Biofilms

A Growing Problem

Dr. Bill Costerton, PhD.
Director, Centre For Biofilm Engineering
Montana State University

Seminar Transcript

September 21, 2000
Kingston, Ontario, Canada

The whole story of biofilms got its start from corruption in the halls of academe, because I needed a way to put mountain climbing expenses on my grant. The only system that I could think of that had any microbiology associated with it was the alpine streams that came down over the rocks, and here was a very tantalizing piece of information. If you look at the microbiological population in the stream, you will find 8 bacteria per ml, possibly 12 bacteria per ml. But if you look at the wet rocks on the side of the stream, you'll find 5 x 10 to the 8^{th} bacteria per square centimeter. Basically the system is almost a pure culture of Pseudomonas aeruginosa, a very common organism in hospital environments. This is where it has lived since long before we evolved, and continues to live in the rocks alongside of the stream for one particular reason . . . biofilms. Biofilms, which the bacteria form very instinctively and naturally, are its way of surviving UV and surviving drying.

So think of this in the hospital environment: Pseudomonas has been around for a very long time. Its strategy, long before we came along, was to get out on the surface, and cover itself with a slimy material. This is what the bacteria do - they grow on surfaces, cover themselves with huge amounts of the polysaccharide material, grow in the slimy mass, survive drying for very long periods of time, and survive UV. That is their native behavior, in their native habitat. If we took Methacrylate plates and put them in this Alpine stream for as little as 15 minutes, you will get a monolayer of Pseudomonas. You will have a monolayer in 15 minutes, and you'll have 5 x 10 to the 8^{th} population in about 4 days.

This is the intriguing thing about Pseudomonas in its natural environments. It gets itself onto a surface, makes a biofilm, which is 15 percent cells with 85 percent polysaccharide matrix, and just sits back and traps nutrients. Now if you are thinking about a distilled water line in a lab or very pure water system in a hospital, what the bacteria are doing is getting onto a surface somewhere in your piping, pulling what little nutrient there is in the water for their nutrition, growing in lovely little communities, sustaining themselves particularly well and shedding occasional bacteria. That is why, in a new building, sometimes you will have a distilled water system and turn on the tap for your first set of experiments and slime comes out of the tap. How does that happen with almost no nutrient around? This is the way the bacteria do it. They extract the nutrients from the passing water so that, in fact, you can add up the nutritional content of the thousands of litres as they run by and that sustains the bacteria population.

So that is the biofilm idea. The idea is fairly encapsulated in this shot from Cam Wyndham. Cam's now in Ottawa, he was working on a river system for me and we should have actually gone further with this picture when we first had it. This bacterium had settled on a surface. It had spun out a fairly large amount of this polysaccharide material and then divided to make two, and then divided to make four, and then divided to make eight, then made sixteen, and what it was trying to tell us is that "we are really happy on this surface". Now, 14 years after this picture was taken, we realize that the natural state, the happy state of bacteria is on a surface in a biofilm. I'm thinking about such things as Legionella in a hospital ventilation system for example. Organisms can be there. If you have condensate water anywhere in the system, you'll find them swimming around in certain numbers, and you'll find about a thousand times that number on a surface somewhere. On an intermittently wet surface, they will be in a biofilm - 85 percent polysaccharide, 15 percent cells - and if you blow hard enough with the air conditioning system, you'll actually blow pieces of that biofilm out. If you aim them directly at patients, they'll get Legionnaires disease. It is just a natural extension, this is the way they grow in streams, this is the way they grow in lakes, and we'll try and make this point in a medical context as we go.

Implanted Medical Device & Antibiotic Resistance

The biofilm idea was fairly hatched in Scientific American Magazine in 1978 and was quite interesting. You know how things work in the medical field . . . quite different in the scientific field. If the chief has an idea, all of the residents have the same idea, and all the interns have the same idea. It is a little bit more regimented than the systems that we are used to. Alan Ronald in Winnipeg had an idea that possibly biofilms had something to do with chronic disease. And so Tom Marrie in Halifax, who was a resident in Alan's system, had the same idea as his boss, but he really didn't believe it. He had a patient and this patient did a lot of good things for us. The patients name was also Tom and he had a severe alcohol problem and lived in a cardboard box by the docks in Halifax. He fell down and hurt his elbow, got a bacteremia of Staphylococcus aureus, felt sick and came into the hospital. Tom noticed that he had a bulge in his chest area, and it was the battery for a pacemaker. But the pace maker battery had long since died and he wasn't pacing anymore. We had a bacteremia and a foreign device in a patient. We asked Tom if it was okay if we just found out how much Cloxacillin he could tolerate. I gave him 14 grams per day of Cloxacillin, which is quite a lot. He was a volunteer in this whole thing. He let himself out and went drinking each night so there was Cloxacillin plus alcohol for three weeks. The alcohol was taken by the traditional route. After a couple of days of this regime of Cloxacillin and alcohol his fever went right down and he was having a great time. He kind of enjoyed all of the attention and a nice dry place to sleep. After three weeks we took him off the Cloxacillin and back came exactly the same Staph. aureus strain - same fever, same symptoms. This is the medical experience: that if you have a device and the device is at all involved in the infection, then of course it will just keep coming back until you do something about it. The intriguing thing was we put him on another three weeks of Cloxacillin and this time it was Rifampicin just to be absolutely sure . . . really clobbered him for another three weeks. We took him off at the end of six weeks and he went right back into the bacteremia. So we took the pace maker out and it was quite a moment for us. This is the metal here and this is the old fashioned cardio pace maker. This crud was all around the outside. After we rinsed the pacemaker, and scraped it off and plated it – we had 10 to the 8^{th} living bacteria at the end of this thing. It was a biofilm of Staphylococcus aureus that we were trying to treat in this particular patient. Here are the cells. Here is the slime that they are embedded in. Clearly, you can really beat on a biofilm like this with huge doses of an antibiotic and it's just not going to get killed. Take the device out. This is now general medical wisdom - if you have a device and the device is part of an infection, it has to be removed before the infection can be cured.

We couldn't take an anecdotal approach - we needed to generate some numbers. For the project we didn't use artificial urine, we used real urine - professorial and graduate student urine. Technician urine was toxic, we're not quite sure why - particularly on Mondays. We got real urine and then we got real bacteria. We decided not to use the strains that had been kicking around in the lab for 1,500 transfers and used on so many experiments. We went straight to the clinical lab and got some “wild” bacteria. Pseudomonas. We got them right back out of a catheter related infection, and ran them down through this device, past catheter latex and made ourselves a biofilm. It is really easy to do. Here are the bacteria clustered on the surface after just two hours and clearly a biofilm is forming. Here we are after eight hours, can't even see the latex anymore. Most of the catheters are milky and cloudy. If you get a clear one - there are two types that are clear - you can actually see the layer of biofilm moving up the catheter wall towards the patient’s bladder. We call it the creeping crud. It's first of all fairly clear, and then it gets a little milky as it traps some crystals, and so on. It actually moves along the surface up into the person's bladder and that is why we have such a problem with urinary catheter associated infections. Here’s the biofilm that we produced - these cells are Pseudomonas. We had some swimming cells and we hit them with 50 micrograms per ml of Tobramycin at a contact time of 8 hours. We killed them completely. But if we had a biofilm on the surface and hit it with a thousand micrograms per ml, at a contact time 12 hours - no really significant kill. What it was telling us is that biofilms are fantastically resistant to antibiotics.

If you want to think about biofilms, think about plaque on your teeth, which is classic biofilm. Not the easy stuff to get off, but the stuff where the dentist almost kills you with that little vibrating thing - the hard plaque. Or if you keep tropical fish, think of the slime that develops on the glass plates. That is the consistency of this material, this biofilm. It’s very hard to kill and just keeps coming back and back at you all the time. Is the answer at all useful in a clinical context or in any kind of a context? Yes it is. If a hip gets infected, some orthopedic surgeons will go directly into the synovial space with huge doses of antibiotics and drain out the far side. They can't prove it of course because you can't go in and scrape the

stuff, but it looks like they are shutting down some orthopedic infections in this way. In sterilization, we do it all the time. We kill biofilms by using huge sustained doses of effective biocide. The critical thing is that when we are testing and making sure what was actually killed, we have to look at the biofilm death and not just the death of the planktonic cells.

Sterilant Resistance

The reason that we are all gathered here today is that they are also fantastically resistant to sterilants. If you have any antibacterial agent, the rule of thumb is that for every unit it takes to kill a planktonic organism, it takes a thousand times as much to kill a biofilm organism. If you have a patient with a foreign body device, you usually recover the bacteria from fluids. We grow them planktonic in the lab and determine the MBC (Minimum Bactericidal Concentration), the planktonic killing dose. It will have a certain value. Then we take the organisms and grow them on a surface to make a biofilm and we get the biofilm killing dose. This could now be done with a kit that is being sold out of Calgary. The biofilm killing dose will be between 1000 - 1500 times as high as the planktonic killing dose.

So whenever you are doing a test to see how a sterilant is working and how an antibiotic is working, you grow them up as planktonic cells. Then you get an answer and that answer is how much does it take to kill a floating cell. Now in a medical context that is really quite a useful answer because in the case of Tom, the guy down on the docks, it was the planktonic bacteria that were giving him his fever. It wasn't the biofilm on his pace maker. So you can quiet down a patient's symptoms, by giving him the planktonic killing dose, but you didn't kill the biofilm. The biofilm will start shedding new planktonic bacteria and this is the explanation for why chronic diseases seem to come back, and come back, and come back - they don't actually come back, they never go away. The biofilm is always there, quietly being the reservoir, shedding new planktonic bacteria. This is evident in a great many different infections that we deal with all the time in the medical context.

Effective Biofilm Biocides

Heat is pretty effective at sterilizing biofilms, and oxidizing agents are pretty effective. Anything that comes down and oxidizes - we are talking hydrogen peroxide and chlorine bleach - will actually dissolve away the polysaccharide matrix. They will kill the bacteria, come right down to the surface, and eat their way through a biofilm pretty effectively. I think I was probably responsible for the largest order of bleach that has ever been on the planet. I was doing a consulting job in the oil business and I had a de-aerator tower, 180 feet high and 30 feet across. When we opened the manholes on it, it was absolutely full of biofilm - 180 feet high. So we got a barge of bleach from Singapore at 15 percent, and we cycled 15 percent bleach through this tower. The stainless steel held up pretty well under the circumstances. We do it in the oil business all the time. With chlorine bleach you are going to destroy or damage things, but it is very effective. My point here is that the biofilm is particularly susceptible to oxidizing biocides because you are actually dissolving the matrix and exposing the bacteria as you go down. It uses up quite a bit of bleach in the process but it is particularly effective. We have actually looked at sterilizing colonoscopes because they've got that biopsy channel down the middle of them, and of course there is your real bomb. We have an electrical method, where we can actually put a DC field across these things and have the biocides work particularly well. Glutaraldehyde is really a tanning agent, which was used for tanning animal hides long before it was used as a sterilant. It gets used up in the matrix material, so you get pickled biofilm. You can actually see it work, but it’s not very effective against biofilms. You put in glutaraldehyde and you can just see it slowly working from the outside killing a few of the outside organisms. It takes an awful lot of glutaraldehyde to get down to the last organism of the system. If you don't kill all of the bacteria in a biofilm before you take away the sterilant, then of course the survivors wake up in the guts of their neighbor that just died. They divide every 20 minutes, and will go right back to a full biofilm within four hours. So if you don't kill a biofilm completely, the survivors do wonders because they are in a perfect, nutrient-rich environment. Glutaraldehyde has never been particularly good for us. We work in the porcine heart valve business. They sterilize with glutaraldehyde and work in baths with ½ percent glutaraldehyde - breathing the fumes and the whole business. Mycobacterium chelonae kept up, became resistant, grew in biofilms and gave us fits. It hasn't been very effective for us. We use glutaraldehyde quite a bit in the oil business and it does quite well for us because we combine it with mechanical cleaning. We take a contaminated line, run a great big three-ton pig on a 60-inch line and a whole slug of glutaraldehyde, followed by another pig. That way we take off the old dead biofilm, scrape it

off, and it works really well. Glutaraldehyde doesn't bother the metal, whereas oxidizing agents do bother the metal and if you combine it with meticulous cleaning, it works. Nobody every said glutaraldehyde didn't kill planktonic bacteria. It kills planktonic bacteria beautifully, like most of the sterilants do. But the question is, does this kill biofilms? Yes it does, slowly by pickling, tanning. Oxidizing agents actually etch away the biofilms, and you expose more cells, and kill them as you go.

Rather than eating your way down through the layer chemically, just remove the layer. Very meticulous cleaning is obviously the key thing. The US Navy did us a great favour because they were looking at cleaning their ships. They would scrub and clean all the outside of the ships and there was this slimy layer of matrix material left, but not many bacteria. It re-fouled at 10 times the rate of a clean steel plate. Then they would use bleach - but any oxidizing biocide will do. It took it right down to clean and then re-fouled at the clean rate. So not only are you cleaning better, your re-fouling rate is much more favourable. Leaving pickled or natural biofilm matrix on a surface just invites a new biofilm back onto the surface. We really should take if off mechanically as best we can and oxidize it right down to clean. That is the best way.

Biofilm Resistant Material

Question from audience. Are there any materials that are biofilm resistant?

That's literally a 100 billion dollar question. Companies are looking for biofilm proof material, and just imagine what they could charge for a heart valve or something like that. We would test their "biofilm resistant" materials and found that their problem was bad microbiology. They would take a bacteria strain that had been transferred 1000's of times in the lab (genetically weakened) - it could barely hang on by its fingernails anyway. The companies produced some Teflon modified, or silver modified material where that lab bug wouldn't stick, and then they would try to extrapolate it into the patient, and immediately everything stuck everywhere. We use wild bacteria, ones that haven't been in a test tube for a long period of time. You can live in a test tube because you have no challenge. There is nobody after you in a test tube, you can lose all your outside layers really fast. You can live dangerously, do whatever you are going to do and nothing is going to hurt you in the test tube. But the moment you get back into nature again, you are dead meat. All we did out in Calgary was, for $5,200 Canadian, we would take anybody's biofilm proof material – on which they spent 1½ million dollars in development in the case of Dupont – and put it in with urine and wild bugs. I once heard a vice president cry over the phone. The wild bugs and natural urine were all over the stuff.

Chronic Infections

We often wondered why chronic infections wouldn't clear themselves - here we are interested in Cystic Fibrosis. Here the Pseudomonas is growing as cystic fibrosis in the alveolus of the lung. Here is the polysaccharide, somewhat condensed by this method, in this crust around the outside here. We wondered what it was for several weeks, and then we stained for IgG. It is actually immune complexes, or antibodies, antigens, reacting in a total crust around the outside ½ a micron thick. Yet these cells, even the ones right next to the antibody crust, are still alive. Antibodies just don't kill biofilm bacteria at all. This is why if you have Osteomyelitis, Cystic Fibrosis, prostatitis**,** or middle ear infections, which are all biofilm-type infections. You can have very high circulating antibodies, and they react perfectly with the antigens, but they don't bother biofilm bacteria at all. There is tragic tale that goes with this one. Pennington, from Brigham and Woman's Hospital down in Boston, decided against a whole bunch of advice that he would immunize kids with Cystic Fibrosis with LPS-based vaccines against Pseudomonas. They were infected with Pseudomonas, and it is classical wisdom that you should get lots of antibodies against the infecting organism. He had four in the treatment group, and three in the control group. All four in the treatment group were dead in four months. What he had actually done was to raise their antibody levels to a point where there was tremendous immune complex reaction around the slime balls in their lungs. Then he refused to write up the paper for them but I think everybody now understands it. We now have some of these patients on Prednisone, which is actually suppressing their immune response so they don't make too many antibodies against the infecting organism. The antibodies don't work against complex disease like this for reasons I'll show you later - you get a lot of damage.

This is a Teflon rod with Teflon disks and some silastic just on the edges of it, and they just got a biofilm started. They only had about 10 to the 3 bacteria per square centimeter, just a few little cells with a tiny bit of slime covering them, little teardrop things. They put them in the peritoneum of a rabbit. The peritoneum is very well defended by phagocytes of all kinds. We were expecting that this thing would get cleaned up by the white cells when it went into the peritoneum. The biofilm progressed right up to 10 to the 8th on these disks in the peritoneum of the rabbit even from a very humble start. So what this means is that a little teardrop shaped blob like this with three bacteria in it is totally phagocyte proof when you put it in a foreign body into the peritoneum. Then what they did was immunize the rabbits against the same strain that they were using to build the biofilm, and for some weird reason, the biofilm did slightly better in the presence of opsonizing antibodies.

Biofilms are really resistant to antibiotics as we said, and antibodies, and also phagocytosis. Here we are looking down on top of a biofilm that is cracked because of the preservative method, and these are the white cells going crazy across the surface. They can sniff that there something is foreign there. The cells are supposed to be going in there and killing these bacteria, they know that they are there, but they can't get at them. This is because of the polysaccharide matrix. If you get planktonic bacteria, they are killed by antibiotics, by antibodies, and they are picked up by white cells. If they get a chance to form a biofilm, which they do preferentially on a foreign material like this, they become antibiotic proof, antibody proof and absolutely phagocyte proof. What happens as the biofilm develops more and more is that the white cells come along and when they can't pick up these bacteria in the biofilm that they know are there, they fire enzymes indiscriminately in the surrounding tissue. If you are in a really bad state where they get really excited and there are lots of antibodies on the surface of the biofilm like this and they know they are supposed to be digesting something, they sit back and fire enzymes, that don't have any effect on the biofilm at all but they kill the surrounding tissue. This is why if you have an infected prosthesis like a plate, or a set of screws, or even an old hip, it starts to loosen quite quickly because the white cells are actually firing enzymes out and destroying a great deal of the tissue. This is also why we are immune suppressing CF kids. Here in Kingston, Curtis Nickel is also immune suppressing prostatitis patient because the prostate gets so irritated and so inflamed that they are doing a great deal of medical damage. So they sort of quiet the whole thing down as best they can. Here is the kicker on this thing. These biofilm infections in this pattern had been slowly building up, mostly in compromised people: diabetics, cystic fibrosis kids, youngsters going to daycare and having a lot of contact with other people - they get middle ear infections. The figure out of the CDC is 65 percent of the infectious problems seen by doctors in the developed world are biofilm infections. We have gotten rid of a whole bunch of the planktonic infections because they were well treated by antibiotics and by vaccines and so on. These are the junky infections and diabetes, diabetic foot, middle ear infections in kids (which is huge), any osteo device-related stuff. If for example a person has a catheter like a vascular catheter going in and you have exit site infection around the catheter, which is a pure biofilm. If you can get enough Vancomycin to it, you can overpower the biofilm sometimes, but only temporarily. Usually you have to take the device out before you can cure the patient.

We had the old infections – we had Diphtheria, we had Scarlet Fever – all caused by planktonic bacteria. Antibiotics worked, vaccines worked, and we don't see those diseases very much any more. What we see now is a diabetic person having lots of diseases that are hard to cure, or a device related infection that is hard to cure, or Osteo. Middle ear is a big one, and as we looked at the hemophilus out of the middle ear we took some tubes out. You can actually hold a tube up and a big blob falls off the bottom of the tube. That's what you're actually fighting against with a middle ear infection.

Question - Has the biofilm always been around or is this something new?

Biofilms have always been there, but they were sort of a minor thing. If you got Diphtheria or Scarlet Fever you had a pretty acute infection. Compromised people didn't live for long periods of time. We didn't have CF kids living into their 40s. People with diabetic infections often succumbed to those infections very early on. Think of an endotracheal tube - the cuff at the end. When it comes out, you have to vacuum around the cuff as best you can. Biofilm heads down into the lungs, white blood cells are not working on the biofilm, antibodies aren't working. We do this with animals right now and we can actually show parts of the lung just fibrose off. In the old days if you had an acute infection you survived or didn't survive - now a lot more people survive, often in a compromised state. Biofilms were always there in

nature. Pseudomonas was always making them in water systems. Legionella was making them in lakes - the edges of the lakes are full of Legionella. This is why we don't have these fire breathing pathogens like Diphtheria any more, it's just Pseudomonas. There is more Pseudomonas on a bunch of wilting flowers in a patient's room. For a very brief period, Pseudomonas was actually used in the First World War to displace anaerobes and gangrene prone serious multiple fractures. People actually used Pseudomonas therapeutically. Until the late 1930s it was never a pathogen, it was always an environmental bug. So what have we got kicking around now? We have the alcaligenes type of organism; we have a lot of strange strains of Streptococcus, which are just skin organisms. We have a lot of Staphylococcus, Pseudomonas, Serratia and other environmental organisms. We don't have the fire breathing pathogens anymore, we just have environmental organisms invading our systems, and they use biofilms as their primary tactic. Biofilms weren't a medical problem before, but we have always had plaque on our teeth and scum on our aquariums.

Question - Has the advent of antibiotics been a factor?

I think it has. Antibiotics left biofilms growing in all of these environments, but they were probably growing even before this. It is just a natural progression that as we solve certain problems, the bacteria had an Ace up their sleeve (if they had sleeves), and the Ace up their sleeve was biofilm. Now when they sit back at the CDC and look at what kind of infections are coming to practitioners, they are most likely a biofilm infection. After all, antibiotics work and vaccines work so most other diseases are wiped out now.

Centre For Biofilm Engineering

In 1990 the Centre for Biofilm Engineering was put in at Montana State University, Bill Characklis was the first director. He died in 1992 and I took over in 1993. It wasn't the medical society in the US, or the microbiology society in the US that actually funded this. It was the engineering directorate, which I find very significant because engineers like to identify and solve practical problems. They voted this to be one of the major problems in computer manufacturing, in the oil industry and in cooling systems - caused really serious problems. The engineers didn't know how to solve these problems at all, but they had this very systematic approach, and voted 22 million dollars over 11 years as a base grant. It was a conservative approach. It was not to just have some microbiologists working on it, but they wanted to have a whole organized approach to the problem. The problem was very serious - the cost of just metal corrosion alone will hit 7 or 8 billion dollars in the country in one year. There are 51 professionals in the Centre and of these only 8 are microbiologists. Some are mathematicians because we do a lot of modeling. The vast majority are different kinds of engineers.

This is a really intriguing approach because this will give you all the weapons of engineering to bear on a particular problem, and engineers just don't subscribe to a whole bunch of the nonsense that microbiologists subscribe to. I failed high school math and that is why I am a microbiologist. I am the Associate Dean right now and I think that I am the only Associate Dean of engineering in the world who failed high school math. You need to get people organized who understand the math, and people who understand the techniques going on in what has always been a microbiological problem. We weren't, as microbiologists doing so good. It is funny that there are 5 percent females in the engineering college, but almost half of the professionals in the Centre are females, and almost all of our PhD students are women. They are all mixed in one area from 11 difference departments, and they are actually in carols with adjoining desks. You have a computer person next to a chemical engineer, next to a chemist, next to a microbiologist, and we are training people that have never been trained this way before. The crossover in training is fantastic. I will show you some of things the engineers did before I came to the Centre that I think were pivotal. Here we have a biofilm under confocal scanning laser microscopy. The confocal scope, which uses a laser to form the image, can look at a living biofilm on an opaque surface - on steel, or any plastic surface of any kind. This was the real clincher. We couldn't keep on doing electron microscopy, because you have to kick around the specimens so badly.

This is in fact a living biofilm. This cell could just be leaving and another could be coming in, and you can look at them in the XY axis or in the XZ axis. You can take the image and roll it over whichever way you want. You could even dissect the image in any way you want. This was the first major discovery in biofilms in the 90s, that biofilm cells are in these slime towers like this with a matrix around them. There

are huge water channels running down through the biofilm. Of course we never saw them on the electron scope because everything is dried out and collapsed. Here is a tower made of slime with gold-coloured bacteria inside. Here the cell is leaving. Here is a mushroom shaped tower. Biofilms have shape. Each cell has its own position, and builds a matrix in a particular way. As we began to see this we were seeing that biofilm is a lot more sophisticated than we originally thought. This is not a random structure. This is a highly organized structure. These are deep canyons. There is water flow. Here is another shallow water channel, and a blind end water channel here. In essence you have sort of like a garden - a bush here, flowers here, and circulation going down around them. We always wondered how the bacteria in the bottom of biofilm could get any nutrition or get rid of their waste. They actually had a primitive circulatory system. When you go to a biofilm in a real river system - here are aggregates, here you can see the different kind of bacterial cells; here you can see the water channels. Here an amoeba actually found the water channel and is going down the water channel trapping any loose bugs it can find and eating them. If they are in a biofilm, the amoeba can't touch them, they sort of polish the outside of each micro colony of bacteria. We are reassured here because this is a natural biofilm in a river, and this is the way they really work. I guess they are not going to worry about white cells because for a couple hundred million years before animals evolved, there were amoebas nibbling at them. They solved that problem a long time ago.

Anaerobes in an Aerobic Environment

We were speculating whether or not oxygen was getting to the bottom of the biofilm, so we took this probe, and made a sensor for chlorine or chloride. We came down under the confocal scope and on sort of a ratchet device taking measurements as we went down through a mushroom like this. We solved a long-standing microbiologic mystery. In a perfectly aerobic environment, sitting in air, in a very thin biofilm, it is totally anaerobic in the head of that mushroom. I always wondered when we were doing skin microbiology - you swab somebody's skin, you pick up about 70 percent anaerobes. What are anaerobes doing on somebody's skin? The middle on every mushroom head is absolutely anaerobic. Now you get an antibiotic and it might work really well against aerobes but not at all against anaerobes. Well, Pseudomonas growing as an anaerobe, which it can do because it’s facultative, isn't going to be susceptible to that particular antibiotic or the other way around. So every biofilm is a mixture of bacteria growing in every physical state you can possibly imagine.

How Biofilms Work

This is a summary of how it works. The bacteria come down onto a surface, and it turns out they rearrange themselves after they get onto a surface and make little stacks here and there. Then they change genetically, start making the slime, build towers like this and water channels **(fig. 1 – CS84495wb.jpg)**. This mass is 85 percent matrix, 15 percent cells. They gradually build higher and higher towers, and that is your biofilm sequence. To give you an idea how long this takes, Pseudomonas will get to be about 25 microns high if you give it six hours on a surface. So biofilm can form fairly quickly. Then the engineers had to look at something called visco-elasticity. So what they did was take biofilms and put it under very high sheer. They cranked up the pumps as high as they would go and what then happened is the biofilm tower deforms and makes a tadpole like thing called a streamer. They start to oscillate back and forth. At some point they break and this mess here containing a couple of hundred cells just takes off in the direction of the flow. So biofilms aren't rigid - they aren't like little crispy things sitting on a surface, they are rubbery. They are rubbery reacting to flow and if they break off at some point and go down the stream, then of course you get the biofilm transferring down the stream at a considerable distance. Taking us back into the medical arena - we are interested in defective heart valves. Clinicians will tell you if you have Endocarditis, or an infected mechanical valve, then you will get down stream effects. You will start to get little peticiae in the lung where a bunch of bacteria comes and jams in a capillary bed and starts growing, and you lose that part of your lung. You also get stroke a lot, because they will break off, go up your carotid into your brain, and hit the capillary bed there. So then we went back and looked at the matrix strength in these biofilm towers, and it turns out that if they are formed at low flow, then they are very breakable. They are very soft and they come off very easily. When they form in high flow they are really tough and rubbery. So why don't you clean something with a pressure surge and knock all of the biofilm off? But if you put a pressure surge through, the next ones that form will get more rubbery. Do another pressure surge and these ones are even more rubbery, and then finally you get them just like hard dental plaque, and they won't move for anything. The bacteria can actually adapt the strength of the matrix that they are living in to the circumstances that they are living in. They live nice and soft if it is a low flow

situation, really hard and rubbery if it is a high flow situation.

Water Filtration

We have a specialist in potable water, Anne Camper, in the Centre and we have been really intrigued with delivering potable water to various hospitals and domestically. You get tremendous biofilms in any water distribution system. If you shock-chlorinate a water distribution system, you can be in worlds of trouble. Basically you can knock off a whole bunch of biofilm at any given point - a backhoe hits a pipe and you get all kinds of stuff coming off. When you get a grab sample you are actually sampling whatever took off - was released - in the last couple of seconds before you took the sample. The biofilm will be there with a thousand times as many organisms in the biofilm as actually were in the catch samples that you are taking. We are looking at the German system now of getting along without chlorine. We put a reactor up front that takes almost all of the nutrients out of the water – a bioreactor. If it grows through, it becomes a bug factory, but if you keep changing it, taking it off line and bleach cleaning it, you can take almost all of the nutrients out of the water. Make biofilm on purpose, deal with it locally, and don't let it come all the way down your pipe. So then you have a whole bunch less biofilm in the distribution system. Our question was, could pathogens still sequester themselves in this biofilm and come out later on and cause some kind of outbreak? We put all kinds of pathogens in including Salmonella, and a great many things including coliforms, and we looked at whether or not they partition in and out of the thinner biofilm. As you go lower and lower in nutrients, the pathogens are less comfortable in these biofilms. Now we are really looking at bio-terrorism in a big way. The bioreactors right up front find all of the viruses. So if the viruses happen to hit, then you can actually isolate them as being all tangled up in the slime in the bacterial biofilm. Then if that bioreactor showed up as positive on a detector for a bad virus, you just engage the other biofilm on the side of a pipe will trap viruses into the biofilm. A biofilm bridging or crossing a whole bunch of sand particles filters the viruses out. If you had a detector on four units and put them all onto one unit and took it off line and then went to exhaust, then you could actually probably protect this system from viruses, if you had a good sensor to do it.

Hospital Ventilation

The system that really intrigues me in the hospital is the ventilation system. If you have an air conditioning system and you have condensation trays in there, they are full of biofilms. When your car air conditioning first starts and a really strange smell comes out, it's anaerobes. It is a stale anaerobic smell. It has been happening all winter - you have had this biofilm growing in your car air conditioner. It is producing anaerobes. It is the only way you get anaerobes in a system like that. You blow the stale smell off of the thing but how many particles do you blow off too? In the ventilation system in the hospital we were working on, we had filters for catching biofilm particles. Biofilm particles are your enemy. Single planktonic bacteria don't cause you that much problem in your lungs, but a lump of biofilm is really bad. So in the case of engineering, just put baffles in. Don't park a patient right under a whole bunch of incoming bacteria. We took animals - five different species of animals with 6 different pathogens. I pre-make the biofilm lump, put it down into their lung, and compare that with planktonic cells. Planktonic cells are clear in four hours because the phagocytes are working. If I put down a biofilm lump, I can still isolate it a month later. The animal is not that sick, it is just cruising along with a biofilm colony in some part of the lung. If it gets stressed - we stress the animals on the steroids - the infection will pop out again. I think we all see a number of these kinds of pulmonary challenges from breathing biofilm, aspirating biofilm.

Water Treatment

The question of cleaning up old, damaged systems is very, very difficult, and Ann is looking at total replacement in most of these systems. She is so busy right now with people putting in new systems, and a question of whether copper pipe in houses is damaging, and a couple of questions like this, that she hasn't been able to get into retro fit. The only thing she has been able to do so far is take a really bad system, heavily overgrown with biofilms, and put a reactor on the front end of it. We actually have an experimental loop in our local water treatment plant, so we can foul it and then put a biotreater and cut down the nutrients coming into the system. It gets on recycle. She has been finding very sad effects, because if you have a well established biofilm, well feed from the nutrients from the source, and you cut down the nutrients, you can have a huge sloughing event. Patches of it will die, and patches of it will come off. You actually get particulates and slime, and as you start to cut the nutrients down, the system goes right to hell. The best you can have in your water distribution system right now (in the absence of a bioreactor) is a

healthy biofilm that is well fed, and tight, and not letting too much go. Never pull the casing on your well and look at it because you have been drinking water that has been coming over a filthy looking mess with all kinds of oscillating slime fibers and so on. The best bet so far, is rather sad - it is to keep your biofilm healthy, don't have it coming off, keep it well feed, don't antagonize it, don't hit with any chlorine. But it is a ticklish situation when you think about it. There is something living down there and you have to keep it happy or will do bad things for you.

With the politics of risk in the US you can't take any risks. It isn't set up for it. They won't accept any risk at all. Getting the US to take chlorine out of water treatment will be the very last thing that will ever happen. So we are looking more at computer plants with our bioreactors. I don't think you would ever get safety minded people to take chlorine off, but we learn how to manage the system better. If we know there is a biofilm there, one of the things we can do is put in a biofilm sampler. Rather than taking a grab sample of the water and trying to figure out what is happening with the biofilms, we can put one of the Robin's Device coupons in and put it into a side stream or a main stream and actually be able to look at the biofilm at any given point to find out what is there. We can do a PCR (polymerase chain reaction technique) on it, find out what organisms are there, and find out if there are any pathogens there. You will never believe what we eventually ended up doing for the computer people. We used glass beads in a little tube, with a fairly narrow diameter and centered them so that there was a 40 micron track through them. We relied on the bacteria making biofilms on those glass beads and at the end of one working shift would just throw them away. We tried to minimize the number of bacteria coming through and then we trapped them as a biofilm on these end-use filters, and then chucked them. We're looking at 75 cents each. That is the best protection you can find.

Think two phases. Any kind of water system has a biofilm in it, and it's got planktonic cells in it. So rather than just measuring the plank's, you measure both, understand both, and you can maintain the system quite well. Ozone has been a real problem for us because if we ozonate the water coming in, it breaks up a whole bunch of nutrients the bacteria otherwise couldn't have used. It makes them available to the bacteria and we get a biofilm bloom right after the ozonater. In some cases we can use that to our advantage and sometimes we can't. So sometimes we ozonate and sometimes we don't - we are looking at both halves of the equation all of the time.

Question from audience - Is it better to have a constant water flow? I know dental offices turn the water systems off overnight, is it better to have a constant flow?

That's a super good question. Letting it stew is going to let planktonics loose, and any chunks of biofilm come off that are loose. Turn it on in the morning and you're going to get a fairly good shower of things coming off. Keep it going at the right sheer rate all the time, just keep re-circulating it. It is then going to give you a steady release of bacteria and pieces but at a lower level. If you have a Reynolds Number of 2400 (that is really clapping along), and a really smooth surface, you think that bacteria aren't going to make a biofilm on that surface when they are going by at that rate and they actually are. They love it. If you look at one of these streams right where a waterfall lands and there is tremendous sheer forces, that is the very best biofilm you'll ever find. But taken that aside, the whole idea of putting pressure surges through and then going off line, or putting pressure surges through and putting in a 3 micron filter that is going to let planktonic through but catch the biofilm pieces is another question. The thing we are doing now is really quite cute - if bacteria are living, they contain co-enzymes called NAD. If the NAD is there and you irradiate infrared light at 606 nanometers, then all of the co-enzymes in the bacteria light up and they re-fluoresce at 636 nanometers. If you have this probe built into the line and there is nothing there, it goes out at 606 and never comes back. Your gauge stays at zero. If bacteria come on and you irradiate at 606, then they re-fluoresce at 632, the gauge starts to show you fouling. Then you hit it with a sterilant, and if you knock them off like you would with a bleach sterilant it goes down to zero. If you kill them with glutaraldehyde and they are still there, the NAD, which is usually reduced, goes oxidized and re-fluoresces at 618. You can actually watch the biofilm form, and watch the biofilm kill with an online machine developed by Intelligence Optical Systems in California. It is actually going to be able to tell you by building a little optical path to a detector into the side of the pipe. If there is no biofilm, there is no fluorescence. If they are alive, they fluoresce at 632. If they are dead, they fluoresce at 618. You can actually start to see them on a gauge.

We are all thinking single species biofilm so far getting into the water lines and there is no such thing.

Question - does ultra violet protect against biofilm?

Much less so than planktonic cells. UV light penetrates biofilms poorly. A lot of it gets trapped in the matrix and the cells are pretty resistant. You will find biofilms forming on boulders in full sunlight in the Antarctic, they call it a varnish - desert varnish - and the UV is not very effective against biofilm bacteria. It kills the first 3 or 4 of the top. It doesn't penetrate very well.

We're getting involved in periodontitis. This is where you get biofilms in the crevice between your teeth and gums. This is one where we got one of the engineers to look at this on the confocal scope. The easiest way to get it is to pull out somebody's tooth when it is going to come out anyway, and keep it nice and fresh, and put it under the confocal scope. You can see these towers coming right back up at you. It looked pretty solid when we were looking down from the top. Then we gave it to our dynamics guy, he put it into a high flow system and flowed it really hard. What happens is that each one of these has an attachment point - they all move independently. It looks like a kelp bed in a storm, and you see them all going back and forth. When they start whipping around in a high flow situation, you realize that if they are disturbed in any way those water channels widen out and circulation is even better. This is how you get your food down to the bottom, and waste out. The whole thing has attachment points, long things coming out of the attachment points, and a great deal of mobility. This is the icon picture of biofilms, of circulation across the bottom, and streamers out the back - sort of our trademark these days **(fig. 2 – Biofilmswb.jpg)**. We wondered about the resistance of biofilms to antibacterial agents. We thought - okay what's happening is there is a matrix there and obviously whatever it is - gluteraldehyde, Tobramycin, whatever it is - is not making it down through the matrix. At least that is what I thought. So I published that this is probably the case, that these things are being extruded by the matrix material. All kinds of mathematically-based people started writing papers I didn't understand, and a formula about this long, saying that – no, the matrix is so full of water that it is not a barrier to diffusion of any kind. They turned out to be right. In fact if we add an antibiotic to the outside of a biofilm 300 microns thick, and then have a detection system at the bottom for when the antibiotic arrives, it takes 90 seconds to come from the outside to the very bottom. We had thought the biofilm was just slab, so then we started calculating how long it took to get into the middle of a mushroom head. It takes about 30 seconds - so diffusion is very, very quick. If the diffusing molecule is chlorine or something like that then it is getting used up as it goes in, but basically there is no real diffusion problem. So we are left with the question - why are these organisms so very resistant to antibiotics and biocides?

Resistance & Phenotypes

Dave Davies developed a new technique - basically the cells are observed with a microscope. They come on to a surface like this - they have been genetically modified so that when they turn on a gene they change colour. On the promoter for the gene, you put a little reactor there that makes them turn yellow or in some cases, blue or green, when the gene turns on. I don't happen to understand how it works particularly, but the cells light up when the gene comes on. We are interested in the gene for slime production because these floating bacteria have slime on their surface left over from a previous incarnation, but they are not making slime. This one lands and at first it doesn't light up. But then it lights up and so all of its neighbors light up and they start making all kinds of slime. We saw that the gene turned on and we can justify this cartoon in which the planktonic come down, settle, turn on probably we thought 6 or 8 genes and start making the slime fibers like this – and this is your biofilm. The next slide was a bombshell, and it is only 2 ½ years old. These are planktonic bacteria growing on various media, planktonic bacteria growing on an iron deficient medium. What we are looking at here are the proteins in their cell walls and membranes. You can see this banding pattern in a large number of proteins formed by these organisms - that would make up their wall and their membrane. This is the planktonic population, and this is the corresponding biofilm population of Pseudomonas aeruginosa, and none of those proteins are the same. We do densitometer traces and there isn't a single protein that is the same between the planktonic and biofilm. We couldn't believe it. We thought we had contamination and what must be a different species of bacteria. Then we went back and nailed it and now there is a PhD thesis. This is the discovery that there is a biofilm phenotype. That is, all bacteria have the same genes in their DNA, but they express different ones in

different situations. The biofilm phenotype, which is the gene expression of the biofilm, is so different from the planktonic cells, it's more different than a spore is from a vegetative cell. It takes 65 genes to make a spore happen and it's 85 where they actually go into a biofilm. Some shut off, some turn on. There is fantastic genetic change – phenotypic expression of change. They become just about like a different organism. Proteins in their walls change. Proteins in their membranes change and they are spectacularly resistant. What experiment would you do the week after you found this out? Take a biofilm, mechanically stir it up, break it all up, and do it so fast that you get suspended biofilm cells. Get the biofilm phenotype even though they are floating - totally resistant. They gradually change over to the planktonic phenotype and become sensitive again.

So it isn't the matrix and it isn't being all stacked on top of each other, it's a totally different phenotype. When we do this we have planktonic cells coming down and here they are growing as fast as they can grow. They are dividing in this case every 18 minutes. This is the amount of messenger RNA that they are making, and then this is the amount they make when they go onto the surface. They turn on a whole bunch of new genes. Maybe penicillin isn't going to work because there isn't any penicillin binding protein anymore. It is totally different. Streptomycin might not work because the ribosomes are all different. I'm thinking now we could probably start developing antibiotics against biofilm bugs. For a long, long time we thought there had to be a diffusion resistance - totally wrong, it's just a different phenotype.

Testing

This is what the engineers have done for us. We don't trust the old microbiological methods anymore. Think about swabbing. You come along with this cotton ball and you might pick up a micro colony of bacteria, you might not. The micro colony of bacteria wrapped around this fibre might let go and it might not. If it does let go and it contains a million bacteria it will show as just one colony. You are not going to get the right number. The biggest thing about the biofilm concept is that it doesn't do really well with traditional microbiological methods - the scrape and plate methods. They are okay for growing planktonic cells. Engineers have these funny vertical lines between their eyebrows. You explain to them how you count bacteria and these lines intensify - finally the guy looks disfigured. Microbiologists have been doing these incantations for a very long time.

What we wanted to do was be able to monitor the biofilm phase of how the problems were developing. We chose worse case locations. We obviously needed to be able to get a biofilm sample. We can't just take grab samples from planktonic populations for reasons I think we have laid out. You need a biofilm sample from the wall of the vessel that you are looking at and it need not be representative. It is often best if it is worst case so that you have the biggest population, the hardest to kill population. If you don't kill it completely of course it will re-grow. A location right after an elbow in the pipe turned out to be a good place to put a biofilm sampler. If you have a dead leg in your system, like a drain plug on the bottom that is recessed slightly, just put your samplers right along the edge of the dead leg and that will give you the highest sample, and the best sample. Don't put them in a completely quiet zone because biofilms in a quiet zone are so loose that they will fall off as you try and take the sampler out. There has to be some flow. If we were looking for particular bacteria, corrosion bacteria in the system, if you have flow operating like this it is just a matter of luck whether or not you happen to catch that organism. Whereas if you build up a biofilm and that corrosive organism is there eating holes in the pipe wall, and you have a biofilm sampler like this, you are going to get a realistic idea of what is happening. Then if you start to treat the system, and you kill off the planktonic bacteria, perhaps the grab sample will be negative but your biofilm sample will be positive because these cells are protected. If you get a complete kill having killed the floaters and all of the bacteria in the biofilm, then your biofilm sampler will be negative. You can take any water circuit and bring it in and run it through a bunch of Robin's devices and get your actual bacterial count. How would this work in a hospital environment? Well, you could take the condensate trays out of your air conditioning system and run water back and forth with a small pump. You could take the water from your showers if you are interested in Legionella in showers or whirlpools. Run it through a system exactly like this and this will tell you what your biofilm population is in any of these given areas. You want an engineer to set up your Reynolds Numbers and your sheer and pipe strength and get it approximately the same as the system you are looking at. This is actually bringing your system in and being able to sample the wall population any time. Do exactly the same thing to the purveyors of sterilants. If you want the system sterilized and you want it sterilized right down, the grab sample will be negative really quickly.

Only when some of the biofilm samplers come back clean do you work out your times and concentrations to clean your entire line right back to clean.

So how does it work? I think we have to get away from a whole bunch of the old microbiological methods. The best method for taking a biofilm off is a sterile scalpel blade, not a swab, because a swab tends to tangle up these pieces. Remember, this large biofilm section is giving us one colony. Smaller bits are giving us still one colony. Single planktonic cells are giving us one colony. So what will happen is that you start to break up biofilm, your counts will actually go up. You are releasing a whole bunch of planks. There is a strange thing about microbiology, it takes 24 hours, or it takes 48 hours to do your cultures, and in a management scheme that is just not good enough. I guess Walkerton showed us this. Having results the next day or two days later just doesn't cut it and these people in these industries want results right away. So what we developed here and developed in Calgary was a hydrogenase test. If bacteria are going to cause metal corrosion it has to have the enzyme hydrogenase. Rather than count the bacteria, we just checked the enzyme levels. If you have lots of hydrogenase present, this colour change happens in 20 minutes, if you have a little bit, it happens in 2 hours, and if it is very little it will change in about 4 hours. So your data is based on sessile samples, biofilm samples. Your data is immediate, or 20 minutes down the line. If you have a problem you know you have a problem - this is where I think the microbiology has to go. Now we can do any enzyme you like, you name it. I think the PCR (polymerase chain reaction) technique is becoming quantitative. PCR and some of the sophisticated detection techniques may start to replace these enzyme tests, but basically cultures don't cut it. You have to have an immediate test, if you are going to actually manage a system.

Chlorine Residuals In Water Pipes

Question – Is this a possible explanation for a very high colony count in a water system with a very high chlorine residual?

You could be working with a biofilm that is really resistant. The only water sterilant that doesn't pay any attention to what proteins are there is chlorine (or other oxidizers like hydrogen peroxide or Accelerated Hydrogen Peroxide – ed). What I think you have is a biofilm that you are not getting to the bottom of. Your residual is okay but you are not getting the chlorine in. Some of the sterilants are fairly protein specific, but I don't think chlorine cares what exact protein pattern you have in there. It is going to come down and digest the matrix and kill the cells anyway. Chlorine residual is pretty simple - you have water going along the pipe. Chlorine is being used up by the biofilms on the wall of the pipe, and you get a certain amount at the end, which you measure as chlorine residual. That doesn't mean that you penetrated down through the layers at all. This is why I like Lewandowski's chlorine probe because you can stick it in the wall of the pipe under the biofilm and say - yes the chlorine did get there, or, no the chlorine didn't get there. We use it when we do a soak. We shut off an area, really slug it with the chlorine and when Lewandowski’s probes light up and says, chlorine arrived, you’ve got there, and you are right down to the surface.

Summary Example

You have an instrument cleaner tank - pretty high organic load. You have water in there. Water is going by at a certain rate. You are going to get preferential biofilm formation on everything that is in that position for a long time like your instrument washer or fish tank. I don't think there is time in a colonoscope that has undergone the procedure to actually develop much of a biofilm itself. It is only being used for a matter of 20 or 30 minutes. That is not very long for a biofilm to develop. You are going to clean up mechanically, clean off those surfaces – being clean is as important as being sterile. Your sterilant is going to get down to the surfaces. What about the walls of the instrument cleaner tank? What about the case where you keep your contact lenses overnight? The second best biofilm I have ever seen is in a contact lens storage case. The lens is perfectly clean when you take it out of your eye and chuck it into the storage case, and I think that is why these continuous wear lenses so as well as they do – they don’t see storage cases. Stale water is going to develop on the surfaces. Your great two weapons that you have are mechanical cleaning and oxidizing biocides. After you have cleaned it, and there aren’t any bacteria left, and you have completely sterilized the system, in four hours you have a pretty good biofilm, and in 12 hours you have a really good biofilm. In four days you have a mature biofilm. If bacteria coming off were your problem, floating off the biofilm and contaminating something, then a planktonic killing dose is okay

because the biofilm is there, perfectly happy by itself. But if you took that scope and rubbed it past the biofilm wall, touched the wall, you are actually going to have a smear of living bacteria in their own slime and that is going to heavily contaminate whatever you are doing. Let's take a different issue in a hospital like an air conditioning system. Start blowing air past it really hard. Start to mobilize pieces of the biofilm off and get them suspended in the room air, aspirate them and you have a dangerous situation. You have to be realistic about it. My point is to look at both populations. We just traditionally look at the planktonic population. If we look at both populations, I think we will understand better.

I was with a company yesterday that has had contamination problems that were just driving them crazy because they would take a sample one minute and they would get a count of a certain amount and take a sample the next minute and it was sterile. They would even take the same sample and run it through two different filters from the same jug and one filter was heavily contaminated and the other had nothing on it. It was driving them around the bend. Explanation, you have biofilm fragments and in one case you went through one filter from the top of the bottle when the biofilm fragments were all settling out, and next time you stirred up the bottom and they all went to the other filter. The big thing is just inconsistency and in all water systems you have massive inconsistencies. You take a sample one minute, you have one count, and in another minute you have another count. What does the count mean in the first place? The count doesn't mean a whole bunch if a biofilm fragment with a million cells show up as a single colony, and it doesn't really represent the right numbers. We have needed to develop a whole new set of methods.

Whirlpool Tubs

Let's take a couple of samples of your day-by-day experiences. Let's take a whirlpool tub that is drained after every use. A domestic one, you fill it up and then you drain it out after every use. Where would you look for a biofilm(?) . . in the lead to the pumps. It is always wet, dark and sludgy. Put Robin's device samplers around that area or take a section of the pipe and scrape it - huge bacterial population. On the walls of the tub itself - you have a window with UV coming in, it is dry – there is not very much bacterial growth. The moment you start to circulate water past that biofilm in leads down to the pump, you have all kinds, sometimes visible, strings of biofilm coming up. What is the danger to human health in a whirlpool? Ingesting it might be a coliform problem, and biofilm on your skin might be a problem, but what is the real worry? The real worry is inhalation - aspiration of the droplets coming off the surface. Take an air sucker and draw it through a filter. We are not trying to see how many bacteria are there - many times they are all over the place. Then you run your sample through a PCR and see what biofilm species are there, and are there any known pulmonary pathogens that are actually bouncing in a haze over the whirlpool as you are using it? Yes there are, and they are quantitative. For a report we purchased and installed a whirlpool bath, and got a student volunteer, his wife and two kids to bath in it regularly. We characterized their skin microbiology, and then did the skin isolation from the bath. Then we did a pick-up of the haze from the top of the bath, to see if we had pulmonary manifestations, and in fact we did. If you want to design a whole bunch of cleaning regimens for the whirlpool bath, tell me how you are going to do that. Probably by using an oxidizing cleaning system - it depends on how the tub is made. Sometimes plastics will take it sometimes they won't, but an oxidizing system for sure. How do you know you got there? How do you know you killed the reservoir? Pull the studs and then chuck them in a respirometer. There's a respirometer that sees whether the bacteria are breathing – It looks at CO2 and it looks at oxygen. If they are dead there will be no gaseous exchange of any kind, so we don't get too fancy with it. Just up the concentration, and up the contact time until you are pretty clean - then reengineer the thing so that there are no dead legs. Shorten up the pipe as much as you can, and a lock out so that the first surge that comes through goes to waste. We have 3 litres going to waste each time

Your weapon is drying, and UV if you can - it doesn't cover the pipes. Shorten up the pipes as much as you can. You could mechanically clean the tub and just bottle brush the pipes, and then get rid of the material. Or do it in the presence of a fair amount of bleach or peroxide and then you would be in pretty good shape. I don't want to be an alarmist about this thing and I really don't like to help the lawyers do these things either, but I have a feeling that a whole bunch of pulmonary damage could be done by inhaling biofilm from whirlpool tubs because you don't get real sick on these pulmonary damages. You just fibrous off a piece of your lung and it shuts down. You could lose a fourth or fifth of your whole lung capacity and never even notice it. I think that in our modern world with air conditioners, we might all be losing a bit of lung capacity and possibly whirlpools are not good for just that reason.

There are showerheads that drain, and showerheads that hold the water and stay stagnant. We are looking for Legionella. Just where the shower comes out and makes the connection we put a little extra pipe in there and put a bunch of studs in. Someone said neoprene grew Legionella really well. We put rubberized studs in there and pulled those out to see how much bacterial growth we could find in a showerhead and it was monumental. So plumbing it so that the head drains and doesn't hold a whole bunch of water would be a good idea.

Caprocco Studs

There are two main classes of commercial testers - a company named Caprocco makes them. They make this Robins stud device which is the high-pressure device and quite expensive. There is also the modified Robins device, which is made of plastic - it is for low-pressure situations, it just has an o-ring around it. You just have to drill a hole in whatever vessel you are interested in and pressure fit in the Robins device.

Question - You see a lot of discussion about disinfectant residual activity. How do you feel that relates to biofilms?

There is a whole bunch of surface-active compounds that are going out onto the surface itself. We know that biofilm is a field of mushrooms, and that a whole bunch of the surface active agents actually get down there between the stems of the mushrooms and linger for a fairly long length of time right in the biofilm. We get a pretty good effect if we start with those kinds of compounds that are surfactants. We will often kill the bacteria in the stem of the mushroom, and then if you put pressure on them, the mushrooms will come off. As long as the mushrooms aren't going to do any damage down stream anywhere, you can actually clean the surface doing that. So yes, surface-active residuals have been very attractive in some of the applications we have seen.

Biofilm Traps Particulates

The easiest way to build biofilms is in streams, particularly in Ontario-type low country streams. There is a lot of clay in the water and biofilms trap clay. Take a boulder, or a stone, out of the stream and it will be slippery. That is 2 percent biofilm and 98 percent clay. Put a new rock in the stream, and then come back in a length of time and you will see that it has started to slime-over. Those are all of the kinds of biofilm we are talking about. If there are particulates in any water system, there will be particulates in the biofilm. A clay platelet cannot stick by itself on a steel surface. It will just keep going. If there are particulates in any water system that you are looking at, then you will find particulates on a steel surface. So if it is sticking then there are biofilms there. Sludge in the bottom of tanks gets visible, not because of the bacteria, not because of the slime, but because of what they trap.

Question - as a microbiologist we used to think when you grow a bacterium on a semisolid agar, that it also is growing on the surface, and that you probably found a biofilm. But Peter Gilbert said that, "no it is not, because nutrients are coming from underneath and that really doesn't qualify". Do you agree with that?

Absolutely, and we just proved it. I was looking for a way to prove it because everybody is saying, okay you are growing it on a metal surface and your water isn't the same if you are growing it on an agar plate. When you first inoculate an agar plate, there going to be a lot of water around. Then you've incubated it all day and in an incubator it goes pretty dry. They are in a dry phase seeing a water trapped phase with a lot of nutrient in below and they can take off and swim down into the nutrient. They can penetrate a certain distance in the agar and they don't turn on LC - that reporter, that light - they keep it turned off. They think they are planktonic on the surface. At some stage when the colony gets really big they might think they are kind of biofilm. It is not clean cut. Don't think of every colony on a surface as being a biofilm colony.

Quorum Sensing

I would like to walk you through the logic on this one because it was one of those slow realizations. An idea comes at you, not in the light bulb function at all, but just very slowly. A bunch of us in the Center just got to thinking about these water channels and saying how can you possibly maintain a water channel when you've got the bacteria of one species here, bacteria of a different species in the adjoining tower. How can you actually maintain that space? Doesn't this look kind of organized? Doesn't it look non

random? If bacteria were just growing, then they would be in an amorphous lump and they would have a certain amount of slime produced amongst them. So we began to think about cell-to-cell communication. This is the most exciting current area. We thought there had to be some kind of signaling or communication going on, and so we went back to a 15 year old discovery on quorum sensing. Bacteria growing intermittently in a fluid will produce signals. They are extruded from the cell, but they never build up any high concentrations and never have any effect. But when the bacteria get crowded in a solution, they get to be what Greenberg calls quorum. Then these signals are there in very high concentrations, they drift back through the membrane. They don't need a pump. They get onto the DNA and they turn on certain bacterial activities and that is quorum sensing. You have to have a quorum before you do something. Almost all of the toxins that we know about are turned on by quorum signals. So when the bacteria are getting started in an infection and make a beach head, look after the housekeeping, get to have enough of them to really have an effect, they don't make the toxin at all. Then they settle down and they have a certain density and start making the toxin.

Cystic Fibrosis

Can we in fact shut off toxin production by blocking the signal? In other words, if we put a counter for the signal in there and blocked it, could we have the bacteria growing, but just unable to make the toxin? In fact that is true and a product will come out in the next three years. It will actually be a blocker of a signal and the organism will grow but just can't make the toxin. Staphylococci aren't just a vaginal situation with menstruating people. There can be a lot of boils and you can get a toxic shock, TSST1 mediated reaction. The question right now is the blocker. Could you ever make them systemic? You could put them in somebody's blood and stop a reaction, and of course you could put them on a tampon? I think that will be coming up. It is a massive discovery if you think about it because we always thought that people have hormones and insects have pheromones. All of our cells communicate, but we didn't know that bacteria could communicate until quorum sensing came up. We thought that bacteria couldn't talk to each other; there was no organized behavior. Well there is. That means we can start putting blockers and manipulating molecules. Pete Greenberg and I are really close friends - we both have kids with Cystic Fibrosis, my son and his daughter - we go back and forth about once a week on the emails. We decided to take some of Pete's mutants in Pseudomonas. He has a mutant that makes this green signal, and a mutant that makes the red signal, and these mutants are "knock outs". Take the knockout mutants and see what kind of biofilm they made. So if we took a wild Pseudomonas, which we have - it settles, produces all kinds of slime fibers and makes a whole biofilm. Here we have knocked out the red signal, it makes no difference, the cells still settle, still make the polymer, still make the biofilm. If we knock out the green with or without knocking out the red, then the bacteria can't make the biofilm. This was a very intriguing thing for us, because we have been looking for biofilm control for a long time. We would try biocides and antibiotics that would knock out biofilms, and here we have a signal that just says, don't form biofilms. Here in this picture we have knocked out the green signal and rather than making these big towers, we just make this log jam - no slime at all. The cells are really close together, and with a little bit of surfactant it comes right off the surface. It never made a biofilm. They never turned on any of these genes, never got the 85 gene change going – it's just like a planktonic cell stuck on a surface. We keep doing this in different iron concentrations and different flow rates and it is a little bit iffy. Sometimes it happens and sometimes it doesn't. We are getting into complications with it - there are environmental factors that control it. This is the model we used to think about it - here is the strand, here is the protein that mediates the whole thing called an R protein. The signal comes in, triggers the trigger like this and then this protein starts to move along the DNA making all of the biofilm enzymes. Then the concentration signal goes down and of course the whole thing stops. What we figure to do is just make a blocker to fit into the slot like this, probably covalently bind so it won't come back out again, doesn't hit the trigger, blocks the whole thing, and the bacteria will be totally unable to make a biofilm.

Biosignal Blockers

The scenario looked like this – a planktonic cell comes down. Under normal circumstances it makes the signal like this. The signal interacts with the R protein, turns on slime production, and turns on biofilm formation. Here is your blocker in a higher concentration - it occupies the site and won't come out. The cell just stays in the planktonic state and since the blocker won't come out, it is never going to make a biofilm anytime during its life cycle. You're not killing it. You're just manipulating its behavior. You know whenever you try to kill something it fights back. That is why we get the resistant strains that we are

into, and all the troubles we currently have. This is not pie-in-the-sky. This is actually here.

Then you get into serendipity and real biologists. In Botany Bay in Sydney, within yards of Stefan's house grow huge beds of this algal species called Delicia Pulchra – "delicious and beautiful". It never develops biofilms on the leaves or fronds. This particular algal species just never finds bacterial biofilms. It's great because you don't trap the clay, you don't stop the photosynthesis, and these critters do really well. Twelve years ago they made the observation that Delicia never develops a biofilm and they found these molecules, called pheromone. You are going to make the automatic connection between that lactone ring, that lactone ring, this asyl chain and this asyl chain. They lack the amino group and they always have a bromine on them like this. This compound is fantastic at stopping biofilm formation. There are 42 varieties - some natural and some artificial. This is a plastic incorporating the C4 pheromone. It is situated near a sewage outfall in Sydney Harbour, and it has a few macrophytes growing on it, but virtually no bacterial biofilm at all growing on the surface. What could we do with a thing like this? You could put it on boat bottoms to start off with. In Australia they do a lot of aquaculture with nets for shrimp and fish and the critters often suffocate because so much biofilm develops on the nets. They now put this into the nets for aquaculture and they don't get any biofilms on the surface. They are starting to put them into contact lens holders. Of course a lot of this is proprietary as you can imagine, these guys have resigned their university tenure positions, they are buying lunch for everybody they know. There is a company called CNBB Biosignal and you might want to buy stock in it. The kicker is that when we put in a pheromone, it blocks the activity of the natural signaling molecules - sets them right off. In other words, nature discovered this blocker long before any chemist synthesized it. Pheromone happens to be a natural blocker of the Homoserine Lactone signals, a wonderful system. This has been happening for millions of years - this algal species has been holding off biofilms, using the pheromones, and resistance cells never developed in that millions of years. It can't have or these things would be covered. That is a beautiful place to grow, on an algal frond. Resistant bacteria would have definitely colonized all of these fronds and there aren't any. The logic is you are not actually killing the bacteria, just persuading them to go somewhere else and do something else. Go and make a biofilm somewhere else, which is a beautiful manipulation. I think we are coming into a new era that is going to be incredibly exciting in bacterial control. We are going to be able to say to Staph. aureus on a tampon, this is an attractive environment, you live as much as you like here, but don't make the toxin.

Periodontitis Biofilm Blockers

I'm intrigued because in dental areas, you have your tooth and your gum, and normal plaque that doesn't do you much damage. When you have periodontitis, a bacteria called Fusarium nucleatum comes in and joins the biofilm, starts to deepen the plaque around your teeth, and you go from 3mm down to 7mm and you are in worlds of trouble. This is not clear-cut, but they think that this particular organism starts the whole process. We are dissecting it to see what its signals are and it has about 14 or 15 signals. It has the richest signal system I have ever seen. Find a blocker for the biofilm-joining signal, and have that organism just live loose in the mouth rather than living in the biofilm and I think you would have the problem solved.

Pheromones in Human Disease Treatment

Right now, our relationship with bacteria is we lob hand grenades at them and they lob hand grenades back at us and we really have a pretty crude, antagonistic relationship. I think we are right on the edge of being able to manipulate these situations. Now what has got Pete and I fantastically excited is a guy in Denmark who decided he would actually just take a chance in an animal with cystic fibrosis - it is a real biofilm disease. He put a pheromone into a bunch of rats that have the animal CF model, and all that he will say right now is that the whole pattern of the disease changes. It becomes very acute. I think that he is probably breaking up the biofilms, and can then treat it then with an antibiotic, or probably treat it with vaccines. Antibodies are going to start to work if you stop the biofilm formation and it is a long, long way from putting it into people. It is going to have all kinds of effects. One of the effects might be that it hits some kind of a signal in your gut that makes all of the particular species of bacteria leave the surface of your intestines. That could be really bad. Or it could stop biofilm formations where you wanted biofilms. It is a complicated signal system. It has only actually been going for two years at this point and these signals have been found accidentally. Of course, the pheromones were found a long time before, but now we are thinking of bacteria in communities. In communities there is embryology, there is a developmental sequence, there is whole bunch of signals happening. As we start to play with these signals we are going to

get some particularly exciting things happen. There is a signal that controls biofilm formation - I wasn't expecting that. I was just expecting a signal that maybe held the water channels open or another one that made the towers go in a particular shape. But in fact there is a master signal for all of the biofilms.

A company that was making puffers with a preventive that was previously made sterile. It is generic and they thought they would save money by not making it sterile - an inhalant does not have to be sterile by FDA standards. So they made it, and held it in a 1000 litre vat, and didn't package it until the chemistry data came back okay. It was about a 24-hour hold in a stainless steel tank. They went from sterile to just having a few bacteria around. But a biofilm developed on the walls of the holding tank and about every tenth unit of the puffer had biofilm fragments in it. Of course in a puffer you are actually driving it right down into your lungs. There were 150 deaths because of it and a huge legal case that it is called the Cockly Pharmaceutical Matter.

Butyryl Homoserine Lactone

We got even more excited about the signals because there is a signal that happens in biofilms quite nicely if you leave the biofilm with no flow for about four days when it has previously had good flow. The red signal comes into its own – Butyryl Homoserine Lactone**.** It starts the bacteria releasing an enzyme like these little packman things here that digest away the matrix, and you get a natural detachment event. We could do this with Pseudomonas all of the time by stopping flow for about four days - watch under the scope and you begin to see all kinds of strange things happening. The first thing that you see happening is in the head of a mushroom - all of the bacteria will start to seethe because Pseudomonas has a flagella on it and it swims. So you have a mushroom that is in tact and a whole bunch of cells swimming around in the middle. They have digested away the matrix and they become planktonic. They switched off Alg C. We can tell that they are planktonic in genetic form as well. This liquefaction of the head of the mushroom gradually spreads until they breach the mushroom somewhere, it collapses and they all swim away. The collapsed bit also kind of liquefies and all of the bacteria can then swim away. So there is detachment in biofilms. I think it is on purpose so that you can go and colonize new areas. This is something we noticed over a considerable length of time. We took a mutant that didn't have the red signal in it and it was unable to do this detachment. It looked like it was the red signals that were responsible for it. Under very carefully arranged conditions, we can actually get a bacterial biofilm to get up and go. If you took this signal**,** Butyryl Homoserine Lactone**,** for the gram negatives, put in a blocker - in fact a locked molecule, wouldn't really be a blocker - covalently linked it, did hit the trigger (sort of reinforced this activity), then of course you could lock it permanently in the on situation. So a bug would always be predetermined to get off, even before it got on. If you already had a biofilm and normal detachment, this molecule would cause a faster detachment. I think that if we put it into a plastic coating on a medical device, the bugs would be in a get off mode as soon as they got on. I think it could be a very interesting coating for medical devices.

Get-Off Signals in Heart Valves

The case that we are into right now is mechanical heart valves. The heart valve is a space age device. The tissue isn't very smooth, so they put a sewing cuff around it so the surgeon can actually place it. Four point five percent of them get infected. You then have bacteria, Staphylococcus epidermidis growing on them. We have identified a get-off molecule in Staph. epi. It would produce an analog (which we don’t have yet), the analog would accelerate the get-off procedure, and you would in fact be taking bacteria off. I can see medical applications for this because you’ve usually only got one species and these get off molecules are very specific. The problem is that you can't take a bunch of bacteria that are somewhere in a biofilm like around the heart valve and suddenly let them all off, because you are going to get bacteremia and it is going to kill people. So you want a slow acting get-off molecule. I would rather never let the biofilm develop. I would rather put it on at the very beginning so that you just never got the biofilm in the first place.

Let's bring up another tragic tale that happens in these areas by getting the microbiology wrong. A company called St. Jude Medical decided that they would buy silver coating from a company called Spire. The silver coating was held to be antibacterial. They put the silver coating on the old heart valves, put them into a culture of Staphylococcus epidermidis, brought them out, rinsed them, scraped them, and then did counts. They swirled them around after they scraped them, and took the fluid and spread it out on a plate. The silver coated one grew many fewer colonies than the old one by about 10 to 1. The FDA approved it and they sold 30 thousand and installed them. Nobody ever looked under a microscope to see

how the colonization of the "antibacterial valve" was. We were working for their rivals, because they thought they wanted an antibacterial valve too. We thought we would look at the gold standard and to our horror and dismay the thing was covered in Staph. epi. biofilms, absolute gardens of Staph. epi. biofilms - at least a 100 times as much as the old valve. The microbiology works out wrong because when you scrape out a crispy, metal covered surface, it has crevices in it and it doesn't scrape really well. What was coming off was a beautiful biofilm, so you get millions of clusters and they are going to give you just one colony per cluster. They actually produced a pro-bacterial mechanical heart valve. The cases are going to start hitting the courts in about the next 2 or 3 months. The infection rate went from 4.5 percent to 11 percent. If you had a valve like that and it is pro-bacterial, you might get it secondarily infected. Are you going to go in for extra surgery and get the old valve put back in? Are you going to take a chance with a new valve? There is a whole bunch of personal tragedy - I think about a thousand deaths, because if you work out 4.5 to 11 percent on 30,000 people, it is a very large number of people. They have now withdrawn this heart valve from the market, gone back to the old valve, and they are back to 4.5 percent infections. So this business of the microbiology is really important. If you do counts and scrapings and don't look at the thing on the microscope, even when the microscope is available, you are back in an old set of microbiologic reflexes. We have to get rid of those reflexes for sure. I think that you can probably look at any system that you guys deal with, and you can get trapped if you are not looking at the biofilm population. If you are just looking at planktonic population with the old microbiological methods, you can make some fairly serious mistakes.

Sterile Water Systems

Totally sterile water systems are going to keep you out of little contaminations – one bacteria at a time. I love them because they are not going to let you have a stealth situation where you had a biofilm that you didn't know you had. Now the system you are thinking about is a water system?

Audience member: It is actually a pressure-measuring device that measures upper or lower intestines and anal rectal pressure - always a wet system.

You are not going into sterile systems in either case are you? NO. And in any case is it a sterile system to start with or just a system you are pouring sterile water into? I think you might be very fastidious in a dirty environment.

Audience member: I have been catching periodic isolates of Pseudomonas and I have been thinking that biofilms are already in the system now.

You may very well have them. It might be better to have it totally sterile. I don't mean to be fanatic about it because I think you guys are thinking in the right direction. There is not much point in cleaning something up absolutely completely and then sticking it into somebody's colon. I don't think it is particularly sterile up there. So I think you have to be reasonable about this. What is happening with the vessel surface is much more important than what is happening in the water phase in the first place. So obviously there are some places where these things make sense.

Phage-proof

There are viruses that live on bacteria - bacteria phage. They kill bacteria very effectively. Each one bursts after it kills, and 30 new phages go and find 30 new bacteria. The world should really have a lot of phages and no bacteria. Biofilms are totally phage proof - one hundred percent phage proof. That is probably why bacteria decided in the early stages, if a virus is coming after you and you are out there swimming, you are vulnerable. If you stay in the biofilm in your home community you are not vulnerable. Phages are probably one of the major reasons that biofilm is so predominant in nature. This is their defensive mechanism - a protected mode where bacteria really like to live is the biofilm mode. They are taking a chance when they become planktonic. The benefit to the planktonic form is that you can go and colonize a new environment, and reproduce and do all those good things. But the safest place is right in the biofilm.

Here is the sequence. When a biofilm is formed, it can do a couple of different detachment mechanisms. One is, just have a piece let go - it happens all the time. A Schwan-Ganz catheter going through the heart into the pulmonary artery, sitting there for too long a length of time may have a biofilm on it. Any pieces

that come off the Schwan-Ganz go immediately to the capillary beds of the lung. You begin to develop biofilms on the heart valves that you are traversing as well. A Schwan-Ganz catheter shouldn't stay in for a really long length of time, but sometimes they do. Basically this type of detachment can give you stroke and pneumonia and so on, by getting involved in the lung. Secondly, all biofilms at all times are returning to the planktonic phenotype. When they do this, of course they will swim away and all biofilms are shedding at all times. We can make them shed spontaneously and detach if we use the right signal.

There are two areas of biofilms that are signal dependent. One of them is from the bacteria on the surface, aggregating and making little stacks that start producing slime to make the micro colonies. That is a new signal control. Also, when you have a biofilm already formed, you get planktonic bacteria reverting spontaneously, and that is under signal control. Those are the two areas that I think we will eventually come to for biofilm manipulation.

Question – Regarding the expression between planktonic and biofilm, bacteria and exotoxin production - is that changed as well or has much work been done on that?

NO, exotoxins are really loose. Exotoxin comes off bacteria even in a biofilm without them coming off, it washes off. So if you have biofilm with a gram-negative organism, it is shedding exotoxins continuously and then sits there and sheds endotoxins as well.

Question - what about extreme heat and extreme cold - what is the effect?

Cold has no effect in biofilms as far as we know. We can take a biofilm and sterilize it in an autoclave, at normal pressures; all of the bacteria end up dead. In cold situations, there is a fairly major effect on ice crystal formation. These organisms that they let loose in the citrus groves to stop ice crystal nucleation do it by producing exopolysaccharides – and then exopolysaccharides freeze at a different rate. If you took a bunch of planktonic bacteria in just water and froze them, most of them would die. However, if you take them in biofilm and freeze it down then most of them will not die because ice crystal formation is quite a bit muted in the polysaccharide.

Biofilms in Well Water Disinfection

Question - Presently when we talk about disinfecting a private well, we say Javex or chlorine will disinfect it. What I have heard today is, yes you may kill the E. coli that is not in a biofilm, but you may have created a whole new problem with the biofilms being released or shedding.

If I was doing this and you can't get at the system right at the bottom of the well, then what your symptom is going to be is that you get negative grab samples for a while then they become positive again really fast. I wouldn't pay any attention to the level that gave you a negative grab sample - that just teased the biofilm a tiny bit. What I would do is leave a long dwell time. Don’t bump up your concentrations too much higher, you may be using about .5 percent concentration - works on my oil wells. Leave a four-day contact time. Buy water from somewhere else for four days. Then when you put it on-line, just waste it for a considerable length of time. Take a filter, any ordinary filter, or even just let the water settle in a bottle a little bit and see if what ends up on the bottom. At first you are going to find most of your biofilm out there pickled and dead in the bottom of the bottle. When you don't have any more slimy, ropey business and biofilm fragments coming out, then put it on line and go. Then just watch your performance on grab samples and see if it comes back again. Just don't pay any attention to a negative grab sample because it is going to make you happy when you shouldn't be happy.

Remember that your bleach going down at 0.5 percent is going to be strong for a little while but the more biofilm it eats the weaker it gets. So then a second soak at the same concentration might be appropriate, and then a third soak at the same concentration. I don't know how to do it, but my oil engineers do it all the time. They tell me how strong my bleach is when it comes back up. I guess they just do wet chemistry for the bleach. You put the bleach down and it comes back up dead - there is no bleach left - there was a lot of biofilm down there. Do it a second time, do it a third or fourth time until your bleach is coming back at you without being used up. That means that there wasn't biofilm down there any longer.

Question - So that is the same type of principle that we should be using in the waterline.

Same principle. Don't go really high in your bleach concentration; go long on your time if you can. Then you know that you are not going to kick your system apart. Even in a filthy looking oil well, I don't go above 0.5 percent bleach.

Concluding Story

I was sitting, minding my own business in Calgary and I got an e-mail from Abu Dhabi. The oil company has two low points in a twin 60" oil pipeline. This line ships out 1.8 million dollars a day worth of oil from the Zadco field. They had two low spots in the line and they put in a smart pig. As well as cleaning the pipe, the smart pig broadcasts back wall thickness, and cost a couple of million dollars each. The company jammed a pig, lost a pig in one of the low spots, and they were in there with a magnet trying to get the pig back out. It was broadcasting 3/5ths wall thickness, so with the pitting, that pipe was just hanging there. That pipe was almost ready to go. This was near the entrance to the Arabian Gulf and a spill in there would have been absolutely murderous. So we left that pig in where it was and built a bypass, and we put it on a bridge so it didn't have a low point on it. The other line was in equally bad shape so we just put gluteraldehyde in there so concentrated that it was practically pure glutaraldehyde. These were all French people, highly trained engineers and really sophisticated people. We built another patch on the other line, shut the whole system down for two days and had to pressure test it to make sure that the wells were okay. What did they pressure test it with? Raw seawater. I had 42 miles of twin 60" pipelines and somebody had just put in raw seawater, not oil and seawater, just raw seawater. They had inoculated the whole system all over again. So we took about 22 million dollars worth of Gluteraldehyde, borrowed it from Saudi Arabia, trucked it over, sealed both ends of the pipe and just held it for a length of time. I don't know what concentration of Glut. we had, but we sterilized the whole thing. Afterward, we went back and had a meeting. These guys had just cost the company 22 million dollars . . . it was a quiet little meeting.

Find out more about Biofilms,
or contact the Centre for Biofilm Engineering at:
http://www.erc.montana.edu

The seminar was organized and sponsored by
Maunco Sanitation and Safety Supplies, Belleville, ON
For more information about Maunco seminars, contact Paul Webber
800-363-5376
paul@maunco.com

Also sponsoring the seminar was Virox Technologies Inc.
800-387-7578
roger@virox.com