a

Continued On Page 43

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protective environment in which to live. In fact the general structure of a biofilm consists of 85 per cent polysaccharide and 15 per cent microorganisms. That's a whole lot of slime!

The bacteria and slime layer can now trap other materials such as clay, organic materials, dead cells or any other particle that floats over the biofilm, which adds to the size and diversity of the biofilm colony. Much like a snowball rolling down hill, getting ever larger, this growing biofilm serves as a magnet for attachment and growth of other organisms, thus increasing its size and diversity.

It is interesting to note, that more than 99 per cent of all bacteria in the world exists as part of a biofilm community although, historically, microbiologists have only studied free-floating (planktonic) bacteria. This may not seem entirely significant, but research has shown that once a microorganism attaches to the surface of a biofilm, it "turns on" a previously unused set of genes. This effectively

makes it a significantly different organism to deal with. Studies conducted to date have shown that an antibiotic dose sufficient to kill free-floating bacteria needs to be increased as much as 1000 times to kill a biofilm colony.⁴

Herein lies the problem. A biofilm colony provides a number of advantages for microorganisms including environmental protection from adverse elements like UV light, drying and antimicrobials. It also acts to attract nutrients based upon its negative charge. Many nutrients (particularly cations) are attracted to the biofilm surface. This provides bacteria cells within the biofilm with a nutrient supply greater than that found in the surrounding water. In essence this verifies the results that van Leeuwenhoek reported in 1684 when he studied dental plaque.

THE RISKS

Biofilms are a hot topic. The first case that moved Biofilms to the forefront of microbiology was in 1994 – involving the

infection of hundreds of asthmatics. The infections were caused by contaminated inhalers, which contained pieces of biofilm containing a bacterium known as *Pseudomonas aeruginosa*. The bacterium, in its biofilm state, was able to survive the disinfection process during manufacturing of the inhalant, and when used by the unsuspecting asthmatics, was transported directly to the lung tissue where it flourished. At least 100 people died from the biofilm infection.

An example closer to home is the May 2000 E.coli O157:H7 outbreak in Walkerton, Ont., that killed seven people and sickened some 2500 residents, many of them children. Walkerton's water system was contaminated with E.coli O157:H7 following heavy rains in early May 2000. The source of the E.coli O157:H7 was traced back to a field where manure had been spread. Because the water treatment system was inadequately maintained and did not have the appro-

Continued On Page 44

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priate levels of residual chlorine that would have killed the bacteria and stopped, or at least lessened, the level of contamination. Following the outbreak, during the restoration program of the town's water-mains, flushing and swabbing of the 41 kilometres of water-mains was completed. It was noted that while the swabbing program was underway, there were increased levels of Coliform and heterotrophic bacteria populations in the system due to biofilm material from the inner surface of the water-mains being

dislodged.⁷

Another area where biofilms are a growing concern is with the use of whirlpool tubs, hydrotherapy tubs or foot spa baths. In North America there is a large number of tubs in use in various healthcare, educational and hospitality facilities, not to mention the number of such tubs used in the Spa industry. The design of many of these tubs allows water to accumulate and pool in the pump and other piping, ideal conditions for biofilm growth.

There are two real hazards from biofilm in one of these tubs or spas. First, bacteria are shed from the biofilm and from other bathers, and are present in the water. Sores or breaks in the skin may become infected as a result of this exposure. The more significant hazard is not in the water at all. When a tub's jet system is turned on, small segments of biofilm can break free and become aerosolised, bouncing along on the haze above the water surface. Inhaling biofilm bacteria from whirlpools could do a significant amount of pulmonary damage. Continuing to use or work around a hot tub could be a downward spiral to possible incapacitation. People feel poorly and try to make themselves feel better by staying longer in the hot tub. Then they feel worse.

EARLY DETECTION

Monitoring the presence of a biofilm

It is interesting to note, that more than 99 per cent of all bacteria in the world exists as part of a biofilm community although, historically, microbiologists have only studied free-floating (planktonic) bacteria.

and its relative size can be difficult. Bacteria will be regularly shed from the biofilm slime. Shearing forces (mechanical or hydrodynamic) applied to the biofilm will literally "shave off" slices or shards of potentially infectious material.⁵ If sudden stress is applied to the location of the biofilm a shower of bacterial shards will be dislodged. If you take a water sample just after disinfection of the tub, it is likely you will find a higher than acceptable bacterial count because the biofilm has been traumatized by the disinfectant. If you take repeated samples over the course of a month your results might display a strange pattern of high counts and low counts. This variation can be a result of a number of factors such as time of



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day the sample was taken, the length of time the tub was run prior to taking the sample, and when the tub was last disinfected. This variation can identify that there is a problem and that a biofilm is present. It does not identify what the actual size or level of biofilm contamination exists. This can only be done by taking an actual sample (scraping) from the surface of the biofilm.

HOW TO DEAL WITH A BIOFILM

The good news is that biofilms can be removed or destroyed by chemical and physical treatments.⁶ Chemical treatments using oxidizing chemistries such as chlorine or hydrogen peroxide have been recognized as being effective at both removing and destroying biofilms. Depending on the level of contamination, mechanical removal (good, old-fashioned scrubbing) can also help to remove most of the biofilm from the surface, but oxidizing chemicals are ordinarily required to completely clean off

the biofilm slime. In a hydrotherapy tub, proper disinfection of the pump, water lines and jets cannot be over-emphasized. Consistent cleaning and disinfecting procedures must be adhered to. Remember, it only takes a very short time for a biofilm to re-establish itself and then you are starting the battle all over again.

CONSIDER THIS...

The next time you stay at a hotel, ask yourself: When was the last time the lines of the in-room coffee pot were cleaned? You may reconsider making yourself a pot of coffee and head for the closest Starbucks or Tim Horton's. I know I do!

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