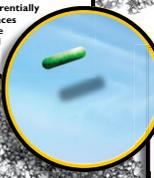


# Essential biofilm concepts & phenomena

Bacteria preferentially attach to surfaces when favorable environmental conditions are available.



## ADHESION

Microbes stick to surfaces. They will stick to plastic, glass, or metal—as well as plant or animal tissues. Adhesion is the initial step of biofilm formation that allows microbes to associate with each other and to establish residence in a particular environment.

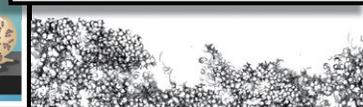
The formation and development of a biofilm occurs in a predictable manner. Initially the attachment of a bacterium to a surface is tenuous and reversible. Within a matter of minutes, the bacterium becomes irreversibly attached and begins to secrete the anchoring EPS.

As bacteria begin to multiply, EPS holds cells in close proximity, which is necessary for cell-cell communication. It also allows the formation of three-dimensional structures that give the bacteria increased access to nutrients and the advantages of multicellular living.



## EXTRACELLULAR MATRIX

Microbes in a biofilm secrete extracellular polymeric substances (EPS) forming a sticky, hydrated gel that holds the biofilm together. EPS constituents include polysaccharides, proteins, and extracellular DNA.



## SPECIES DIVERSITY

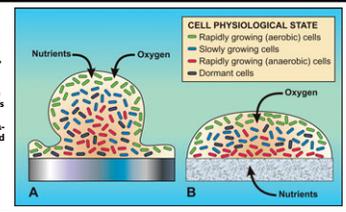
An astonishing diversity of microbial species often coexist in real-world biofilms. Hundreds of phylogenetically and metabolically distinct species have been found in environments ranging from the human mouth (dental plaque) to hot springs in Yellowstone National Park (right).



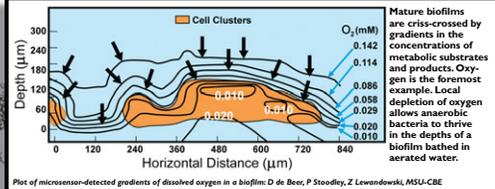
Phylogenetic and clone library imaging reveal gene diversity using color to designate different genes and bandwidth to indicate representation in the community sample; data imaging provided by K. De Lencastre, MSU-CBE

## PHENOTYPIC HETEROGENEITY & DIFFERENTIATION

Cells of a given species can occupy a wide variety of phenotypic states in the same biofilm, from rapidly growing to dormant to expressing a unique activity. Mechanisms of diversification include nutrient gradients, mutation and natural selection, and genetic regulatory switches and signaling pathways.

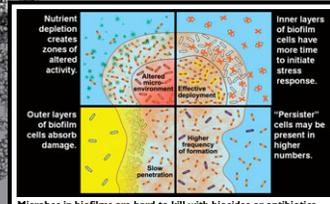


## OXYGEN GRADIENTS



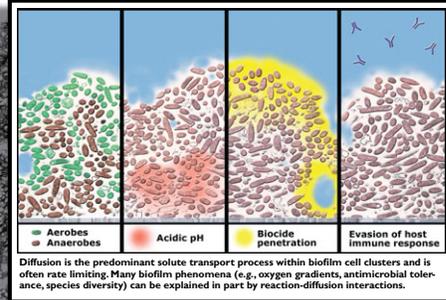
Plot of microsensor-detected gradients of dissolved oxygen in a biofilm of *B. de Beer*, P. Stoodley, J. Lewandowski, MSU-CBE

## ANTIMICROBIAL TOLERANCE



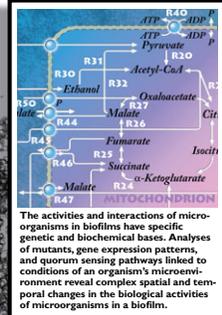
Microbes in biofilms are hard to kill with biocides or antibiotics. Protective mechanisms include poor penetration of reactive agents, non-growing cells whose inactivity makes them less vulnerable, and implementation of adaptive responses.

## DIFFUSION LIMITATION



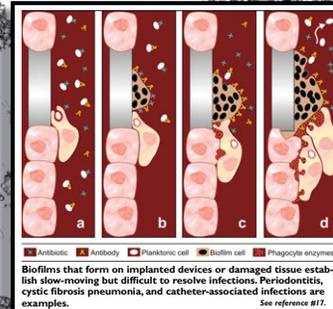
Diffusion is the predominant solute transport process within biofilm cell clusters and is often rate limiting. Many biofilm phenomena (e.g., oxygen gradients, antimicrobial tolerance, species diversity) can be explained in part by reaction-diffusion interactions.

## GENETIC & BIOCHEMICAL BASES



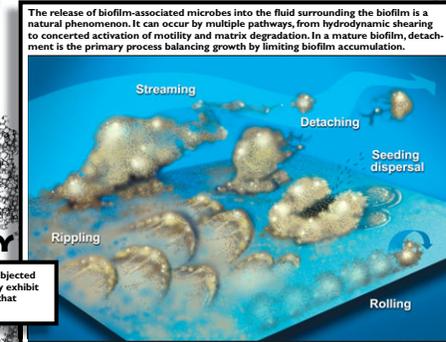
The activities and interactions of microorganisms in biofilms have specific genetic and biochemical bases. Analyses of mutants, gene expression patterns, and quorum sensing pathways linked to conditions of an organism's microenvironment reveal complex spatial and temporal changes in the biological activities of microorganisms in a biofilm.

## PERSISTENT INFECTION



Biofilms that form on implanted devices or damaged tissue establish slow-moving but difficult to resolve infections. Periodontitis, cystic fibrosis pneumonia, and catheter-associated infections are examples.

## DETACHMENT & DISPERSAL

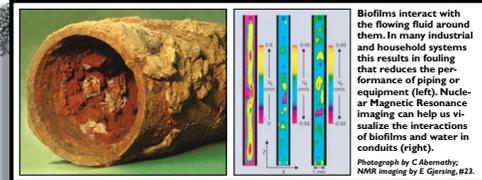


The release of biofilm-associated microbes into the fluid surrounding the biofilm is a natural phenomenon. It can occur by multiple pathways, from hydrodynamic shearing to concerted activation of motility and matrix degradation. In a mature biofilm, detachment is the primary process balancing growth by limiting biofilm accumulation.

## VISCOELASTICITY

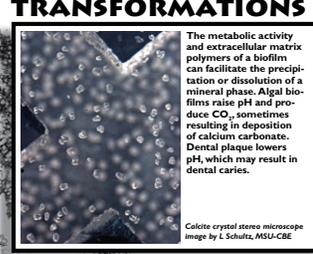
Biofilms stretch, deform, and oscillate when subjected to an applied force, such as flowing water. They exhibit a combination of elastic and viscous behavior that makes them resilient to physical challenges.

## HYDRODYNAMICS



Biofilms interact with the flowing fluid around them. In many industrial and household systems this results in fouling that reduces the performance of piping or equipment (left). Nuclear Magnetic Resonance imaging can help us visualize the interactions of biofilms and water in conduits (right).

## MINERAL TRANSFORMATIONS



The metabolic activity and extracellular matrix polymers of a biofilm can facilitate the precipitation or dissolution of a mineral phase. Algal biofilms raise pH and produce CO<sub>2</sub>, sometimes resulting in deposition of calcium carbonate. Dental plaque lowers pH, which may result in dental caries.

## SELECTED PUBLICATIONS

**ADHESION**  
 1. Camper AK, Hayes JT, Sturman PJ, Jones WL, Cunningham AB. Effects of motility and adsorption rate coefficient on transport of bacteria through saturated porous media. *Appl Environ Microbiol*. 1993; 59(10):3455-3462.  
 2. Hall-Stoodley L, Costerton JW, Stoodley P. "Bacterial biofilms: From the environment to infectious disease." *Nat Rev Microbiol*. 2004; 2(2):95-108.

**EXTRACELLULAR MATRIX**  
 3. Clark ME, Edelman RE, Duley ML, Wall JD, Fields MW. Biofilm formation in *Desulfotributis vulgaris* Hildenborough is dependent upon protein filaments. *Environ Microbiol*. 2007; 9(11):2844-2854.  
 4. Hornemann JA, Lysova AA, Codd SL, Seymour JD, Busse SC, Stewart PS, Brown JR. Biopolymer and water dynamics in microbial biofilm extracellular polymeric substance. *Biomacromolecules*. 2008; 9(9):2322-2328.

**SPECIES DIVERSITY**  
 5. James GA, Swigger E, Wolcott R, deLancey Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS. Biofilms in chronic wounds. *Wound Repair and Regeneration*. 2008; 16(1):37-44.  
 6. Taffs R, Aston JE, Briley K, Jay Z, Klatt CG, McGlynn S, Mallette N, Montross S, Gerlach R, Inskeep WP, Ward DM, Carlson RP. In situ approaches to study mass and energy flows in microbial consortia: A syntrophic case study. *BMC Systems Biology*. 2009; 3:114.

**OXYGEN GRADIENTS**  
 7. de Beer D, Stoodley P, Roe F, Lewandowski Z. Effects of biofilm structures on oxygen distribution and mass transport. *Biochim Biophys Acta*. 1994; 43(1):113-138.  
 8. Xia KD, Stewart PS, Xia F, Huang C-T, McFeters GA. Spatial physiological heterogeneity in *Pseudomonas aeruginosa* biofilm is determined by oxygen availability. *Appl Environ Microbiol*. 1998; 64(10):4035-4039.

**PHENOTYPIC HETEROGENEITY AND DIFFERENTIATION**  
 9. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J Bacteriol*. 2002; 184(4):1140-1154.  
 10. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. *Nat Rev Microbiol*. 2008; 6(3):199-210.

**DIFFUSION LIMITATION**  
 11. Costerton JW, Lewandowski Z, de Beer D, Caldwell RB, Korber D, James G. Biofilms: the customized microclimate. *J Bacteriol*. 1994; 176(8):2137-2142.  
 12. Stewart PS. Diffusion in biofilms. *J Bacteriol*. 2003; 185(5):1485-1491.

**ANTIMICROBIAL TOLERANCE**  
 13. de Beer D, Srinivasan R, Stewart PS. Direct measurement of chlorine penetration into biofilms during disinfection. *Appl Environ Microbiol*. 1994; 60(12):4339-4344.  
 14. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet*. 2001; 358(9276):135-138.

**GENETIC & BIOCHEMICAL BASES**  
 15. Davies DG, Parsek MR, Pearson JP, Gjellevoll C, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*. 1998; 280(5361):295-298.  
 16. Mah T-P, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature*. 2003; 426(6964):306-310.

**PERSISTENT INFECTIONS**  
 17. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. *Science*. 1999; 284(5481):1318-1322.  
 18. Jessia AI, Franklin MJ, Greenberg E, Sasaki M, Lord CI, Bleazard J, Duffy JE, Beyenal H, Lewandowski Z. Compromised host defense on *Pseudomonas aeruginosa* biofilms: Characterization of neutrophil and biofilm interactions. *J Immunol*. 2003; 171(8):4329-4339.

**DETACHMENT AND DISPERSAL**  
 19. Peyton BM, Characklis WG. A statistical analysis of the effect of substrate utilization and shear stress on the kinetics of biofilm detachment. *Biotech Bioeng*. 1993; 47(7):729-735.  
 20. Sellam A, Al-Niem T, McInerney K, Brunfield S, Nantel A, Suci P. A Candida albicans early stage biofilm detachment event in rich medium. *BMC Microbiol*. 2009; 9(1):25.

**VISCOELASTICITY**  
 21. Klapper P, Rupp CJ, Cargo R, Puredorff B, Stoodley P. A viscoelastic fluid description of bacterial biofilm material properties. *Biotech Bioeng*. 2002; 80(3):289-296.

**HYDRODYNAMICS**  
 22. Cunningham AB, Characklis WG, Abeden F, Crawford D. Influence of biofilm accumulation on porous media hydrodynamics. *Environ Sci and Tech*. 1991; 25(7):1305-1311.  
 23. Seymour JD, Codd SL, Giering EL, Stewart PS. Magnetic resonance microscopy of biofilm structure and impact on transport in a capillary bioresactor. *J Magn Reson*. 2004; 167(2):322-327.

**MINERAL TRANSFORMATIONS**  
 24. Sani RK, Peyton BM, Annette JE, Geesey GG. Reduction of uranium (VI) under sulfate-reducing conditions in the presence of Fe(II) (hydr) oxides. *Geochimica et Cosmochimica Acta*. 2004; 68(12):2639-2648.  
 25. Mitchell AC, Dideriksen K, Spangberg LH, Cunningham AB, Gerlach R. Microbially enhanced carbon capture and storage by mineral-trapping and solubility-trapping. *Environ Sci Technol*. 2010; 44(13):5270-5276.

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