BIOFILM

THE NOW

"An Ounce of Prevention is Worth a Pound of Cure"

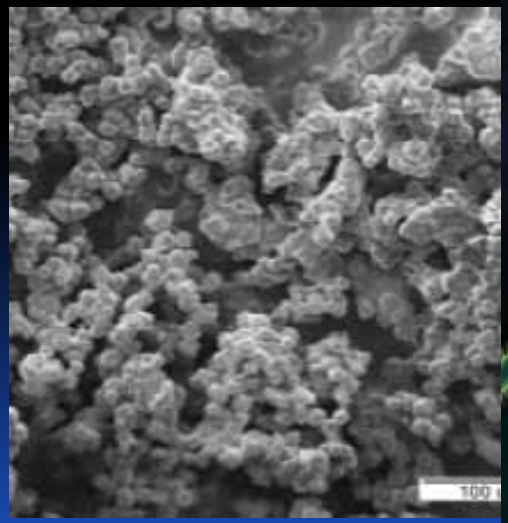
The Future

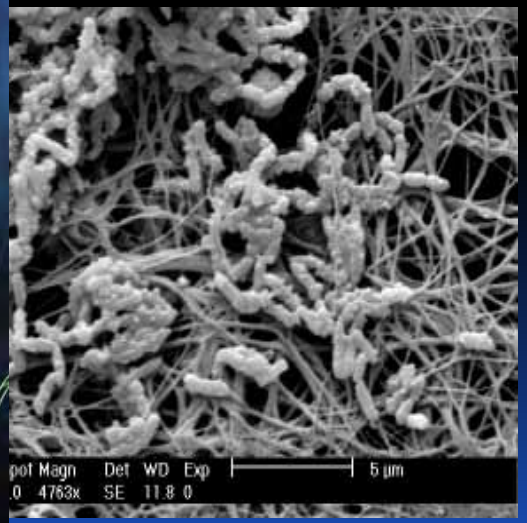
NANT 29th Annual Symposium March 7, 2012,Las Vegas, NV

Jo-Ann B. Maltais, Ph.D.
Maltais Consulting

What on Earth is a Biofilm?

- Survival mechanism
- Community of bacteria
- Symbiotic relationships
- Slimy Matrix





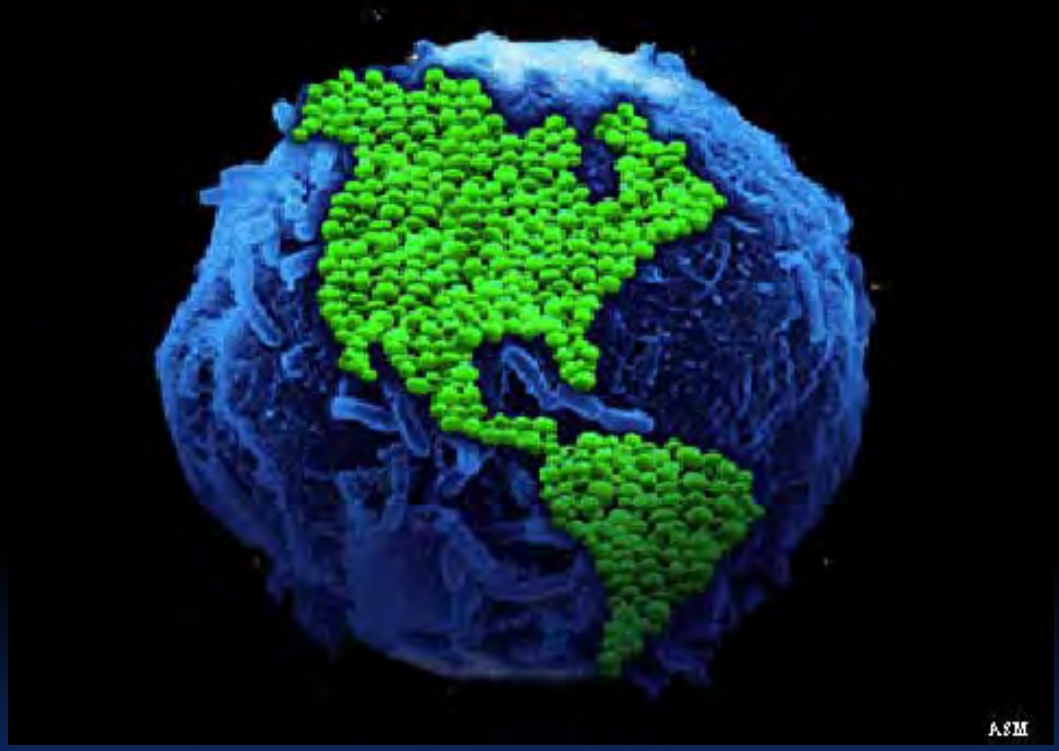
Ryder, M. Medical Biofilm Research
TargetBSI.com Webinar 7/28/09

Donlan, RM. Biofilm Laboratory. CDC

Where Biofilm Can Be Found?

Everywhere!

Why?



Bacteria Are Everywhere!

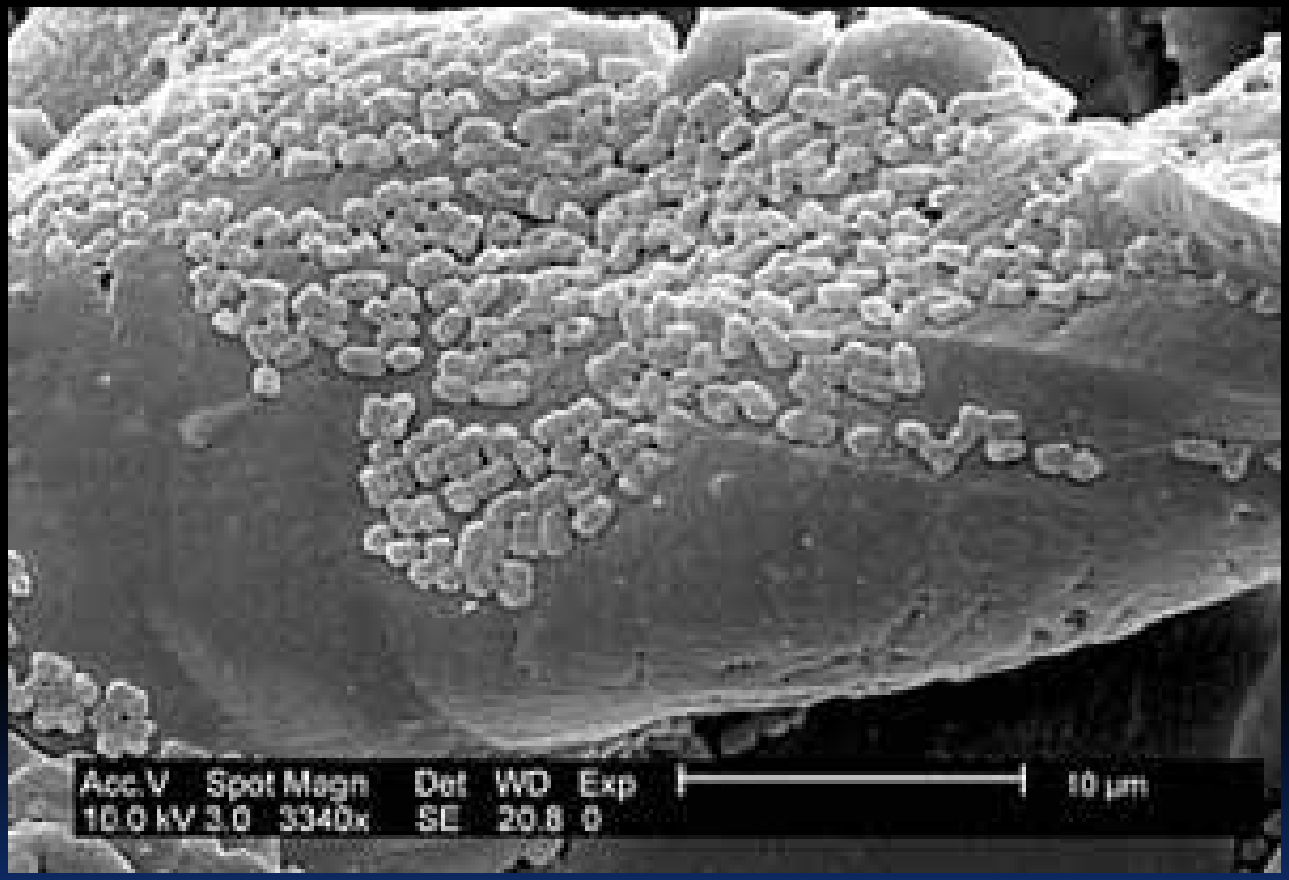
Bacteria Have Been Here a Lot Longer Than We Have

- Bacteria were here 3.6 million years ago
- Man came 100,000 years ago
- Bacteria first discovered in 1670's by Ludwig van Leeuvenhoek
- Biofilm first described by Costerton (1978)
- >60% of human infections estimated to be caused by biofilms

Benefits of Living in a Biofilm

- Built to suit the specific environment
- Food co-op
- Modern plumbing
- Security System
  - Reduced effects of UV and disinfectants

Where Biofilms Are Found

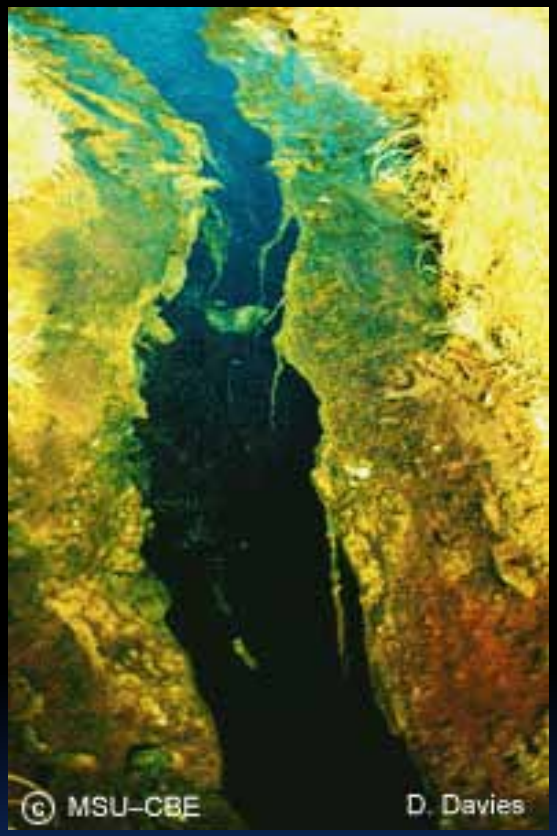


A biofilm on a piece of lettuce

http://bacteriality.com/2008/05/26/biofilm/

Permissions

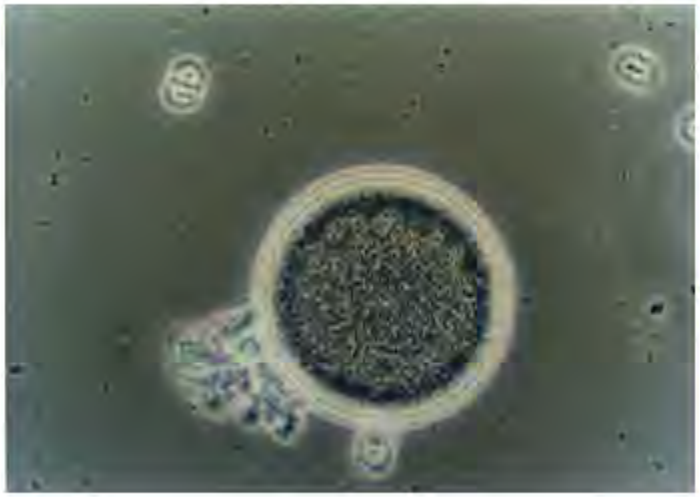
Figure 4. Microscopic View of a Shower Curtain



Biofilm in acidic pools at Yellowstone National Park

http://bacteriality.com/2008/05/26/biofilm/

Oil Drops Suspended in Water



Permissions

Figure 2. Bacteria growing on and near an oil droplet suspended in water

It is fortunate that many microorganisms are capable of metabolizing hydrocarbons. Due to natural and human caused contamination large amounts of hydrocarbons are annually contaminating soil, fresh water and marine environments. Many genera of bacteria including *Pseudomonas*, *Alcaligenes*, and *Flavobacterium* are capable of mineralizing oil and other HCs to carbon dioxide and water. This image shows a population of bacteria actively degrading a droplet of oil suspended in water.

Did You Know?

- Biofilm can develop & survive on the surfaces of a jet plane
- Biofilm forms faster on plastic pipes than on metal pipes

Biofilms—Economic Impact

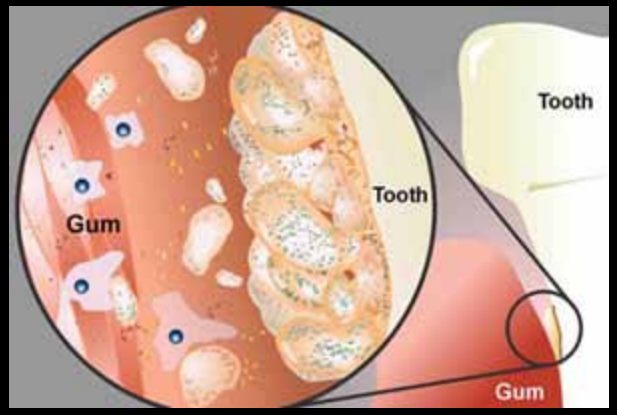
Biofilm & Barnacles on Navy Ships

- Reduces Vessel Speed by 10%
- Increases fuel consumption by 40%
- Cost for removal
  - Time in dry dock
  - Labor
  - Hull damage

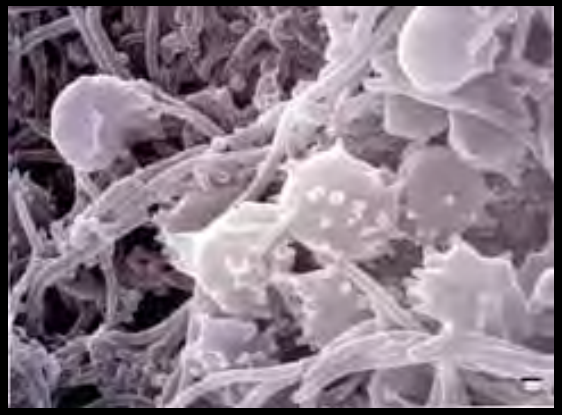
Biofilms

AND

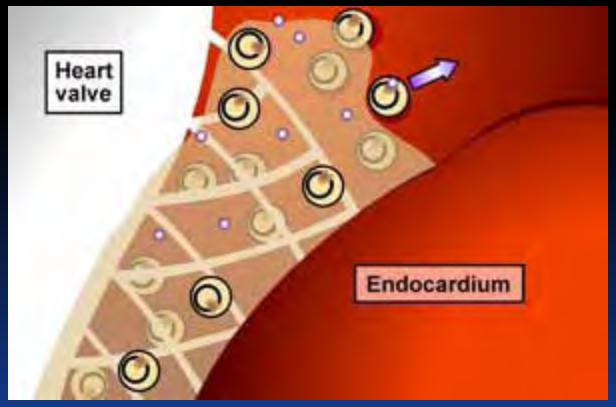
Patient Safety



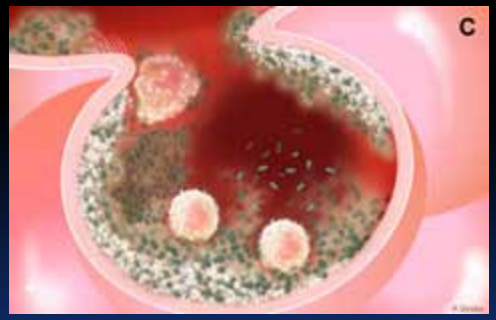
"Hundreds of microbial biofilm colonize the human mouth, causing tooth decay and gum disease"



"Dental plaque as seen under a scanning electron microscope"



"Cells of Staphylococcus epidermidis *causing devastating disease as they grow on the cuff at a mechanical heart valve"*

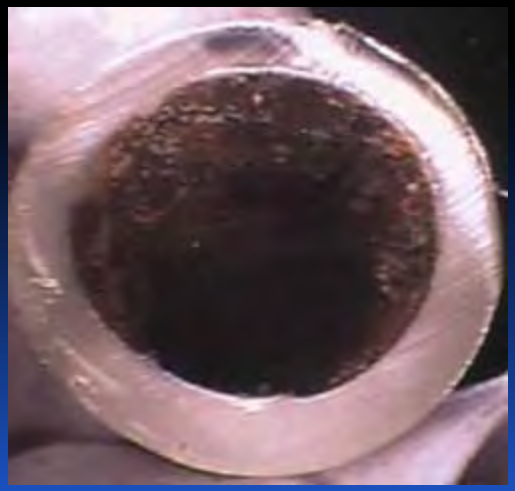


"When the immune response is compromised, Pseudomonas aeruginosa *biofilms are able to colonize the alveoli, and to form biofilms"*

http://bacteriality.com/2008/05/26/biofilm/

Biofilms in Dentistry

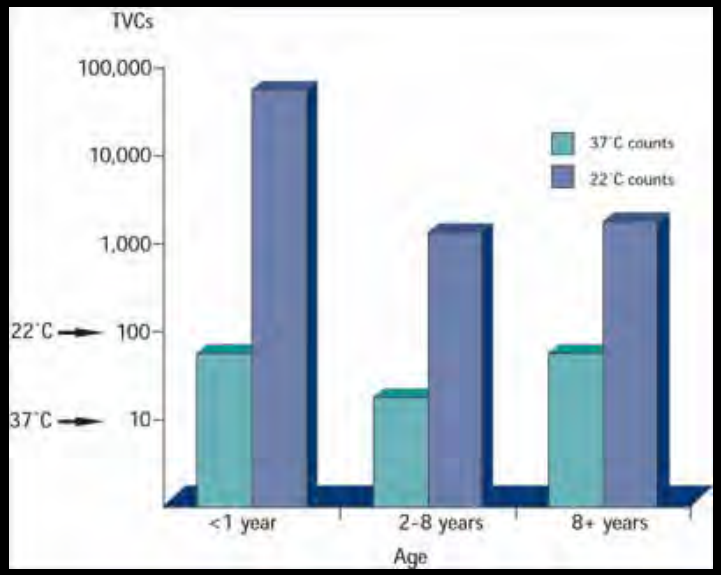
Dental waterline

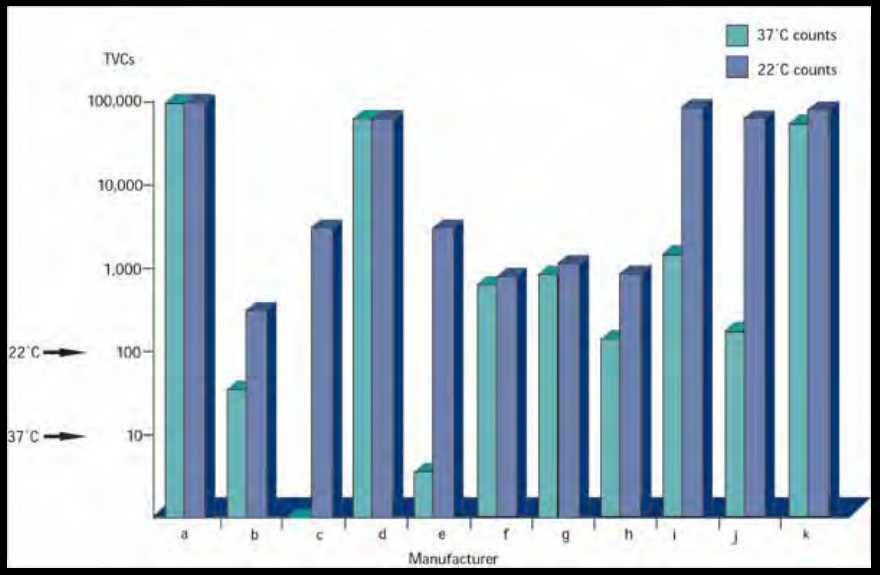


Biofilm in Dental office tubing after 3 weeks

Dental Vaccum System

Biofilms in Dentistry





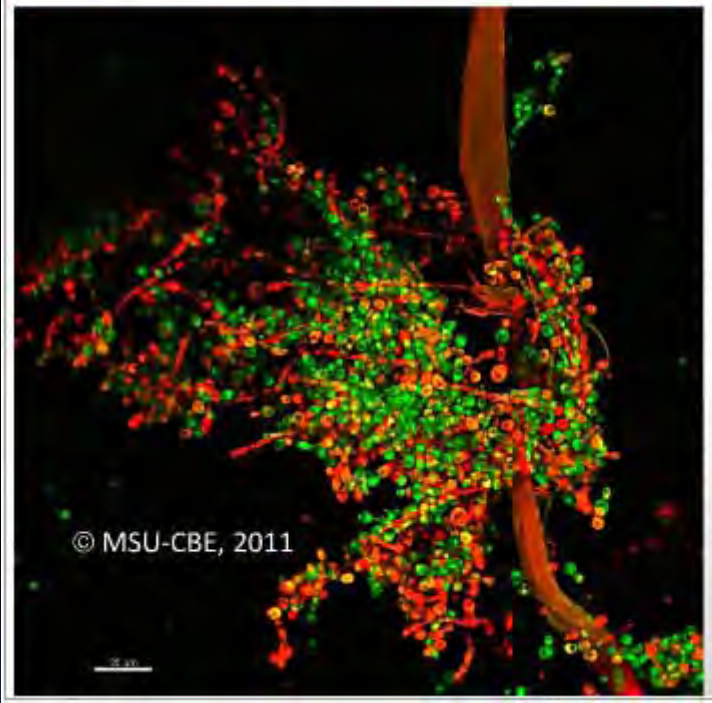
High Speed Drill Waterlines

Table 1. Total counts of bacteria (cfu/ml) in water samples from the 40 dental units of Barretos, São Paulo, Brazil, counted according to ADA recommendations, between January and July 2005.

Site of collection	Number in samples	
	0-200cfu/ml	201-2000cfu/ml
Tap water	25	15
Reservoir	17	23
Air/water syringe	12	28
High-speed drill	8	32

1 million bacteria per ml
Equals
5 million bacteria per teaspoon

Confocal Scanning Laser Microscopy **MSU Center for Biofilm Engineering**



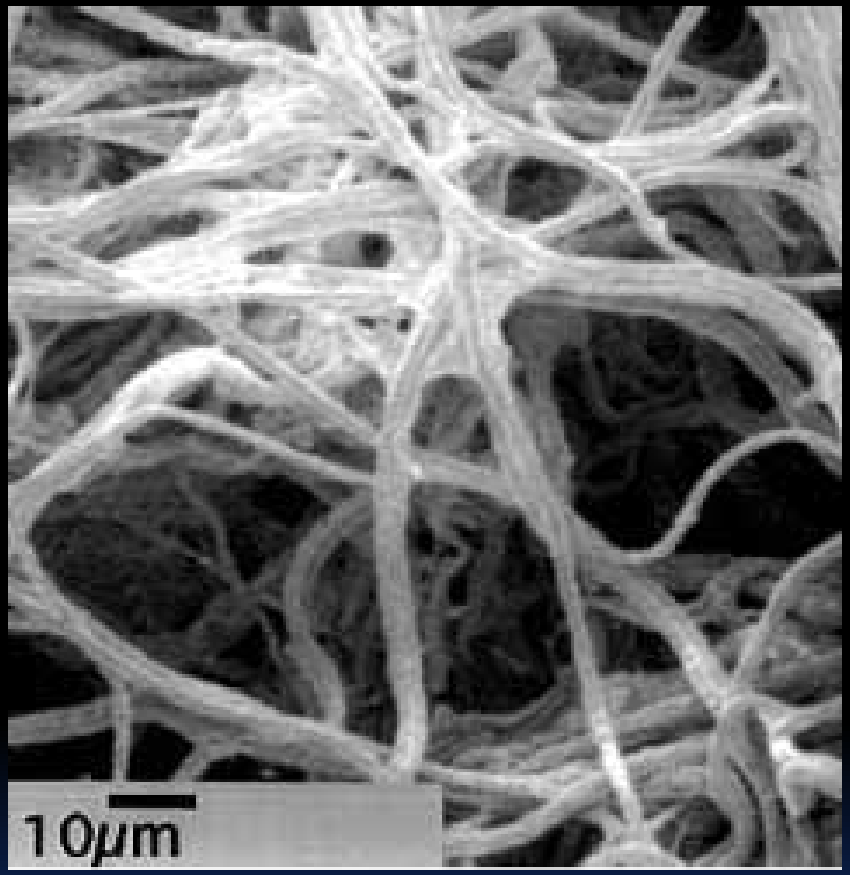
Alessandra Agostinho, DDS, CBE research scientist

"This *Candida albicans* biofilm grown on a PMMA coupon is stained to show live cells as green and non-viable cells as red. PMMA—poly (methyl methacrylate)—is used in dental fillings, denture material, and bone cement."

Confocal Scanning Laser Microscopy **MSU Center for Biofilm Engineering**

Betsey Pitts,
CBE microscope facilities manager

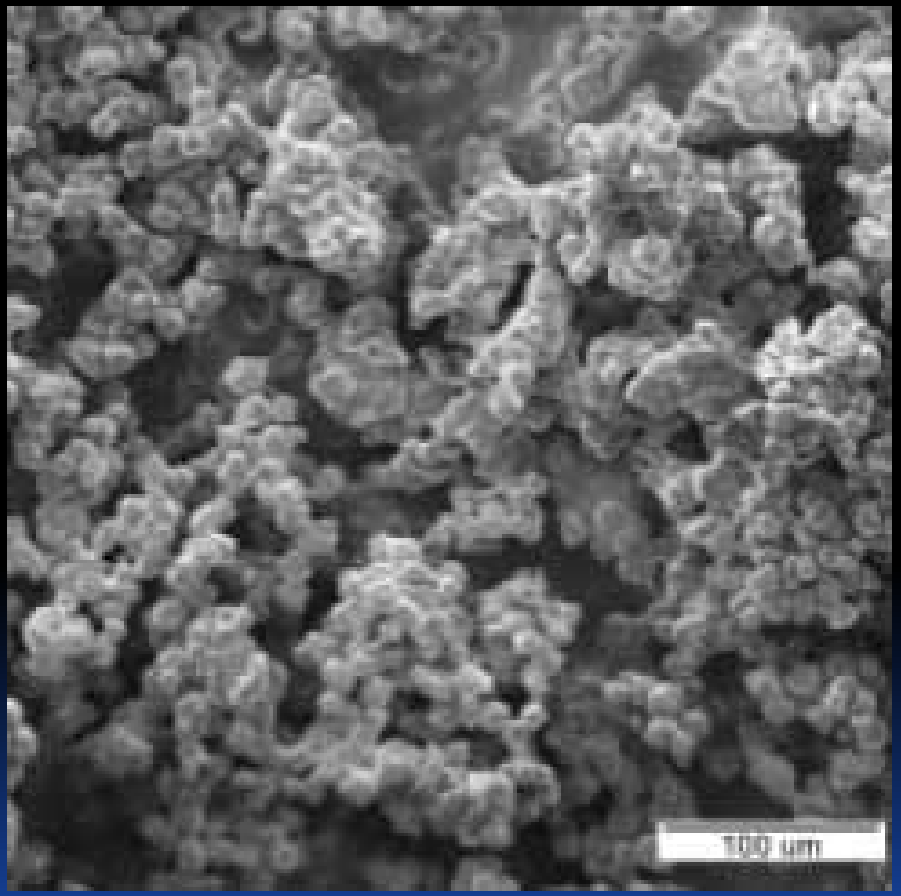
"Part of my job as facilities manager involves finding new fluorescent stains to use on biofilms. Stains that give information about the physiological state of bacteria in a biofilm are especially valuable. This one is an activity stain (Calcein AM Violet) which indicates that all the *Staphylococcus epidermidis* bacteria visible in this biofilm have intact membranes and are likely alive."



After antibiotics are applied to a biofilm, a number of cells called "persisters" are left behind

http://bacteriality.com/2008/05/26/biofilm/

Biofilms in Water & on Medical Devices



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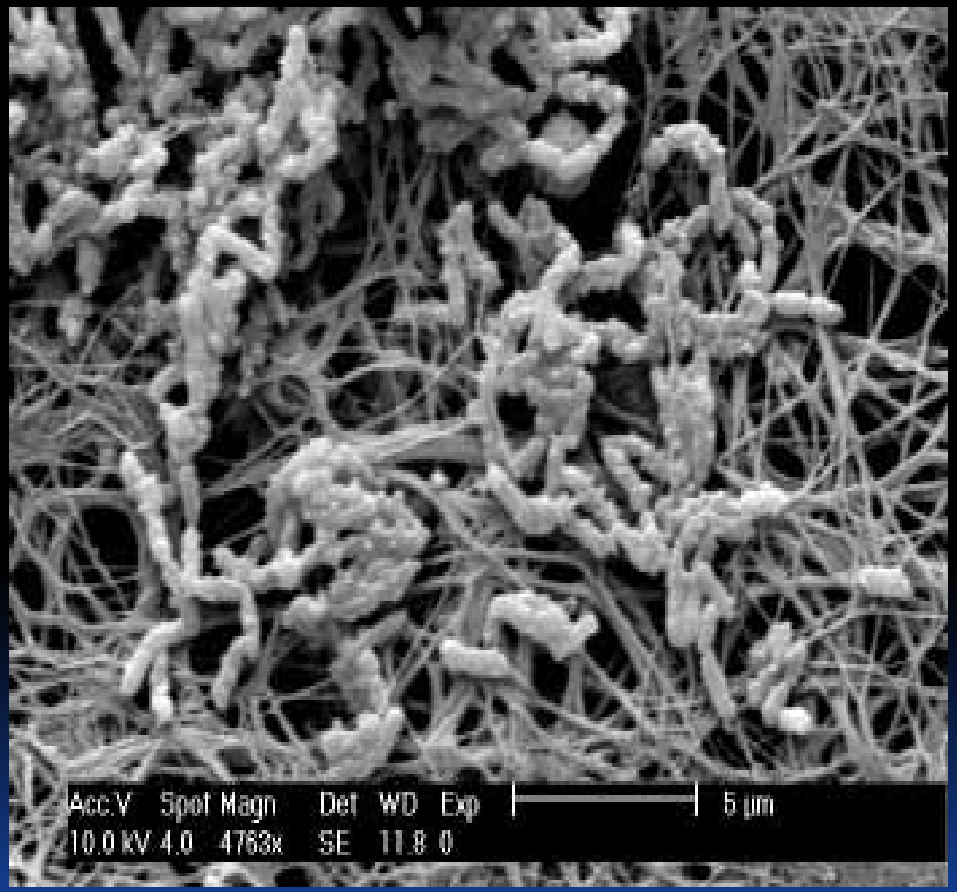


Donlan, RM. Biofilm Laboratory. CDC

How Does Biofilm Form?

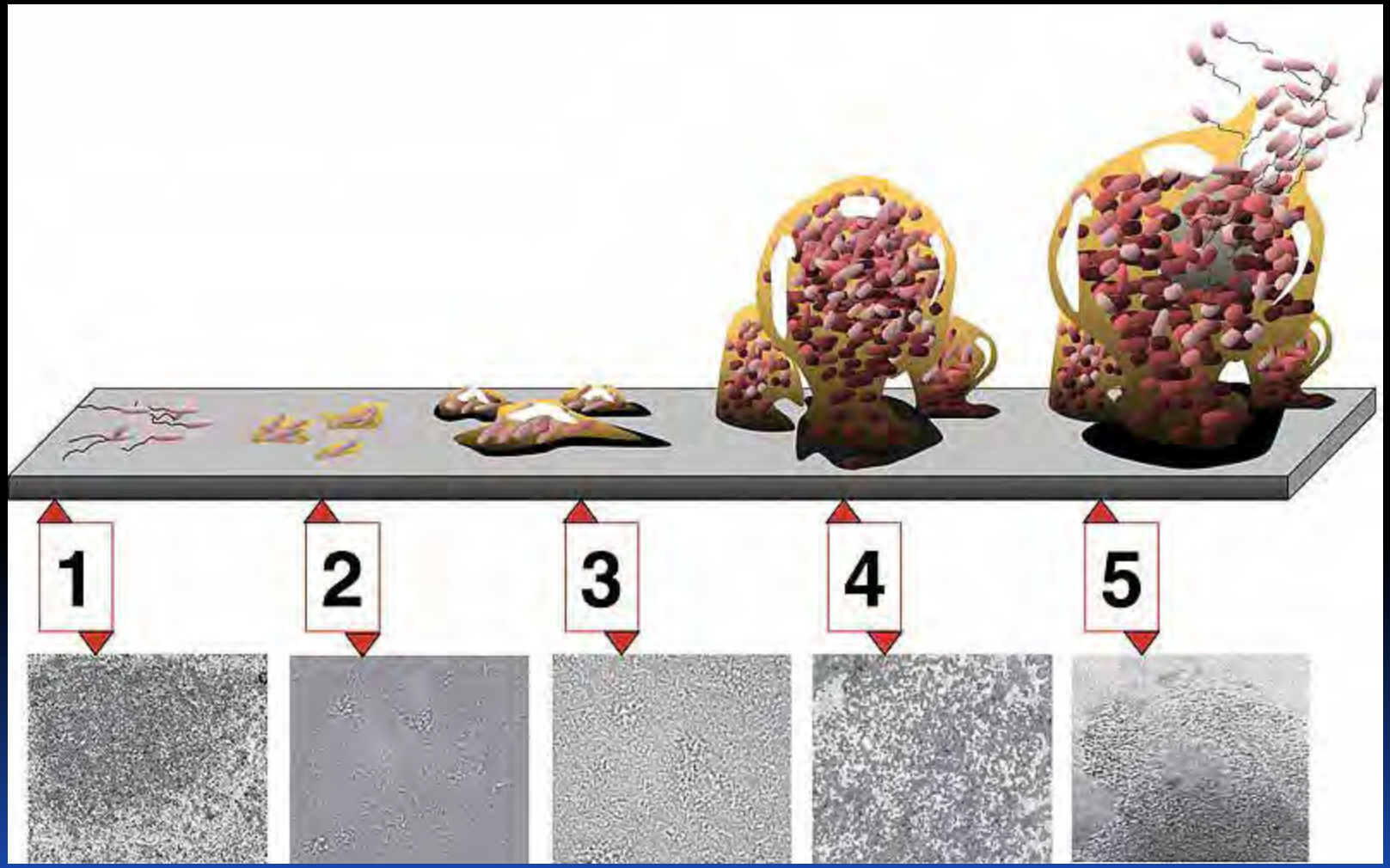
Water

The Primary Source of Bacteria

Bacteria

Are the Primary Source of Biofilm Formation

Stages of Biofilm Development



http://bacteriality.com/2008/05/26/biofilm/

How Biofilm Happens

- A solid surface is submerged or exposed to a fluid such as water
- Free-floating, planktonic bacteria adhere to the surface to begin biofilm development
  - Only certain species can attach on their own
  - Weak, reversible adherence
  - More permanent adherence if not immediately flushed off
- A slimy matrix is excreted to protect residents
- Other bacteria adhere to initial colonists or to the matrix
- Growth of bacteria in the biofilm & recruitment of more residents occurs

Factors Affecting Biofilm Formation

- Environment
  - pH
  - Temperature
  - Presence of nutrients
- Microbial Interactions
- System materials of construction
  - Surface properties
  - Corrosion
- System hydraulics
  - Flow rates
  - Dead legs

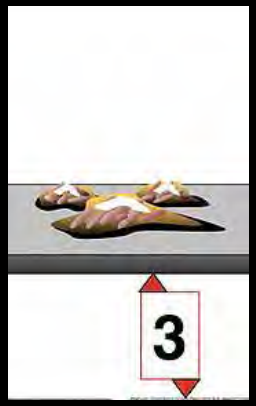
Stage 1: Attachment to Surfaces

- Low flow, laminar areas of surfaces
- Surface conditioning
  - Dead cells
  - Protein
- Bacteria touching hard surface
  - Fimbriae, pili, flagella, adhesion proteins
- Biofilm residents sends out signal molecules to attract other bacteria to join them
- Reversible process at this stage

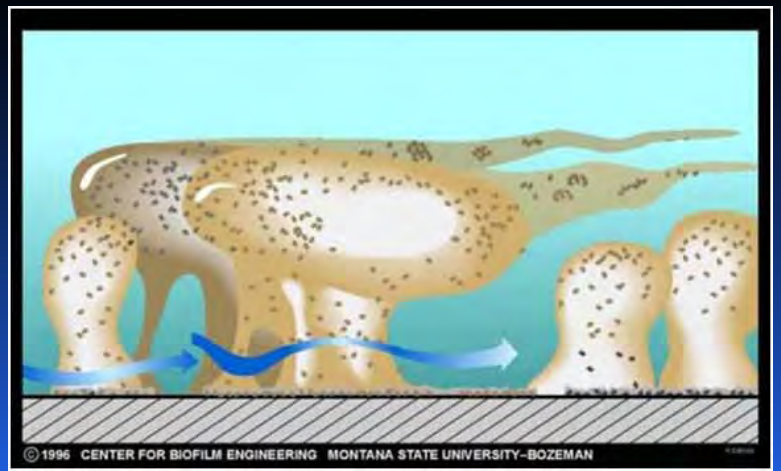
Stage 2: Irreversible Adherence

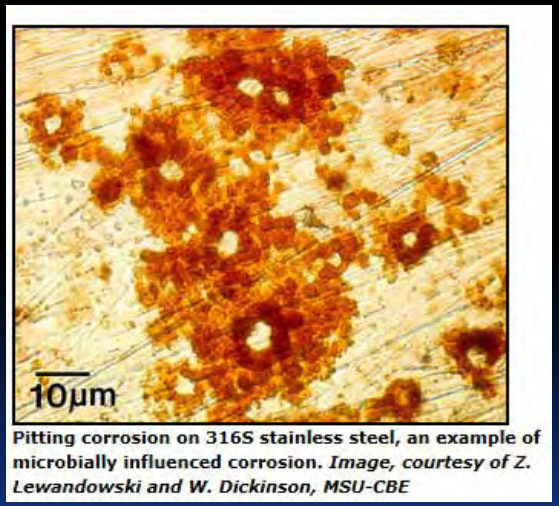
- In 12 minutes, attached bacteria increase
  - Production of proteins
  - Excretion of polysaccharides (slime layer)
  - Rapid cell division—exponential bacterial growth
- Slime layer prevents dislodgement of biofilm
  - Resistant to shear forces of flowing water
  - Keeps bacteria attached to surface

Stage 3: Aggregation



- Location in Biofilm = Specific Responsibilities
  - Outermost Layer = Defensive, aerobic bacteria
  - Higher Layers = Food Gathering
  - Lower Layers = Waste Removers (Sewage Tx), anaerobic bacteria
  - Bottom Layer = Adherence of Biofilm to Surface
- More slime production
  - Creates water channels
  - Allows diffusion of nutrients to inner layers of the biofilm

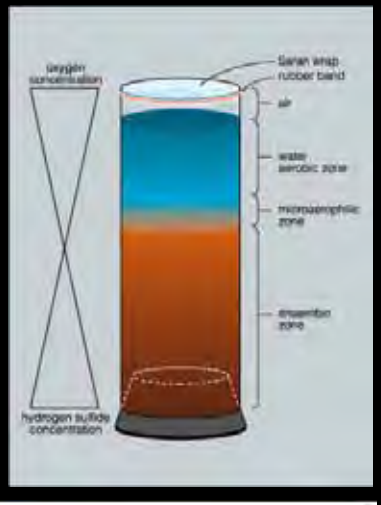


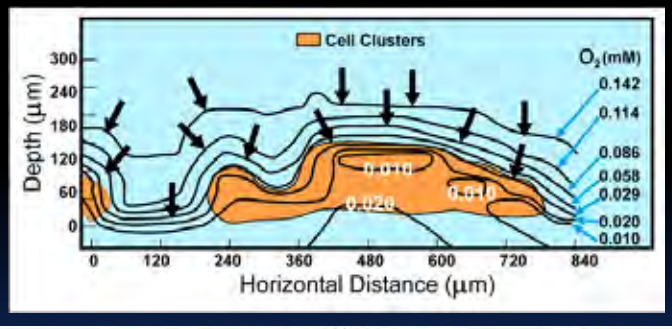


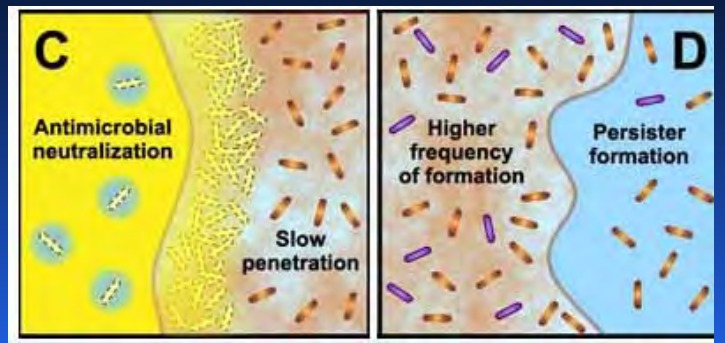
Pitting corrosion on 316S stainless steel, an example of microbially influenced corrosion. *Image, courtesy of Z. Lewandowski and W. Dickinson, MSU-CBE*

Stage 4: Maturity--Composition

- Biofilm Composition
  - 10-75% Bacteria
  - 90-25% Slime
- Oxygen gradient
- 1000x More Resistant to Disinfectants

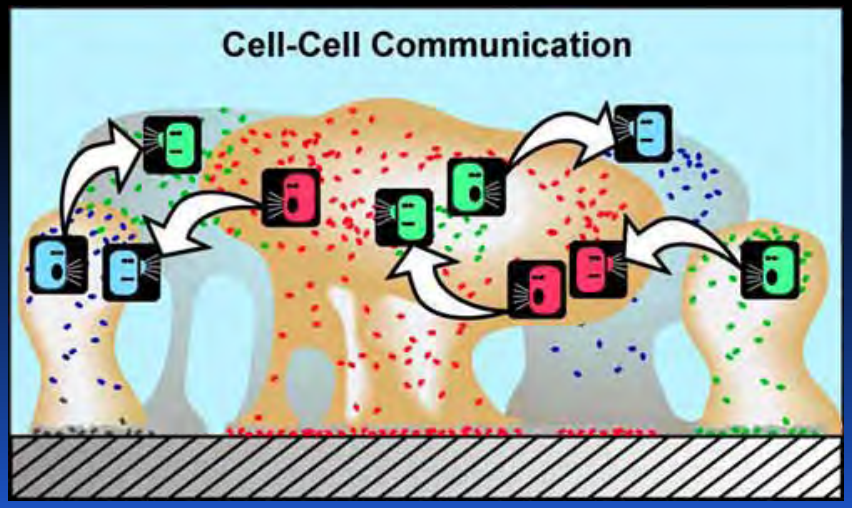


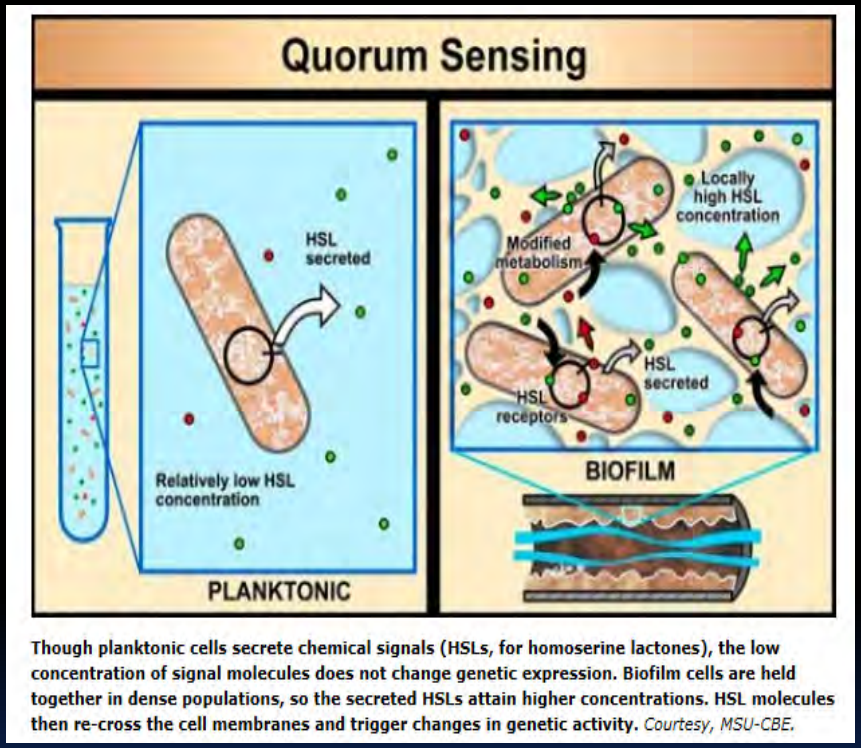




Stage 4: Maturity—Biofilm Communication

- Quorum sensing
- Communicate changes in environment
- Alter behavior

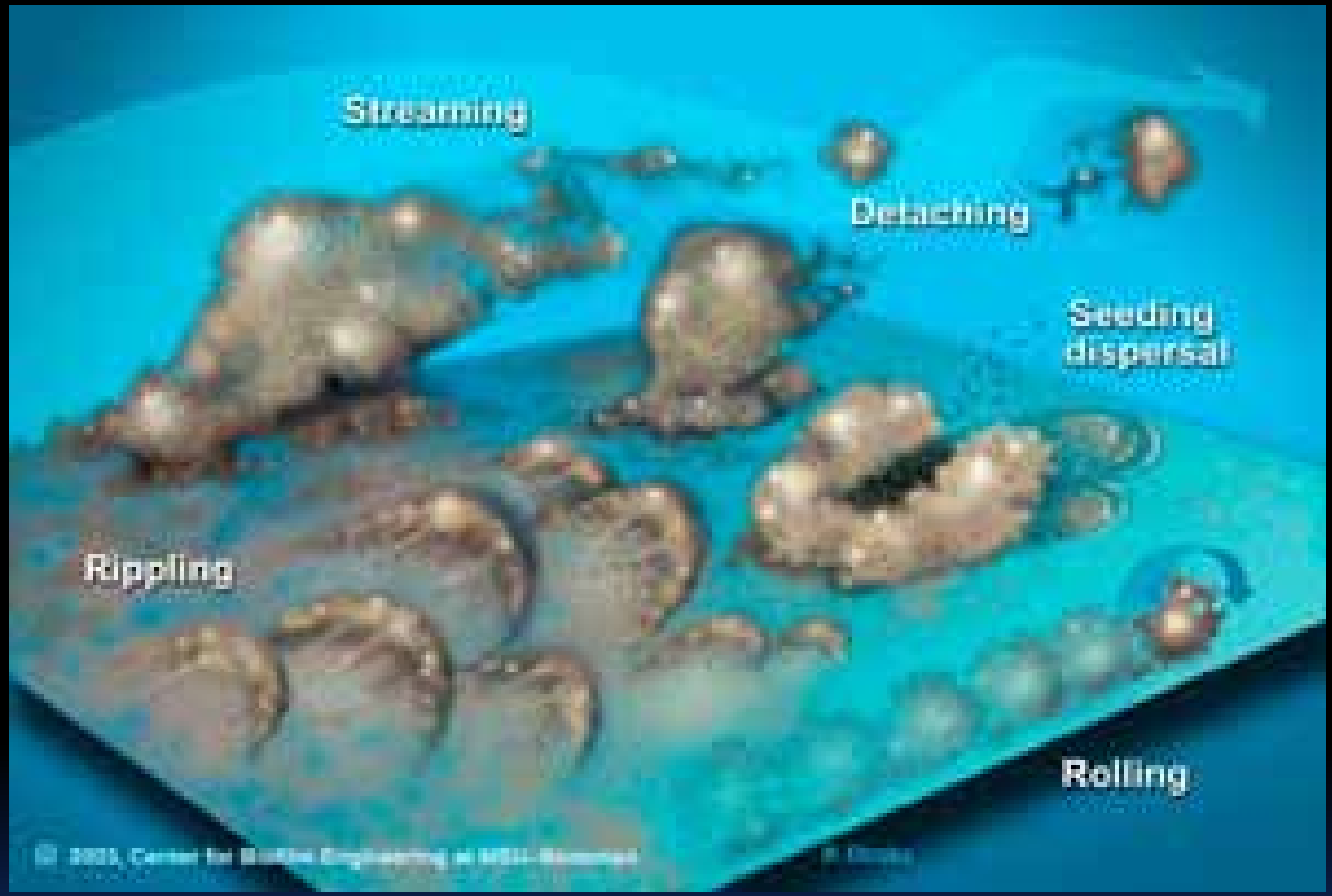




Though planktonic cells secrete chemical signals (HSLs, for homoserine lactones), the low concentration of signal molecules does not change genetic expression. Biofilm cells are held together in dense populations, so the secreted HSLs attain higher concentrations. HSL molecules then re-cross the cell membranes and trigger changes in genetic activity. *Courtesy, MSU-CBE.*

"…Bacteria use at least four of the five senses. In addition to smell, the organisms respond to light (sight), to physical contact with others of their species (touch), and to direct contact with chemicals (taste)."

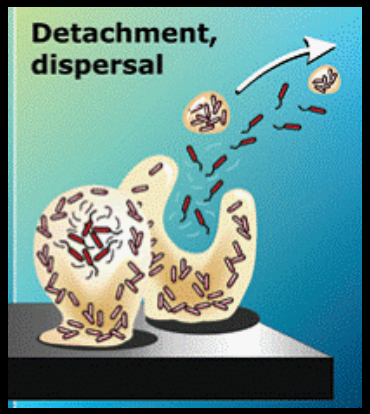
Brian Handwerk
Published August 18, 2010

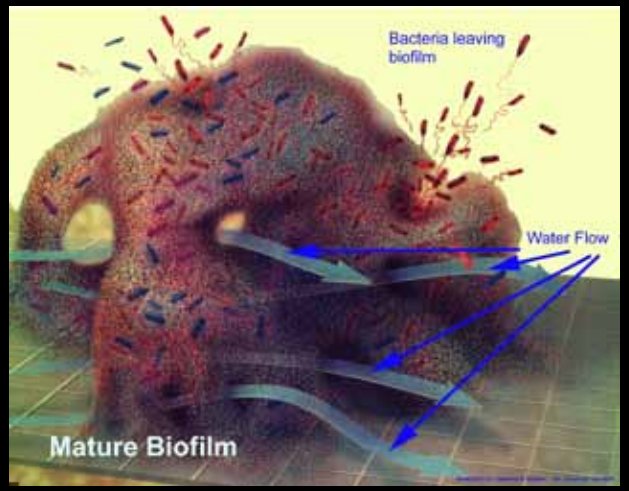


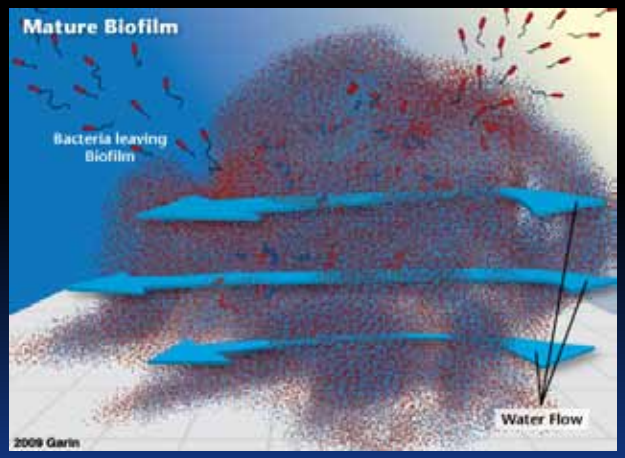
"Biofilm bacteria can move in numerous ways: Collectively, by rippling or rolling across the surface, or by detaching in clumps. Individually, through a "swarming and seeding" dispersal."

http://bacteriality.com/2008/05/26/biofilm/

Stage 5: Dispersal







This is the Biofilm's Most Vulnerable Time!

- Releases Single Cell Bacteria or Cell Plaques
  - Start new biofilm colonies
- Releases cytokine inducing substances
  - Endotoxin, peptidoglycans, DNA fragments

Biofilms In Dialysis

Primary Source of Bacteria For Biofilm Formation in Dialysis

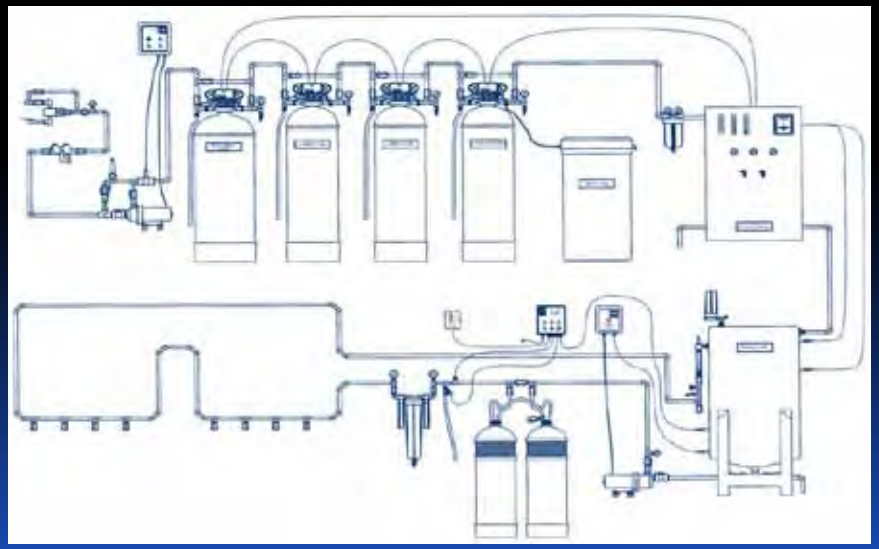
WATER

Importance of Water in Dialysis

- Large volumes of water are used in dialysis
  - Concentrate preparation
  - Dialysate proportioning
  - Rinsing dialysis machines
  - Disinfection of water treatment system components and dialysis machines
  - Reprocessing of dialyzers for reuse
- Water -- a major component of dialysate
- Water is also the main source of contaminants
  - Bacterial
  - Endotoxin
  - Biofilm

Where Biofilm Can Develop in Dialysis H2O Treatment Systems

- Feed water
  - Well Water vs Surface Water
- Water Softener Brine Solution
- Softener exchange resin
  - Provides large surface area for bacteria to attach
  - Captures nutrients for bacterial growth
- Carbon Bed
- Ion Exchange Resin Beds
- Membranes
  - RO
  - Filters
- Break Tank



Post H2O Tx System Biofilm Sites in Dialysis Settings

- Permeate loop
  - Piping
  - Joints
  - Taps
  - Storage Tank
- Dialysis Machine Water Inlet Line
- Dialysis Machine Hydraulic Path
- Bicarbonate Concentrate Mixing System
- Bicarbonate Concentrate Jugs

BIOFILM IN PIPES & TUBING

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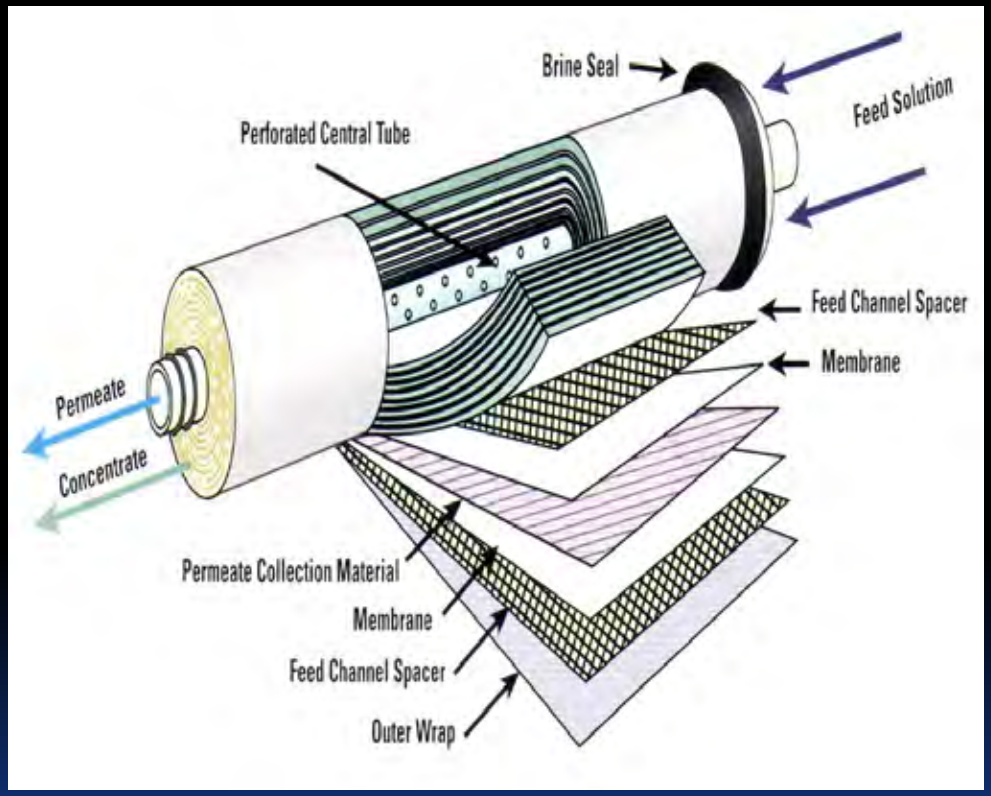
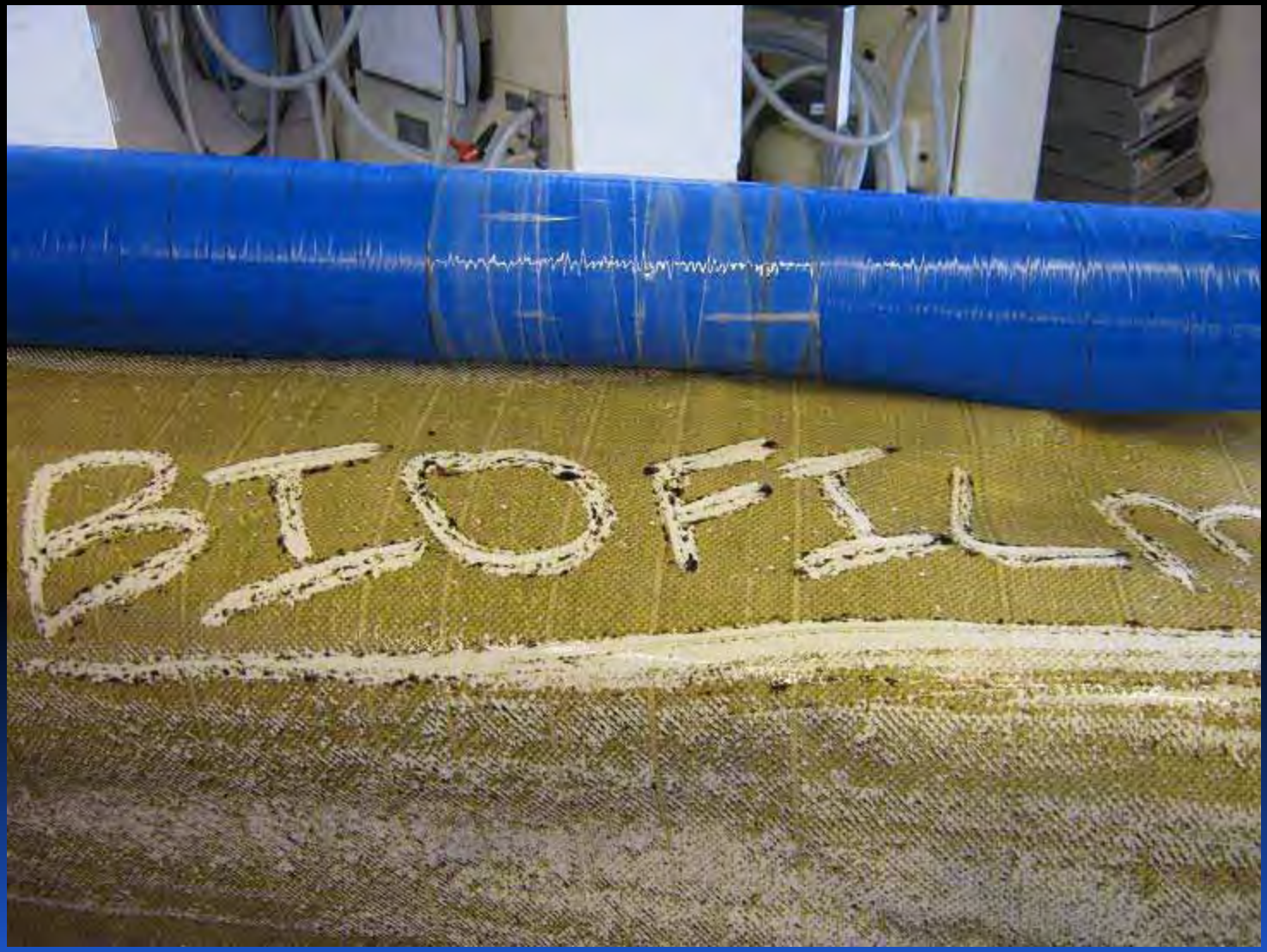
Inside An RO Membrane



RO Membrane Biofilm



RO Membrane 5 Day Biofilm

http://www.youtube.com/watch?v=xJJ_Gw7PzWY

WHY IS CONTROL OF BIOFILM IN THE DIALYSIS ENVIRONMENT SO IMPORTANT?

Effect of Biofilm Presence on Dialysis Patients

- Biofilm contains contaminants that can be transferred to patients thru dialysate
- Most undetectable with current testing methods
- Results in chronic micro-inflammation

- Contaminants
  - Bacteria
  - Debris
    - Endotoxin
    - Exotoxin
    - Peptidoglycan
    - LPS, Lipid A
    - DNA & RNA fragments
    - Low molecular weight by products of bacterial metabolism
    - Carbohydrate slime layer
    - Matrix Proteins
    - Cytokine inducing substances

THE GOAL

Where We Are Today → Where We Want To Be

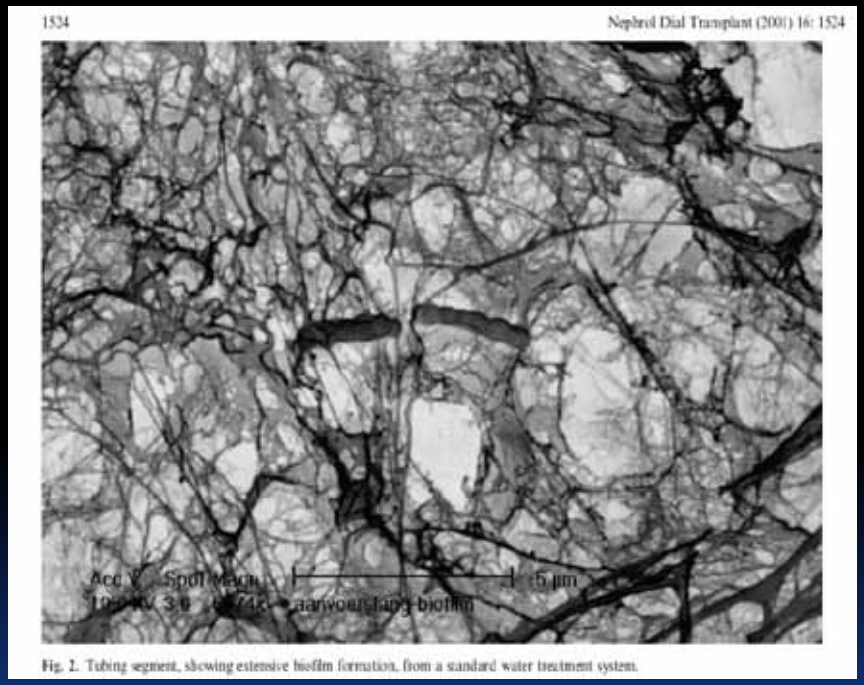


Fig. 2. Tubing segment, showing extensive biofilm formation, from a standard water treatment system.

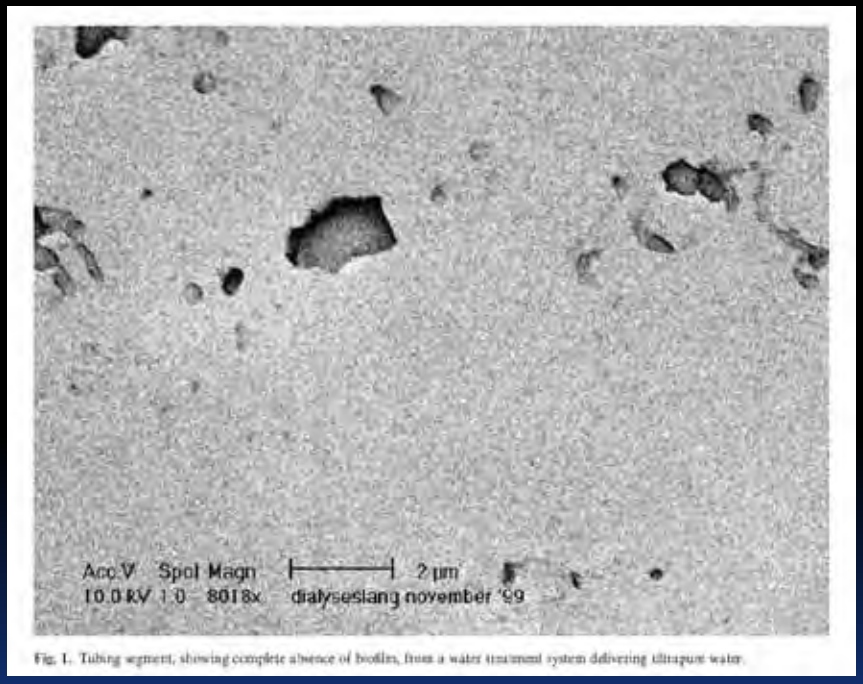


Fig. 1. Tubing segment, showing complete absence of biofilm, from a water treatment system delivering ultrapure water.

Long-term Effects Attributed to Chronic Micro-Inflammation

- Malnutrition
- Low albumin
- Muscle protein wasting
- Protein catabolism
- Increased CRP
- Atherosclerosis
- Low cholesterol synthesis
- Increased ferritin levels
- Resistance to EPO therapy
- Bone disease, cysts, fractures
- Sleep disorders
- Anti-endotoxin antibodies

How Do We Deal With Biofilm?

“The Ounce of Prevention”

- Proper Water Treatment System Design
- Proper Operation & Maintenance of Systems & Equipment
- No oversized piping or dead legs
- Routine Monitoring and Trending
- Routine Flushing, Cleaning & Disinfection
  - Water treatment & distribution systems
  - Storage tank
  - Hemodialysis machines
  - Valves
  - Line between distribution system & dialysis machine
- Use of in-line concentrate generators
- Use of ultrapure water &/or dialysate

Know the Limits & Limitations

Microbiological Limits Water & Dialysate

	AAMI RD52:2004	ISO
Bacterial Limit Action Level	<200 CFU/mL 50 CFU/mL	<100 CFU/mL 50 CFU/mL
Endotoxin Limit Action Level	<2 EU/mL 1 EU/mL	<0.25 EU/mL 0.125 EU/mL

Plate Counts

- Detects only viable & replicating bacteria
- Only 0.01%-30% of viable bacteria are recoverable by conventional methods

Understand The Risks

Water Tx System Component Microbiological Risk Assessment

- Water Softener—High Risk
  - Cannot be disinfected
  - Provides nutrients to captured bacteria
  - Back-flushing does not remove all adhering bacteria
  - Brine solution selects for salt-loving bacteria

Water Tx System Component Microbiological Risk Assessment

- RO—Medium Risk
  - Short interval flushing & frequent disinfection reduces bacteria
  - Endotoxin not inactivated
  - Bacteria, endotoxin, cytokine inducing substances can pass through to permeate side

Water Tx System Component Microbiological Risk Assessment

- Break Tank & Permeate Loop—Low Risk
  - Frequent disinfection can help control biofilm

Have A Plan

Strategies to Control Bacteria & Biofilm

- Design and operate Water Treatment Systems to consistently produce
  - Hemodialysis Quality Water
- Monitor, trend and disinfect regularly
  - To Keep Biofilm under control
- Use Ultrafilter(s) to remove/reduce contaminants especially endotoxins
- Ideally – Use Ultrapure Water & Ultrapure Dialysate
- Use bicarbonate concentrate generators if possible

Additional Control Measures

- Frequent regeneration of softener
- Flushing the RO
- Periodic chemical and/or thermal disinfection
  - Don’t forget to disinfect the RO
- Replace contaminated contents of water softener &/or carbon filters
- Filter out endotoxins from permeate
- Repair all leaks in system immediately
- Disinfect after repairs or breach of the system

RESPOND
EARLY AND OFTEN

Respond to Data

- Bacteria &/or Endotoxin results >action level

OR

- Trending indicates a problem is developing
  - Notify Medical Director
  - Re-disinfect
  - Use a combination of cleaning & disinfection
  - Repeat sampling (bacteria & endotoxin)
  - Weekly sampling thereafter

    Until acceptable results achieved
  - Replace components if necessary

Know What Solution to Apply

- Cleaning
- Disinfection
  - Chemical Disinfection
    - Chemical types, concentrations, temperature & dwell time
    - Certain chemicals may be incompatible with system materials
    - Bacterial tolerance to disinfectants increases within biofilm
    - Biofilm matrix may inactivate or reduce the effectiveness
  - Heat disinfection
    - Can help prevent biofilm formation if done daily
    - Once biofilm exists, heat disinfection will not remove it
    - Endotoxin and CIS remain active
- Combination treatments

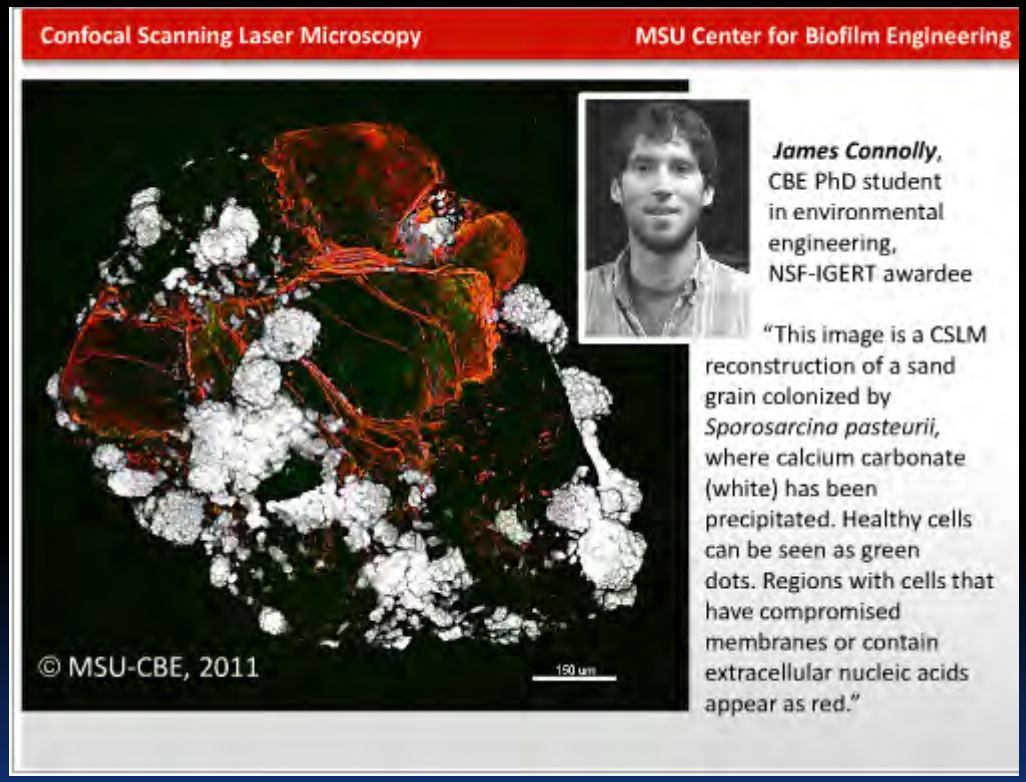
Disinfection

- **What should be disinfected?**
  - Water Treatment & Distribution Systems
  - Hemodialysis machines
  - Line between water distribution system & dialysis machines
  - Water storage tank
  - Bicarb jugs
  - Bicarbonate Concentrate Mixing Systems
- **When?**
  - At Least Monthly for Water System & Hemodialysis machines
  - After maintenance or replacement of components
  - Indications of biofilm
  - Bacterial & endotoxin levels rising (>action level)

Bicarbonate Concentrate Disinfection

- When to disinfect
  - Bicarb jugs—Rinse daily, disinfect **Weekly**
  - Concentrate mixing & distribution systems
    - **Weekly** or as per manufacturers instructions
    - Facility designed system-cleaned & disinfected by validated procedure
    - Routinely meets AAMI/ISO requirements
    - Record data per disinfection cycle in dedicated log

Biofilm and Carbonate



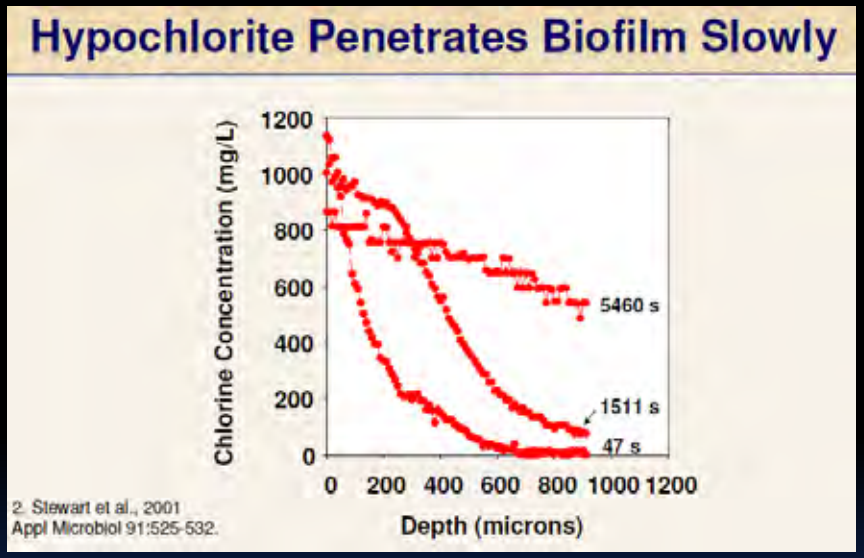
Choosing a Disinfectant is Important for Success

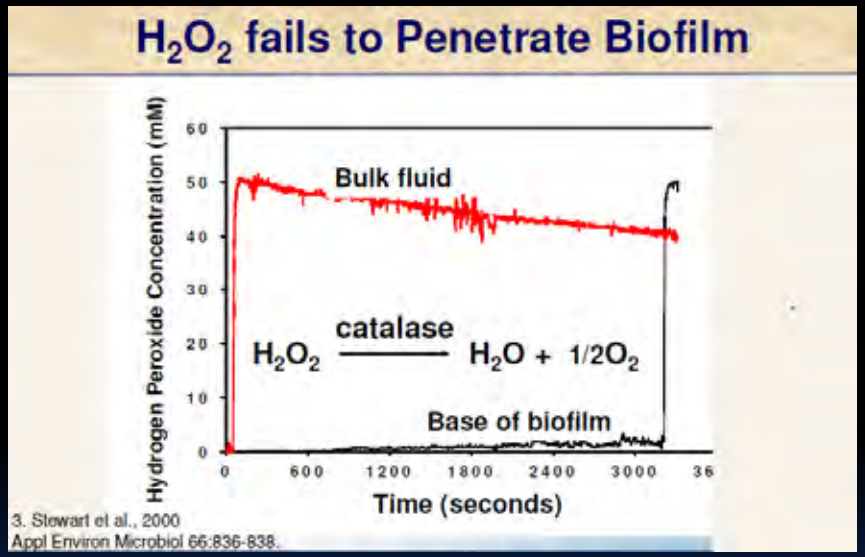
- Established Biofilm or Routine Disinfection?
- Materials compatibility
  - (Table 2 of AAMI RD52)
- Hazards
- Personnel properly trained?
- Design of System
- Effectiveness of disinfectant
- Frequency required
- Cost

Disinfectant Choices

- Ozone
- Acidified Bleach
- Bleach 1:10
- Peracetic Acid
- Formaldehyde
- Glutaraldehyde
- Bleach 1:100
- Heat
- UV

Factors to Keep in Mind





Other Things to Remember About Disinfection

- Effectiveness depends on
  - Adequate concentration
    - Test for Potency
  - Adequate Dwell Time
  - Correct choice of disinfectant for the problem
  - Biofilm presence or not
  - Design of System
  - Getting disinfectant to all surfaces
- Summer months may require more frequent disinfection

Remediation

Criteria for Initiating Remediation

- Routine disinfection is inadequate to keep bacteria and endotoxin levels below the required action levels
- Culture results erratic
- Culture results negative or below action level, but endotoxin levels increasing

Remember

Negative Cultures

DO NOT MEAN ABSENCE OF BIOFILM

Remediation Process

All steps must be performed in detail & in sequence for the highest probability of success

YOU MAY NEED TO CHANGE YOUR DISINFECTION PROGRAM AFTER REMEDIATION TO MAINTAIN CONTROL

IF ALL ELSE FAILS REPLACE ALL OR PART OF THE WATER SYSTEM

MYTHS

WATER TREATMENT SYSTEM MYTHS

- Once monthly disinfection is adequate for all water treatment systems
- No detectable bacteria and/or acceptable levels of endotoxin = No Biofilm
- Turbulent flow in the loop prevents any biofilm formation
- Filters only need to be changed at manufacturer recommended intervals

DIALYSIS MACHINE MYTHS

- The dialyzer prevents transmission of bacteria and endotoxin to patient so water and dialysate levels are irrelevant
- Post disinfection sampling tells you the machine meets AAMI acceptable levels
- As long as monthly monitoring results are OK on a % of the dialysis machines, all of them are within limits and I don't need to be concerned or have to trend my data

CONCENTRATE & DIALYSATE MYTHS

- It is OK to mix old and new batches of bicarbonate concentrate
- Biofilm doesn't form in bicarbonate concentrate
- Containers don't need to be disinfected once emptied of bicarbonate concentrate
- Water quality doesn't matter for Acid Concentrate
- Dialysate contaminants don't cross dialyzer membranes

Summary

- Biofilms are organized communities
- Once established they are difficult to eliminate completely
- Currently, prevention and understanding of how, why & where biofilms develop is the best approach to removal & control
- Lint, debris, pieces of biofilm, organic material & particulate matter act as “crystals” for additional biofilm development
- Bacteria can survive a long time even on dry surfaces, but best in biofilms
- Lack of cleaning, proper disinfectant concentration,dwell time, pH, water quality and temperature can affect biofilm formation, growth and control
- Start with a good system design, keep system cleaned and disinfected, monitor and control feed water quality and respond to data--It is the best we have for NOW!

Things We Can Do Now

- Increase use of ozone disinfection & daily heat disinfection
- Use of ultrapure water and dialysate (Penders, 2001)
- Better permeate loop materials
  - Reduce or eliminate joints (e.g. PEX tubing)
- Dechlorit filter to remove chlorine
- Calcium sulphite– in softeners--bactericidal
- New disinfectants
  - H2O2 + Silver (Accepta 8101)
- Combination treatments
- Creating conditions unfavorable to biofilm formation & survival

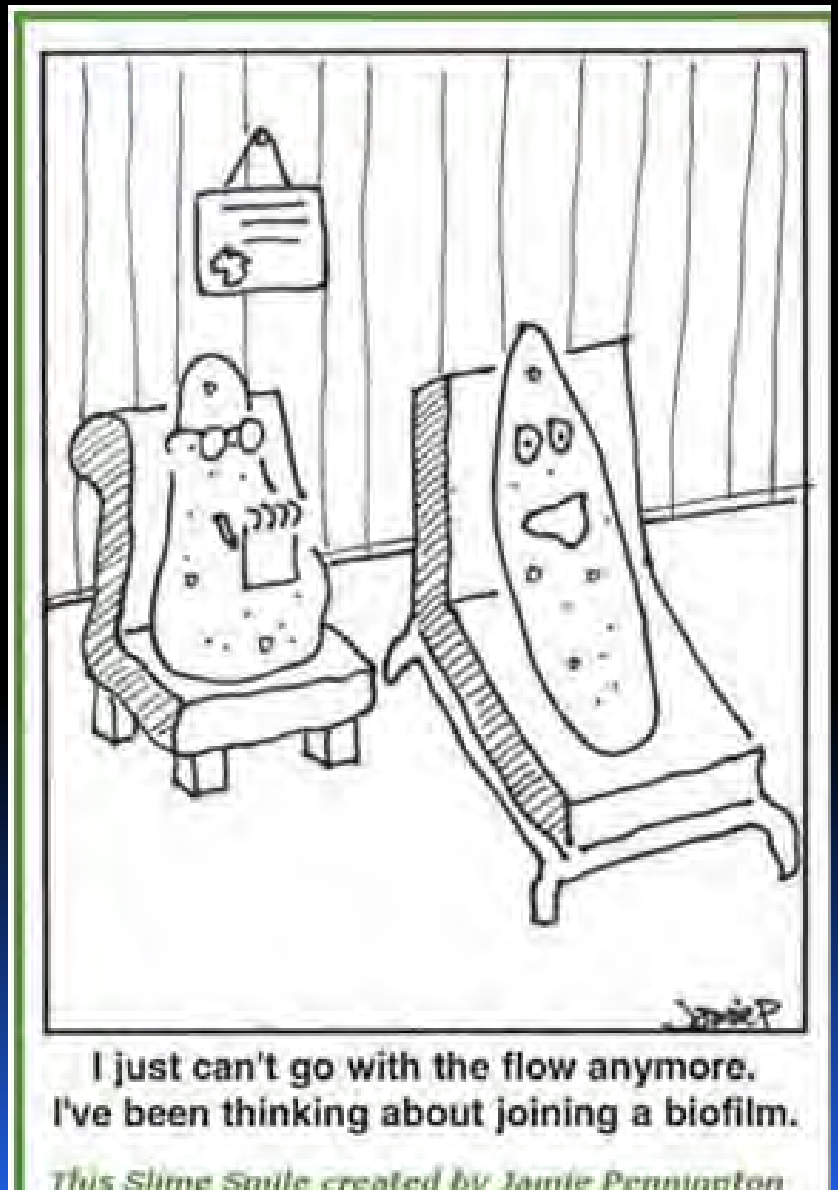
The Future

Areas of Research

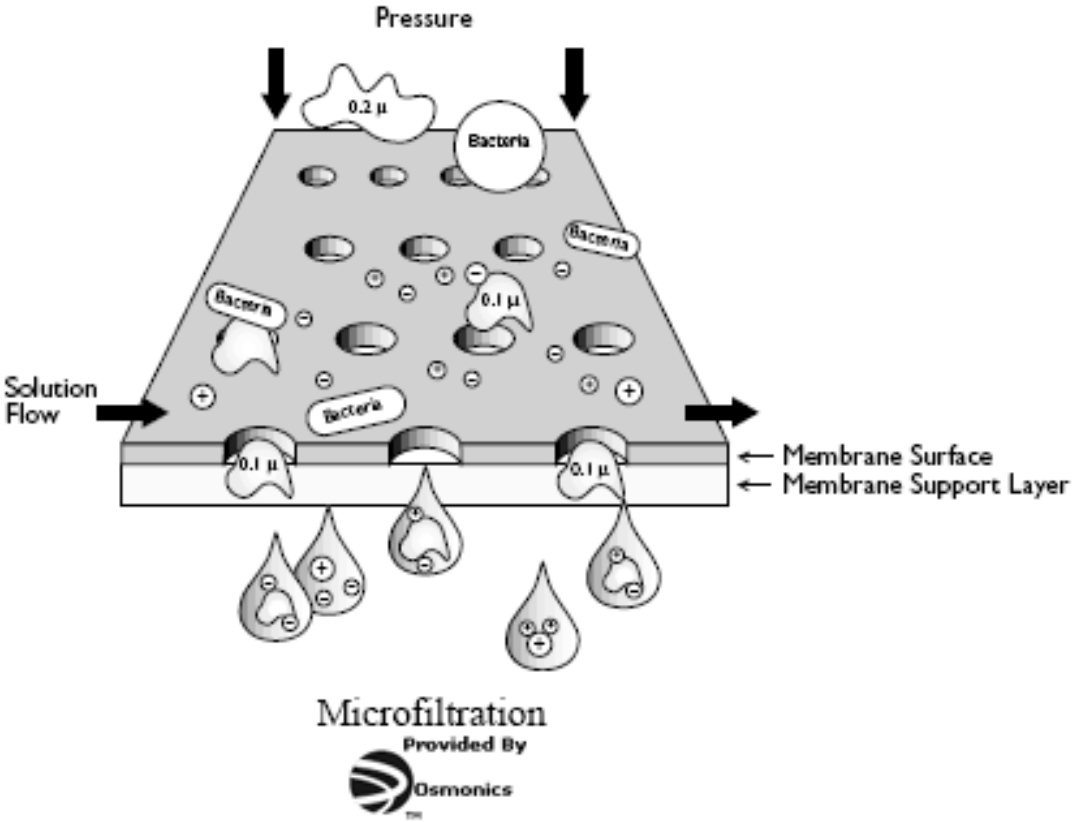
- Understanding the system's microbial ecology & bacterial phenotypes
- Modeling of the biofilms to determine best approach to removal & prevention
- Ways to change conditions of the ecosystem to prevent biofilm formation
- Materials to prevent bacterial adherence & biofilm formation
- Better rapid (minutes-hours), practical, low cost bacterial and biofilm detection methods
- Agents to better penetrate, dissolve & remove matrix material
- Disrupting the metabolic activities of the biofilm residents
- Adding enzyme producing bacteria to remove and prevent biofilm formation
- Application of genomics and proteomics
- Elucidation of genes specific to biofilm organisms
- Treatment of surfaces with anti-microbial agents
  - Prevent colonization
  - Remediate biofilm already present
- Nanoparticle based antibacterials and disinfectants

THE ULTIMATE GOAL

PREVENT THIS!



Achieve This!



More Information About Biofilms

http://www.biofilm.montana.edu/biofilm-basics.htmlEngineering

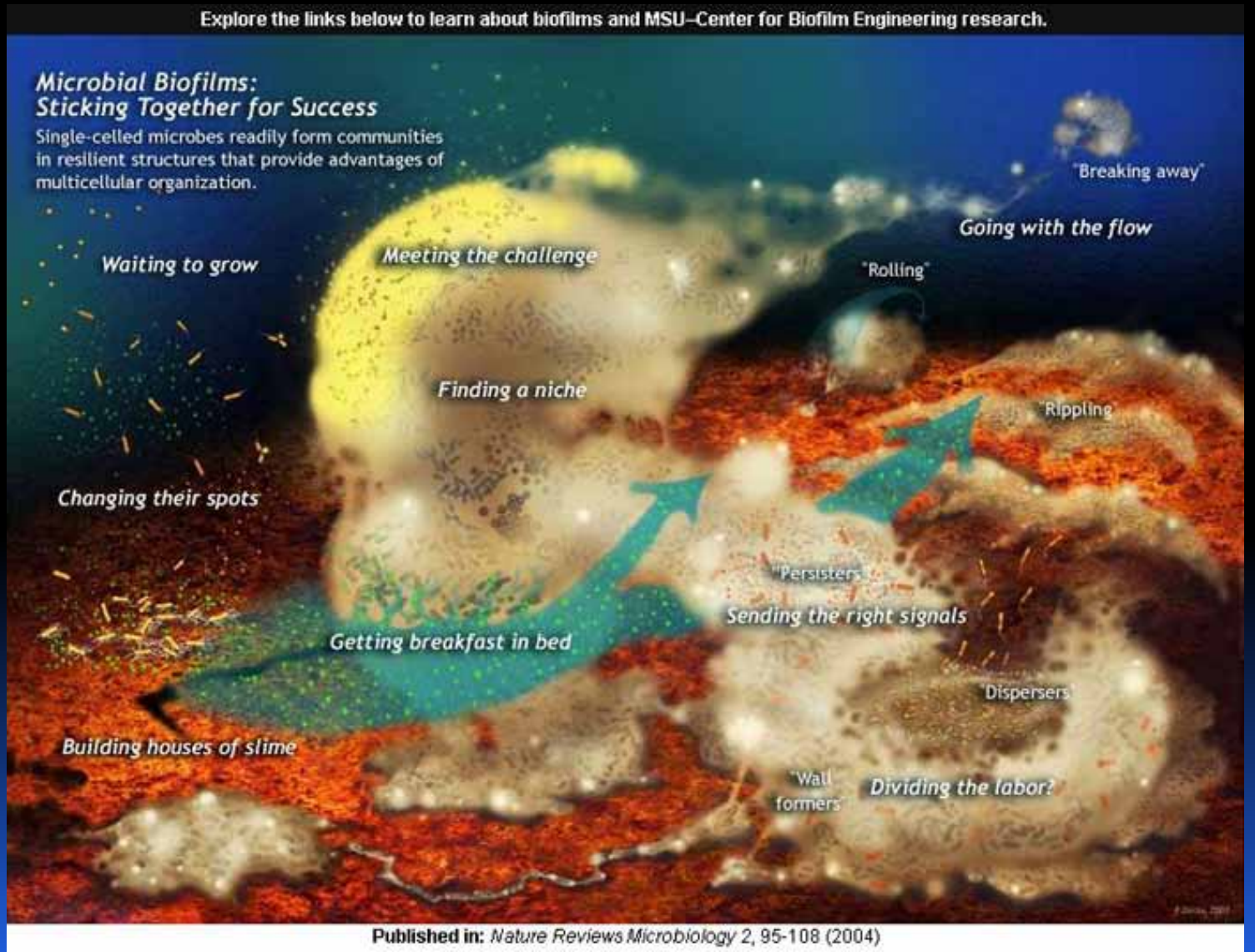
http://www.infectioncontroltoday.com/webinars/2011/12/biofilms-in-medicine--patients-threatened-by-highly-organized-militant-pathogens.aspx

http://www.medscape.com/viewarticle/441355

http://www.nesc.wvu.edu/pdf/dw/publications/ontap/2009_tb/biofilm_DWFSOM124.pdf

http://www.microbialfuelcell.org/Publications/CEB/Where%20are%20we%20biofilm%20now%20Where%20are%20we%20going.pdf

Thank You!


Explore the links below to learn about biofilms and MSU–Center for Biofilm Engineering research.
Microbial Biofilms:
Sticking Together for Success
Single-celled microbes readily form communities in resilient structures that provide advantages of multicellular organization.
Waiting to grow
Meeting the challenge
"Breaking away"
Going with the flow
"Rolling"
Finding a niche
"Rippling"
Changing their spots
"Persisters"
Sending the right signals
Getting breakfast in bed
"Dispersers"
Building houses of slime
"Wall formers"
Dividing the labor?
Published in: Nature Reviews Microbiology 2, 95-108 (2004)