

The background of the slide is a black field filled with numerous small, bright green, rod-shaped fluorescent bacteria. Some bacteria are in sharp focus, while others are blurred, creating a sense of depth and movement. The overall appearance is that of a microscopic view of a bacterial culture.

# Introduction to Biofilms and Biofouling in Pharmaceutical Manufacturing

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# Outline

- Introduction
  - Biofilms: Opportunities and problems
- Biofilm problems
  - High level manufacturing problems
- Biofilm definition
  - Biofilm problems: chemical, physical
- Sequence of events
- Resistance
- Control
- Intro to conference
  - Topics & speakers
- Selected Resources
  - CBE
  - Journal of Biofouling
  - International Biodeteriorization and Biodegradation Society

# Introduction

- Biofilms represent both problems and opportunities in the pharmaceuticals and biomedical industry
- Opportunity: product development in biomedical devices, targeting anti-biofilm coatings and materials or biofilm-removal formulations
- Problems: biofilm-related contamination in finished product and/or manufacturing product transfer lines, tanks, raw materials streams, and water systems
- This presentation is an overview of general biofilm science and characteristics of biofilms in manufacturing processes

# What is a biofilm?

- Unwanted adhesion of bacteria or other organisms onto surfaces of solution-handling systems
- Not necessarily uniform in space & time
- May contain significant amounts of inorganic materials held together by the polymeric matrix

\*(Charackis & Marshall, Biofilms, 1990)

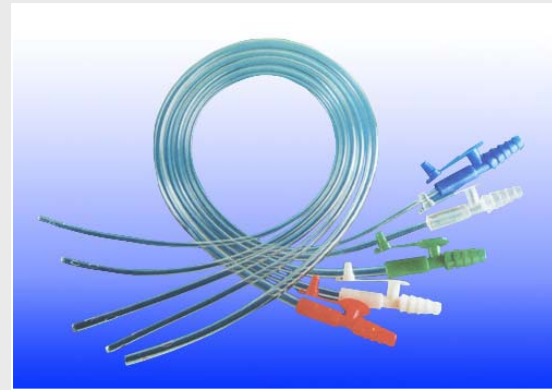
# Environmental biofilms

- Marine and freshwater hull fouling
- Wastewater treatment
- Hot springs
- Deep sea vents
- Pollution remediation
- Ocean energy capture: thermal, mechanical
- Deep earth rock
- Underground caves
- Arctic and Antarctic



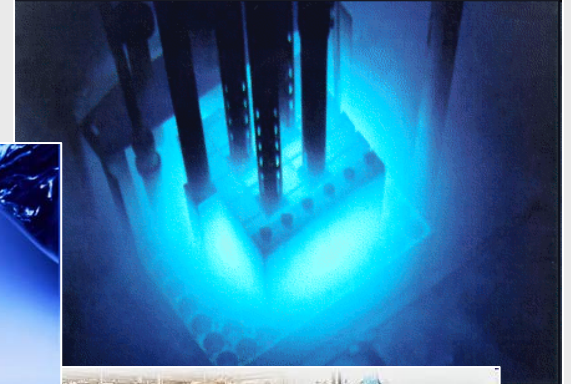
# Biomedical biofilms

- Indwelling medical implants (MRSA)
- Surfaces in the surgical and hospital environment
- Home and hospital bathroom surfaces
- Air-handling and water-handling systems (*Legionella*)
- Biological fluid-handling machines (dialysis equipment)
- Chronic wounds (diabetic ulcers), infections (ear infections) and diseases (cystic fibrosis)
- Studies now show that the majority of chronic infections arising from hospitals is biofilm related



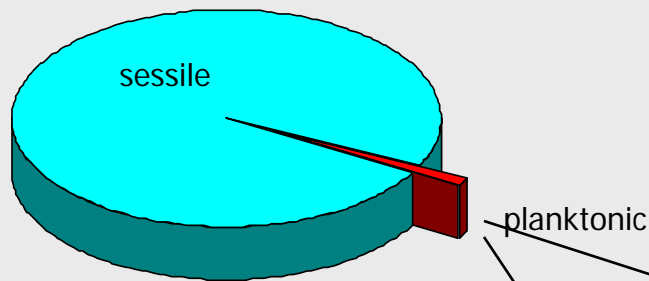
# Industrial biofilms

- Cooling towers
- Nuclear reactor cooling water
- Water systems, including ultrapure, RO, DI
- Pulp and paper mills
- Petrochemical: jet fuel, oil pipelines, metalworking fluids
- Food and beverage processing
- Pharmaceutical manufacturing
- Cosmetics manufacturing
- Bulk and fine chemicals



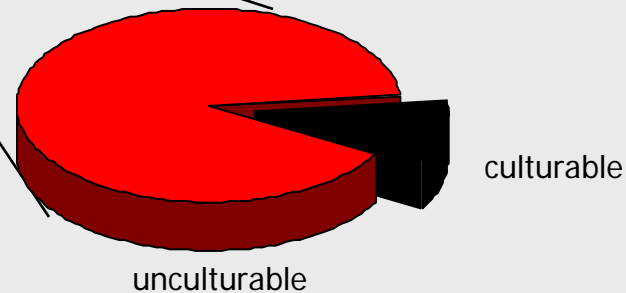
# Life Inside a Pipe: Planktonic vs Sessile Populations

Total Bacteria



Potera, *Science*, 273:1795 (1996)

- Sessile organisms (i.e., biofilms) are dominant form in a system
- Organisms recovered from a system may not grow in culture
- Result: grab samples may underestimate the true population of a system



White, *Manual of Environmental Microbiology*, 91-101 (1997)

# Biofilm Problems

- Product quality
- Process problems
- Sampling problems
- Lab analysis problems
- Control problems

# Product Problems

- Pathogens
- Shelf life
- Product recalls
- Loss of market share

# Process Problems

- Process downtime
- Filter plugging
- Viscosity increase (due to number of cells) or decrease (product hydrolysis)
- Microbially induced corrosion of metals and plastics
- Product degradation during processing
- Chemical contamination:
  - Metals
  - Anion contamination (acetates, formates, nitrates, nitrites)
  - Biosurfactant contamination
  - Enzyme contamination

# Sampling Problems

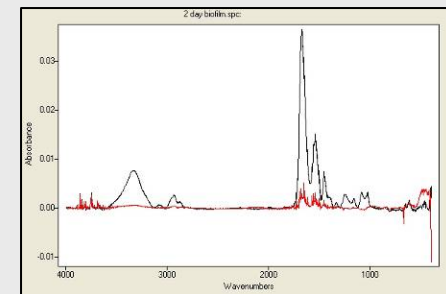
- Non-uniform biofilm distribution in time and space
- Contamination events can be random
- Limited process access, both in process sampling location as well as in available time for sampling
- Wall sampling required
  - Swab methods can be difficult to reproduce
  - ATP methods may suffer from chemical interferences

# Analysis Problems

- Plating/culturing methods require growth of the biofilm organisms, and some biofilms simply will not grow in standard lab conditions
- Biofilm cells must be released from the surrounding exopolymer in order to be free to grow in culture
- Mixed species biofilms recovered from a process may not grow in a similar fashion in the lab
- PCR-based methods require a minimum number of detected cells; biofilms can contain few cells with a lot of exopolymer and may not generate enough signal for PCR methods

# Biofilm Detection

- Microbiology
    - Plating
    - PCR
    - FISH
    - Microscopy
  - Engineering
    - Flow
    - Pressure
    - Heat exchange
  - Chemistry
    - Spectroscopy
    - Electrochemistry
    - Vibration dampening
- Requires growth or minimum cell density
- Requires relatively thick biofilms
- Requires specially designed sampling devices, instruments

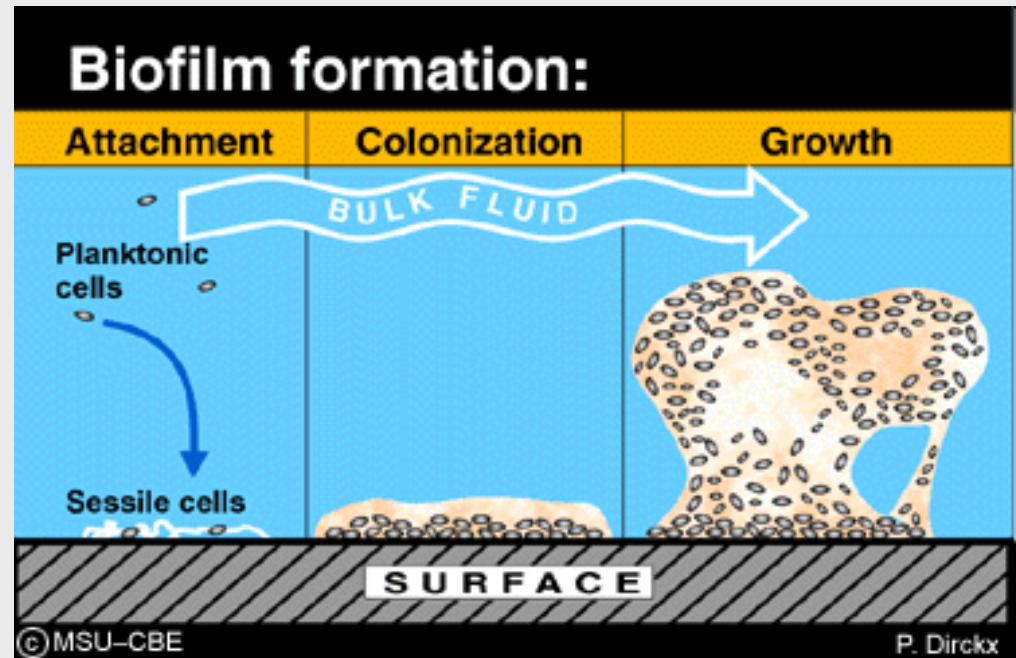


# Control Problems

- Resistance to antimicrobials/preservatives and cleaning solutions increases with biofilm age
- Response to random biofilm contamination problems is aggressive “shock” cleaning, which is costly, time consuming, and usually only a temporary fix
- Underlying causes to biofilm problems:
  - System design
  - Contaminated raw materials
  - CIP/SIP not effective

# Sequence of Events

- Conditioning film
- Initial colonization
  - Reversible
  - Irreversible
- Maturation
  - Exopolymer production
- Degradation and release



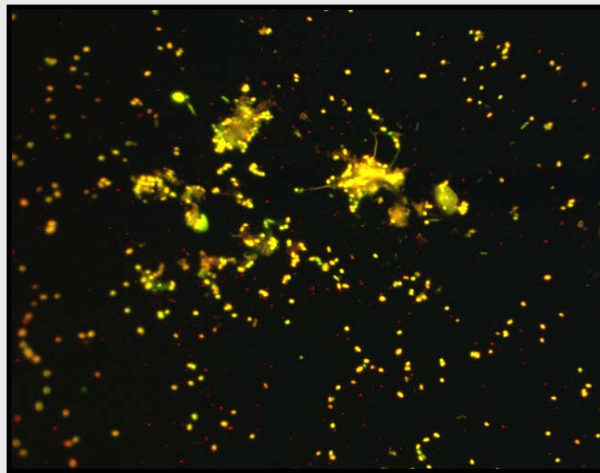
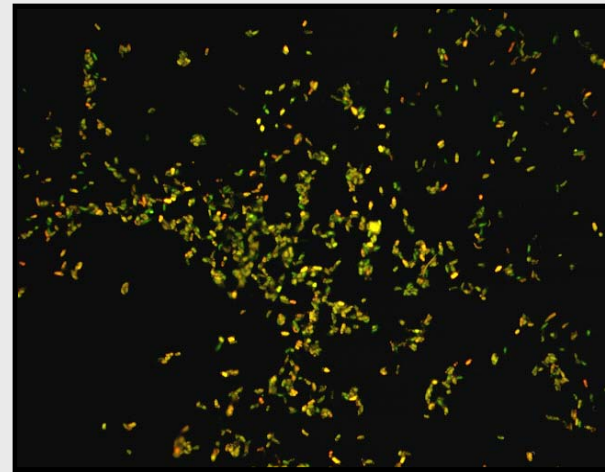
[www.erc.montana.edu/biofilmbook/default.htm](http://www.erc.montana.edu/biofilmbook/default.htm)

# Biofilm Structure: 45°C Ultrapure Water Biofouling

1 day

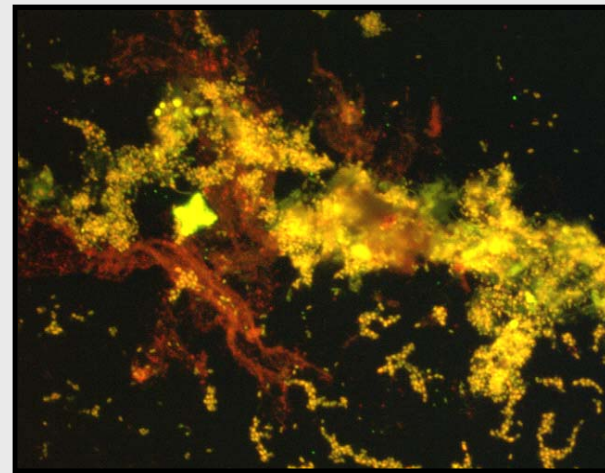


2 days



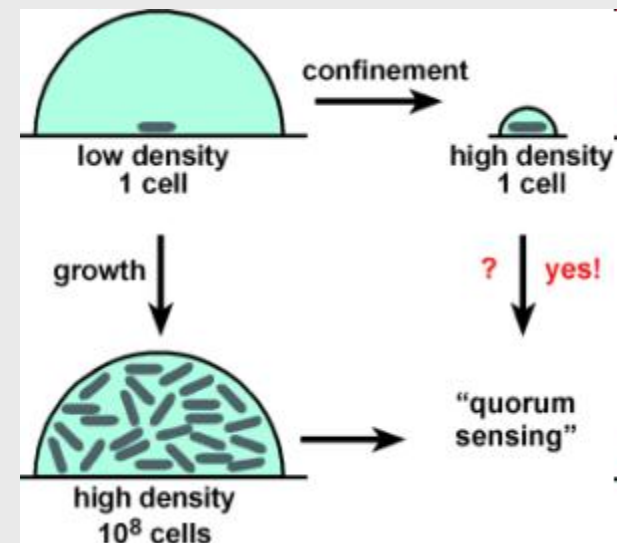
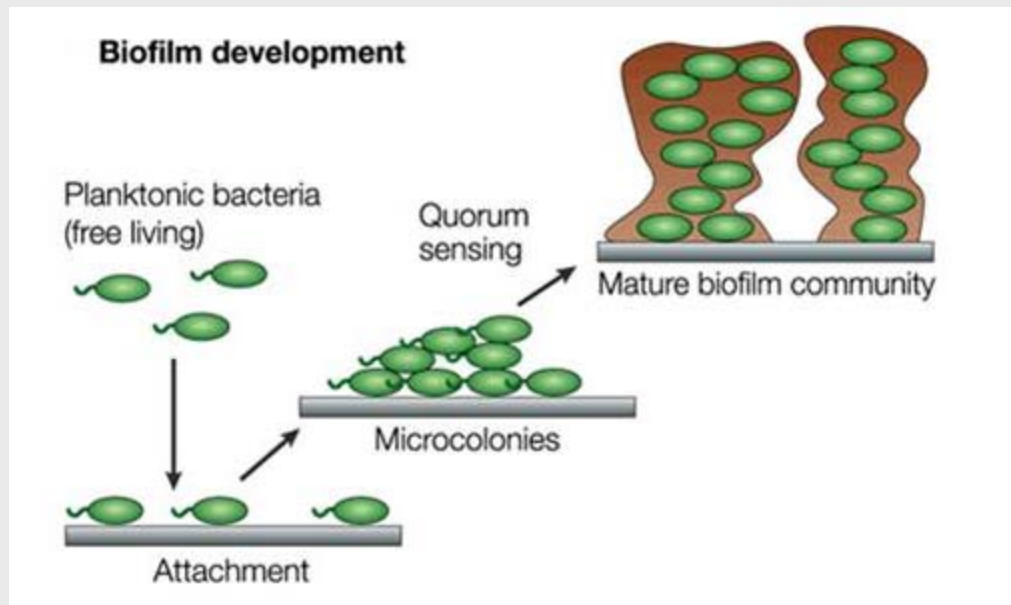
4 days

[www.biofouling-science.com](http://www.biofouling-science.com)



9 days

# Quorum Sensing



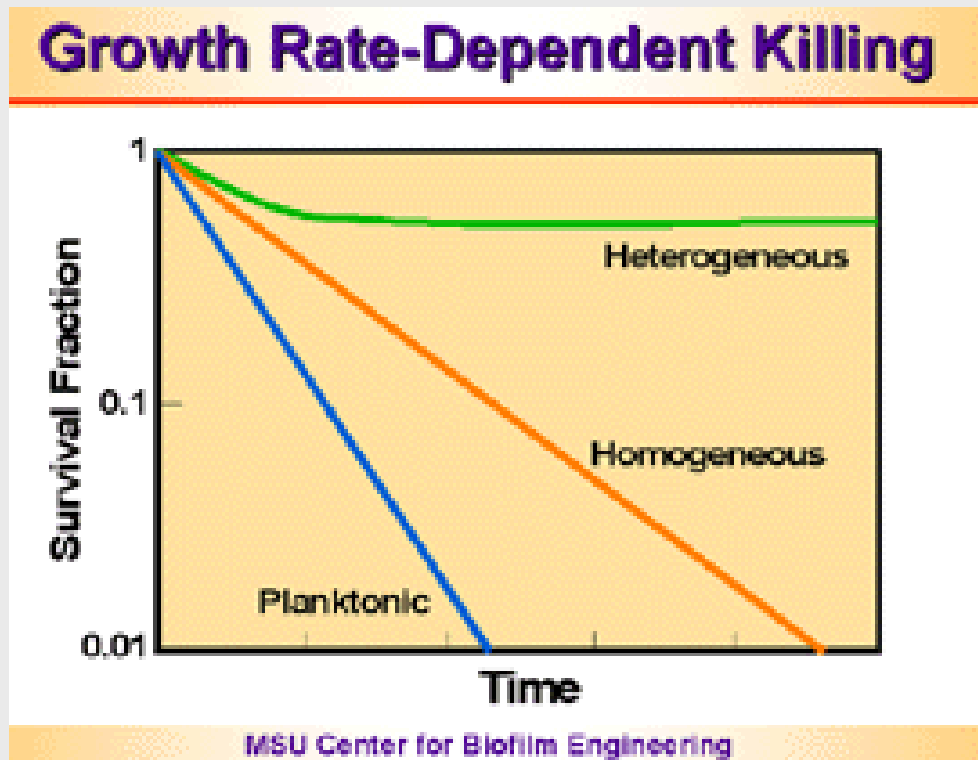
Clay Fuqua<sup>1</sup> & E. Peter Greenberg, Nature Reviews Molecular Cell Biology 3, 685-695 (September 2002)

<http://www.nature.com/nrm/journal/v3/n9/abs/nrm907.html>

*Researchers have demonstrated that the absolute number of cells is irrelevant to the process of bacterial quorum sensing; only the number of bacteria in a given volume plays a role. (Credit: Copyright Wiley-VCH)*

<http://www.sciencedaily.com/releases/2009/07/090710101452.htm>

# Biofilm Antimicrobial Resistance

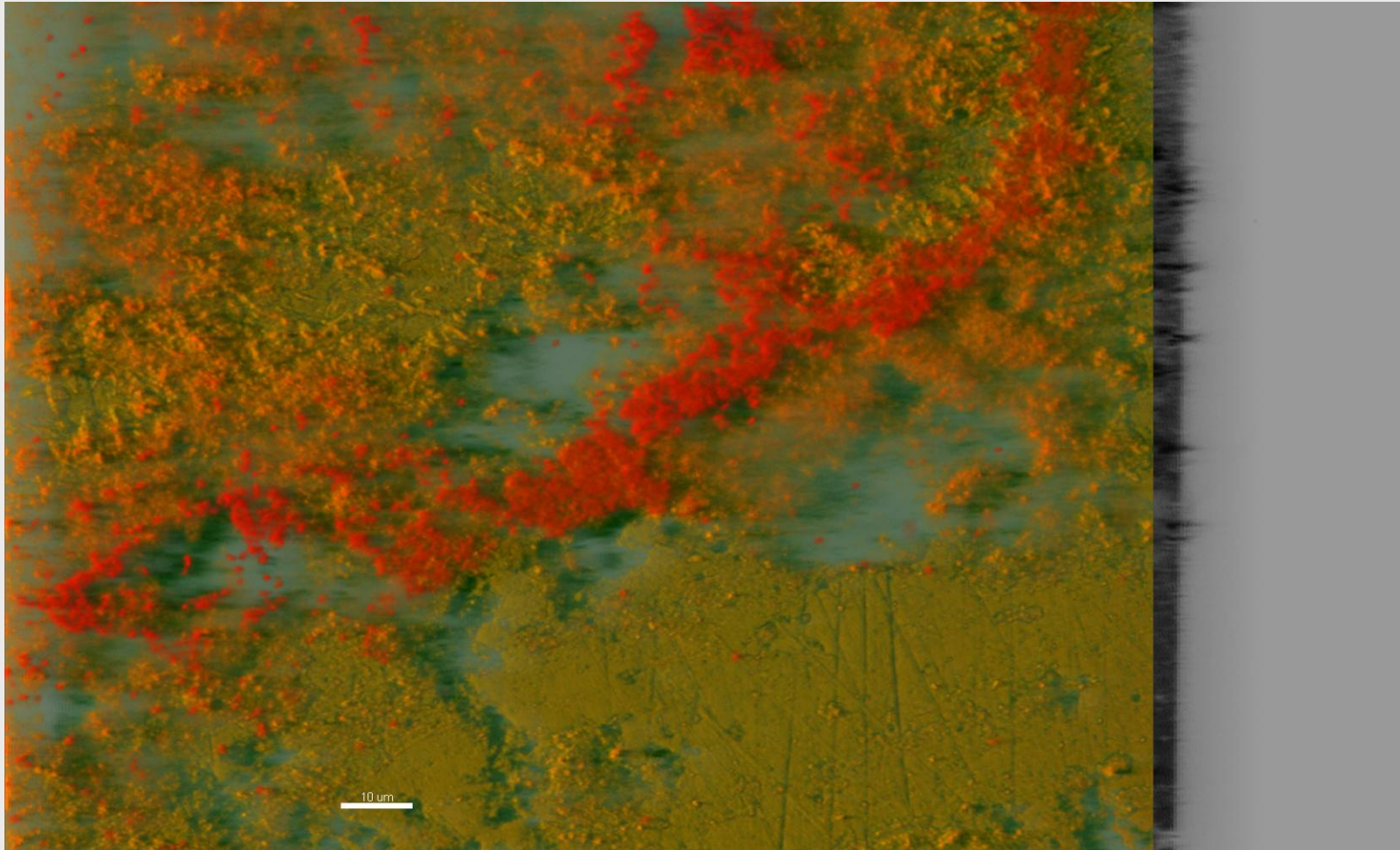


Planktonic organisms are more susceptible to biocides and cleaning chemicals than sessile organisms

# Biofilm Resistance to Cleaning



# Biofilm Resistance to Heat: Steam-in-Place (SIP) System Biofilm



# Hurdle Technology

In food manufacturing, hurdle technology is the application of a series of “barriers” or “hurdles” in the manufacturing process that reduce the level of organisms at each barrier, that ultimately lead to a microbe-free final product.

# Hurdle Technology for Biofilm Prevention

- Process maintenance
- Sanitary equipment, sanitary layout
- Process water flush parameters and fluid dynamics
- Process surface cleaning
- Process sanitization
- Raw material cleanliness
- UV treatment, filtration
- Antimicrobials, preservatives

# Final Thoughts

- Biofilms can occur on surfaces in any liquid-handling environment and in any liquid
- Biofilms can cause various forms of contamination
- Biofilms grow more resistant to cleaning and sanitation with age
- Sampling and analysis techniques must be optimized for biofilms
- Biofilm control is broadly based on process cleaning procedures and frequency, system design, and bio load of the product and raw materials

# Selected Resources

- Academic:
  - Center for Biofilm Engineering (<http://www.erc.montana.edu/>)
  - Industry/University Center for Biosurfaces (<http://wings.buffalo.edu/iucb/>)
- Books
  - Biofilms, edited by William G. Characklis and Kevin C. Marshall, Wiley Series in Ecological and Applied Microbiology, Series Editor Ralph Mitchell, Wiley-Interscience (1990).
- Journals
  - Biofouling (<http://www.tandf.co.uk/journals/titles/08927014.asp>)
  - International Biodeteriorization and Biodegradation Society (<http://www.ibbsonline.org/index.html>)
  - Biofilms Online (<http://www.biofilmsonline.com/cgi-bin/biofilmsonline/index.html>)
- Groups
  - LinkedIn Biofilm Interest Group (<http://www.linkedin.com/groups?home=&gid=1800307>)
- Publications
  - Costerton, J.W., Geesey, G.G. and Cheng, K.-J., "How Bacteria Stick," *Scientific American*, 238:86-95 (1978).
  - Flemming, J. and Kemkes, D., "Biofilm Contamination Issues in Pharmaceutical Fluid-Handling Tubing," *Pharmaceutical Engineering*, 19(5):1-6 (1999).
  - Kulakov, L.A. et al., "Analysis of Bacteria Contaminating Ultrapure Water in Industrial Systems," *App Envir Micro*. 68(4):1548–1555 (2002).
  - Riedewald, F., "Biofilms in Pharmaceutical Waters," *Pharmaceutical Engineering*, November/December, pp 8-19 (1997).
  - Alfa, M.J. and Howie, R., "Modeling microbial survival in buildup biofilm for complex medical devices.," *BMC Infectious Diseases* 2009, 9:56 (2008).
  - Hall-Stoodley, L. and Stoodley, P., "Evolving concepts in biofilm infections," *Cellular Microbiology* 11(7), 1034–1043 (2009).

# PMF Biofilm Conference

The PMF biofilm conference will be held in Philadelphia on April 12-13, 2010, and will look at the different aspects of process and contamination control with an eye to discussing practical measures of biofilm monitoring and control.

# Speakers

- **Paul Sturman**, Center for Biofilm Engineering, Montana State University, “Biofilm growth, measurement, and visualization”
- **Anne E. Meyer**, Industry/University Center for Biosurfaces, SUNY/Buffalo, “Life at Interfaces: Macromolecules and Bacteria on Surfaces”
- **J. Stephen Richards**, R&D Microbiology, Johnson & Johnson, “Micro Flora of the Human Skin and the Biofilms They Form”
- **Mark Pasmore, Sr.** Principal Engineer, Baxter Healthcare Corporation, “Biofilms: Investigating the link between system colonization and the effects on patients”
- **Lucia Clontz**, Technical Services, Microbiology & Training, Diosynth Biotechnology, “Contamination Control by Design: Preventing Biofilms in Pharmaceutical Manufacturing”
- **T.C. Soli**, Soli Pharma Solutions, “Successful Water System Biofilm Sanitization”
- **Mark Fornalik**, Industrial Biofouling Science, “Early, Direct Detection of Biofilms and CIP-Related Problems in Liquid Process Systems”