The Biofilm Matrix: A Complicated Problem

OEST 740

013008

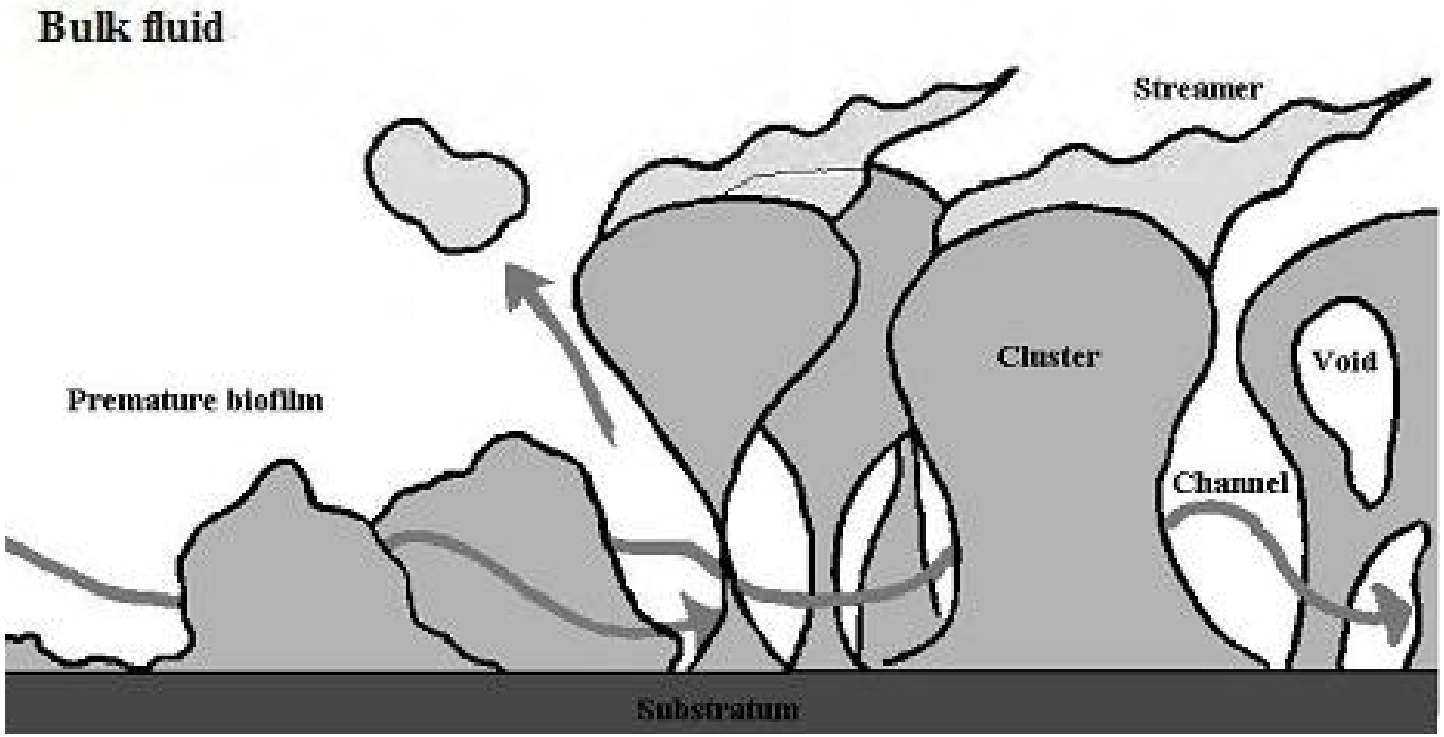
Outline

- Underlying complications in predicting biofilm structure
- Heterogeneity of structure
- Individual factors
  - Limited diffusion
  - Matrix components
  - Environmental
  - Genetic
  - Gradients
- Important implications

Complications

- Infinite range of constantly changing microenvironments
- Immobilized enzyme system – where environment and enzymatic activities are constantly changing
- Enormous diversity in composition and timing of synthesis in exopolysaccharides
- Varying matrix components (EPS, cells, protein, cellular detrius, etc.)

Heterogeneity of Structure



Limited Diffusion

- Planktonic
  - Agitation
    - fluid flow transport - convection
  - No net convective flow of fluid across membrane
    - Molecular diffusion important
- Biofilm
  - Reduced fluid flow
  - Molecular diffusion distance increased
    - Diffusion equilibrium time increase 3 to square distance
  - Throttle effect of substratum and biofilm
    - Convection transport
  - Cell clusters

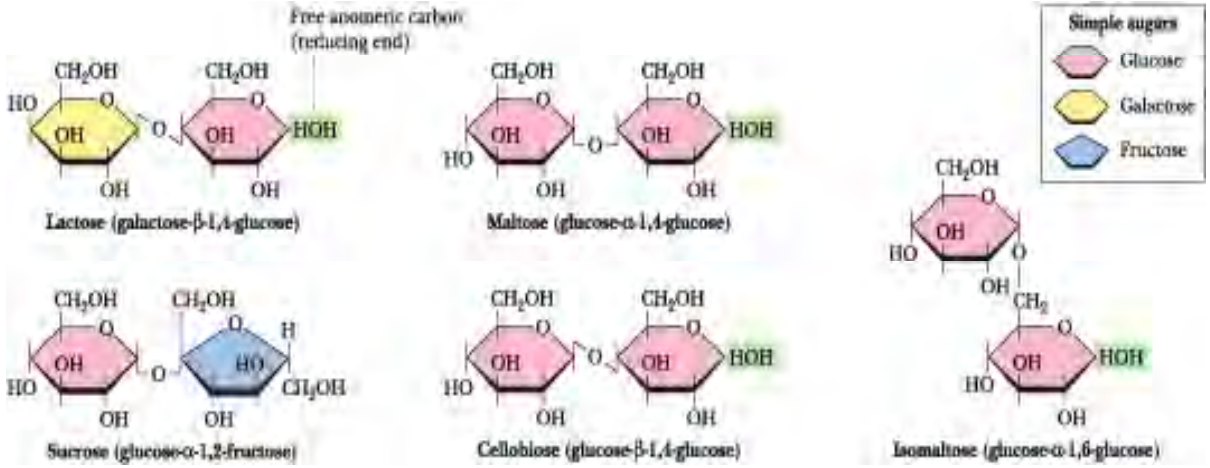
Role of EPS

- Mutant strains unable to synthesis EPS are unable to form biofilms
- Play roles in determining morphology, structure, coherence, physio-chemical properties and activity of matrix
- vast number of microbial species, interactions, and range of polysaccharides = infinite number of permutations

EPS

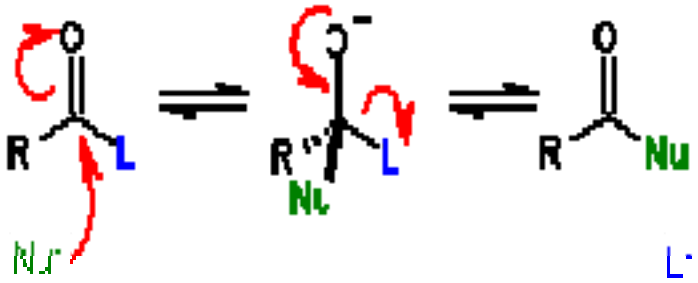
- Primary conformation is determined by structure and composition of polysaccharides

Sucrose

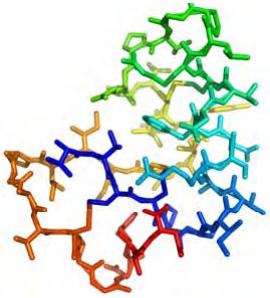


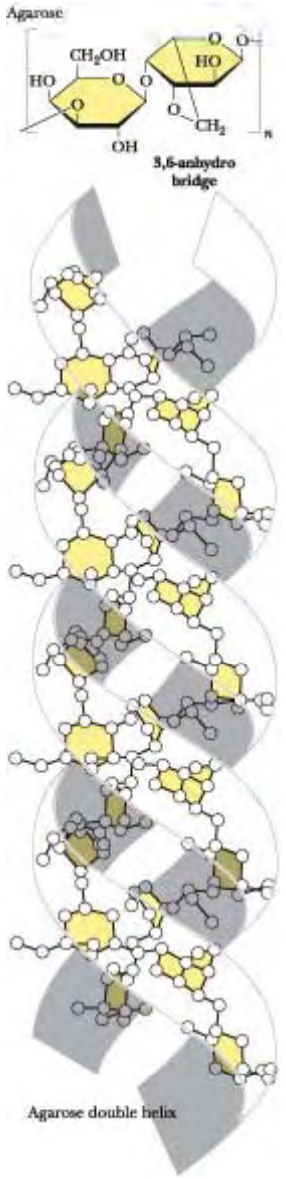
Secondary Conformation

- Aggregated helices
- Influenced by Acyl substitutions



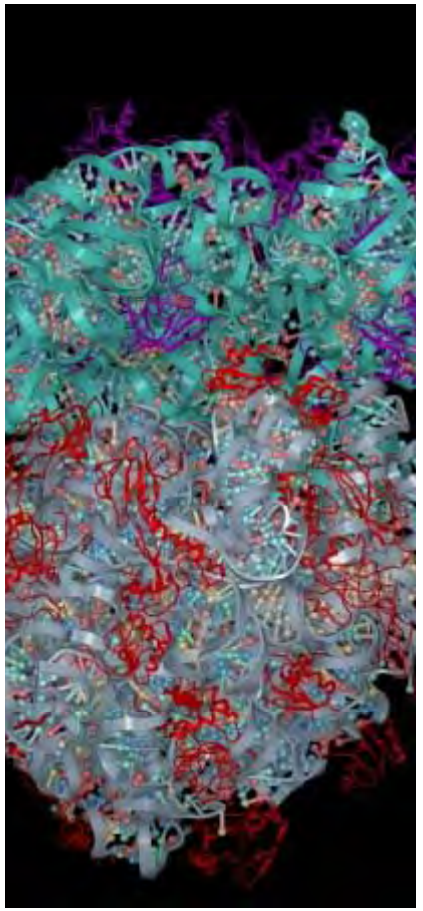
- Backbone composition effects elasticity
  - (1-4)β or (1-3)β linkages
    - *Xanthomonas campestris*
  - (1-2)α or (1-6)α linkages
    - dextans





Tertiary Structure

- Interact with wide range of other molecular species (lectins, proteins, lipids, ions, etc.)
- Dominant forces
  - Electrostatic and hydrogen bonding
- Macromolecule ‘fits’



Contribution of EPS

- Relatively soluble, large molecular mass, yields highly viscous aqueous solutions
  - Weak gels
- Effect binding of water
  - Hyaluronic acid - 1kg water g^{-1}
  - cellulose, mutan, etc.
- Contribute to mechanical stability
- Affect hydrophobicity
- Interaction with ions

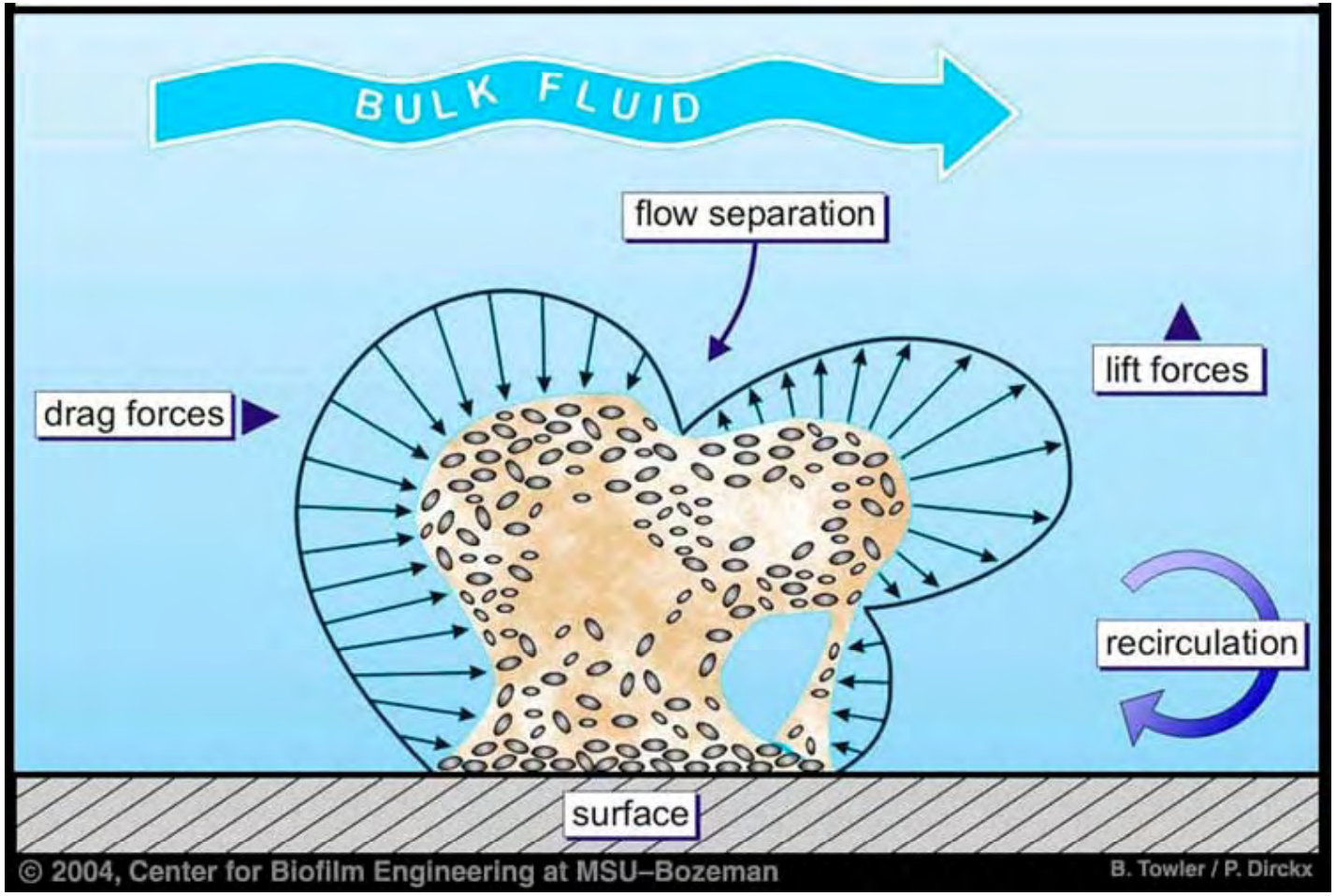
Extracellular polymeric material

- Comprise 50 – 90% of total organic matter in biofilms
  - Includes carbohydrates, proteins, nucleic acids, lipids/phospholipids and humic acids
  - Hydrophobic/hydrophilic regions and distribution
    - Ion-exchange potential
    - Sorption properties
  - Vary in response to environmental conditions and nutrient availability
  - Only 1-2% required to retain 98-99% of water

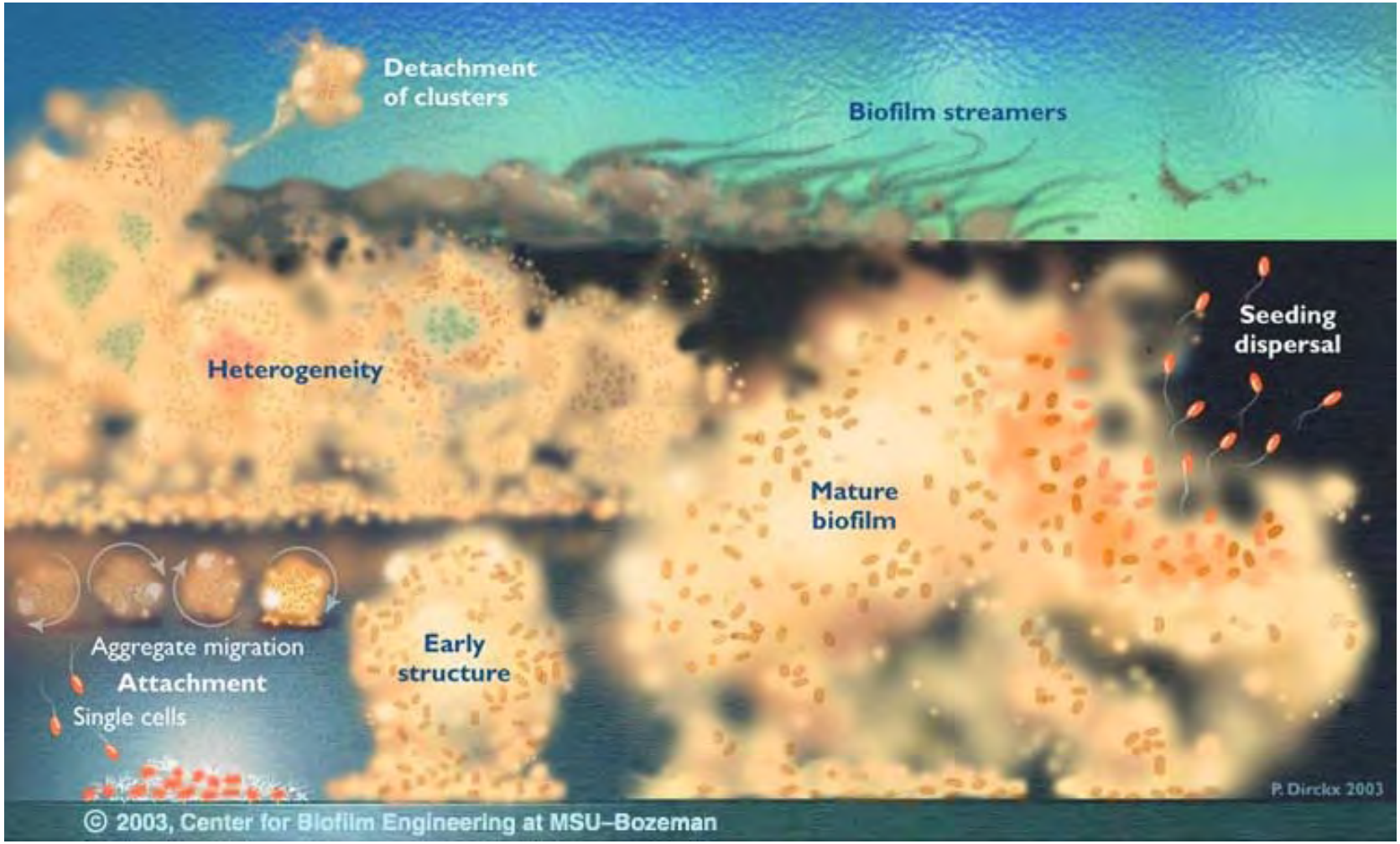
Environmental Determinates

- Surface chemistry
- Surface roughness
- Physical forces
  - Hydrodynamic shear
  - Nutrient conditions
  - Chemical conditions

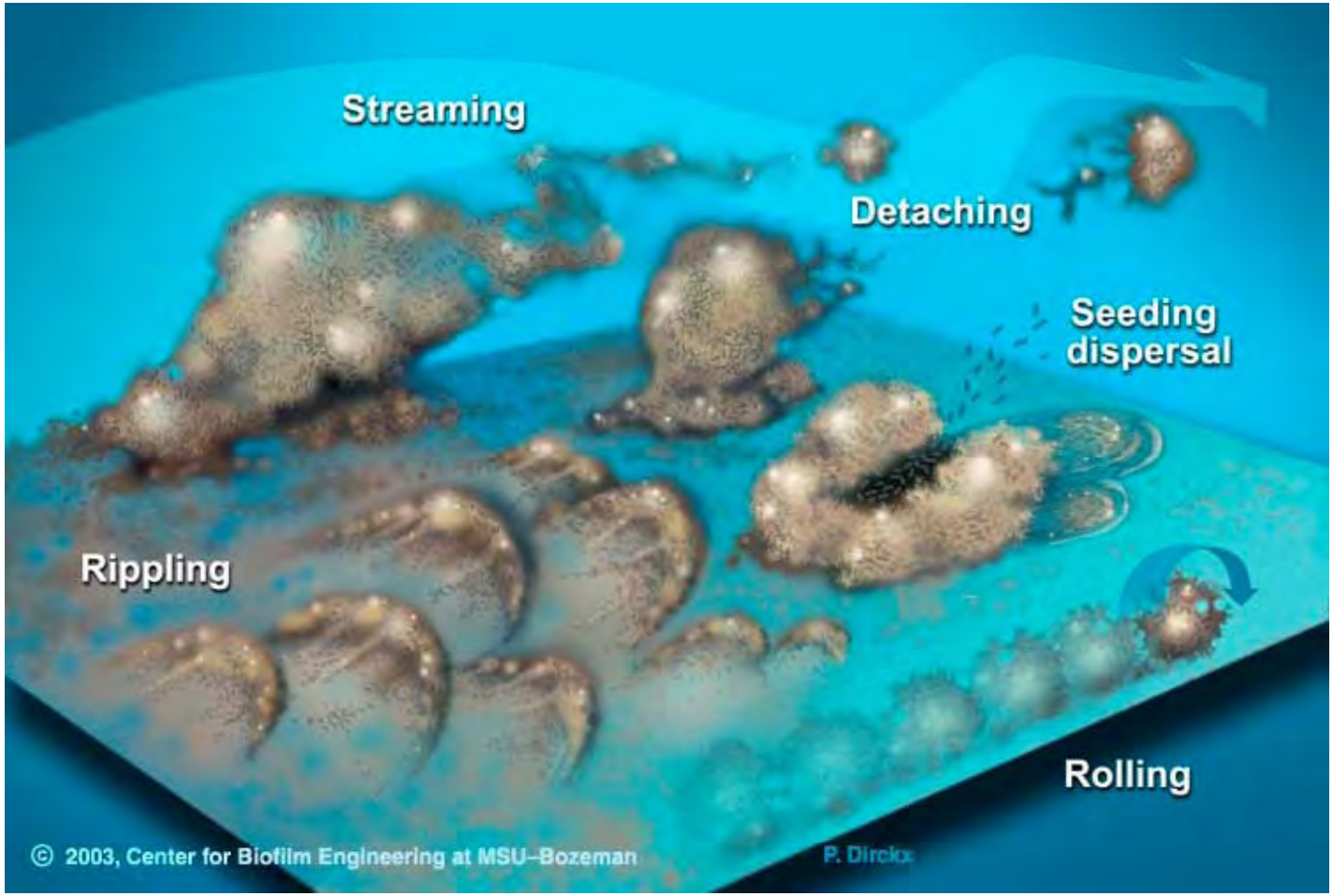
Hydrodynamic



Effect of Shear Stress



Motion


Streaming
Detaching
Seeding
dispersal
Rippling
Rolling
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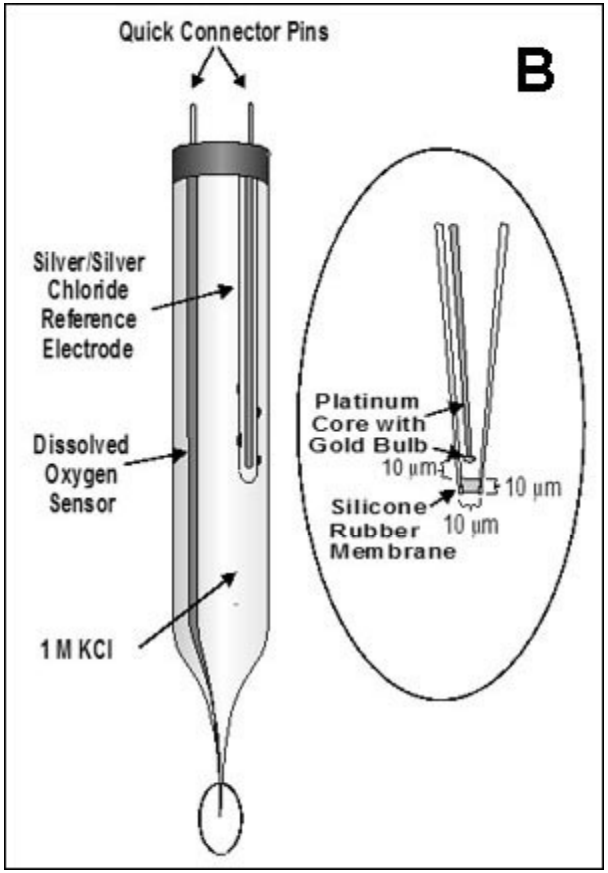
Genetic

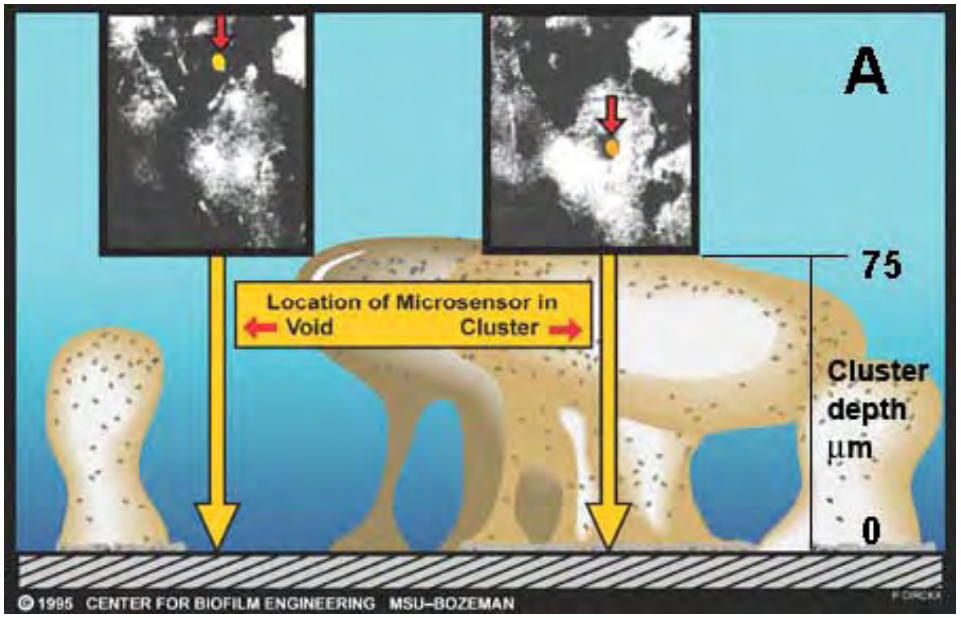
- Structure could be controlled through cell-signal production
- Quorum sensing – found in Gram positive and negative bacteria
  - Regulates gene expression
- Some bacterial species unable to produce QS signal molecules are unable to achieve complex biofilm structure
- Some QS inhibitor molecules – halogenated furanones – have been shown to inhibit the production of complex structure in biofilms
- Can be affected by hydrodynamic properties in/and around biofilm

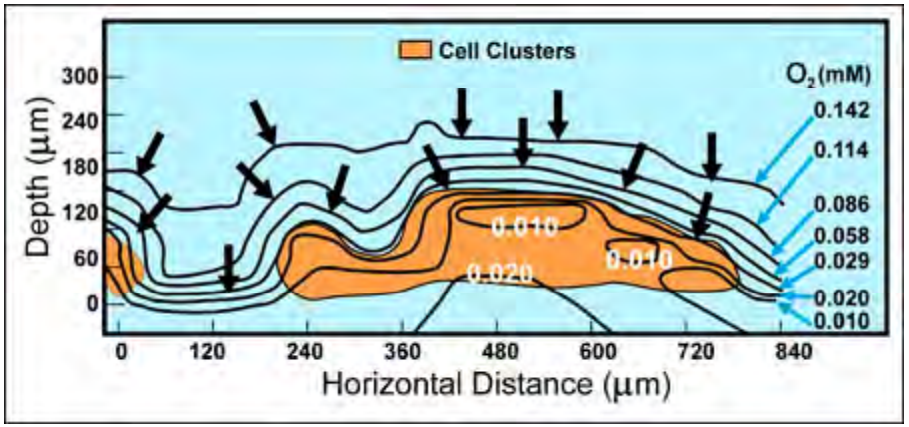
Gradients

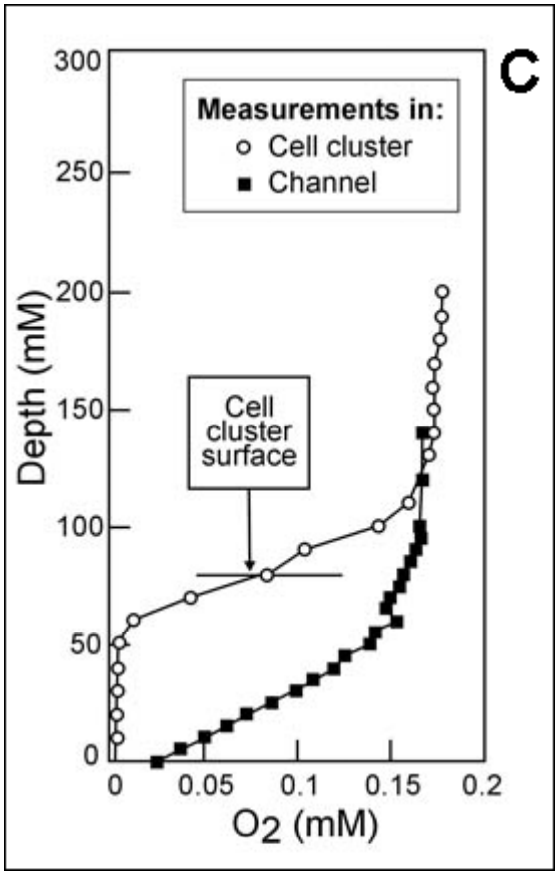
- Metabolic substrates and products
  - Oxygen
  - Nitrate
  - Nitrite
  - Ammonia
  - pH
  - Sulfide methane
- Microbial species
- Metabolic activity



Oxygen gradients

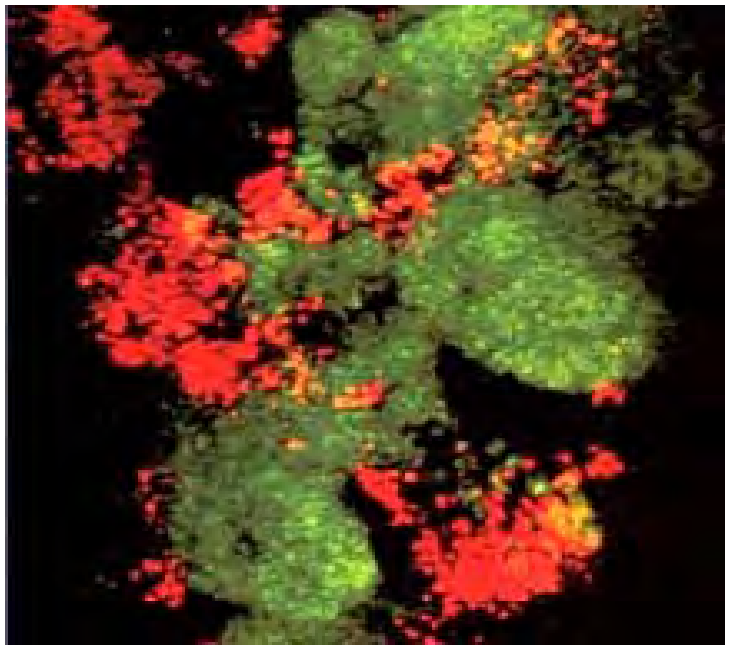




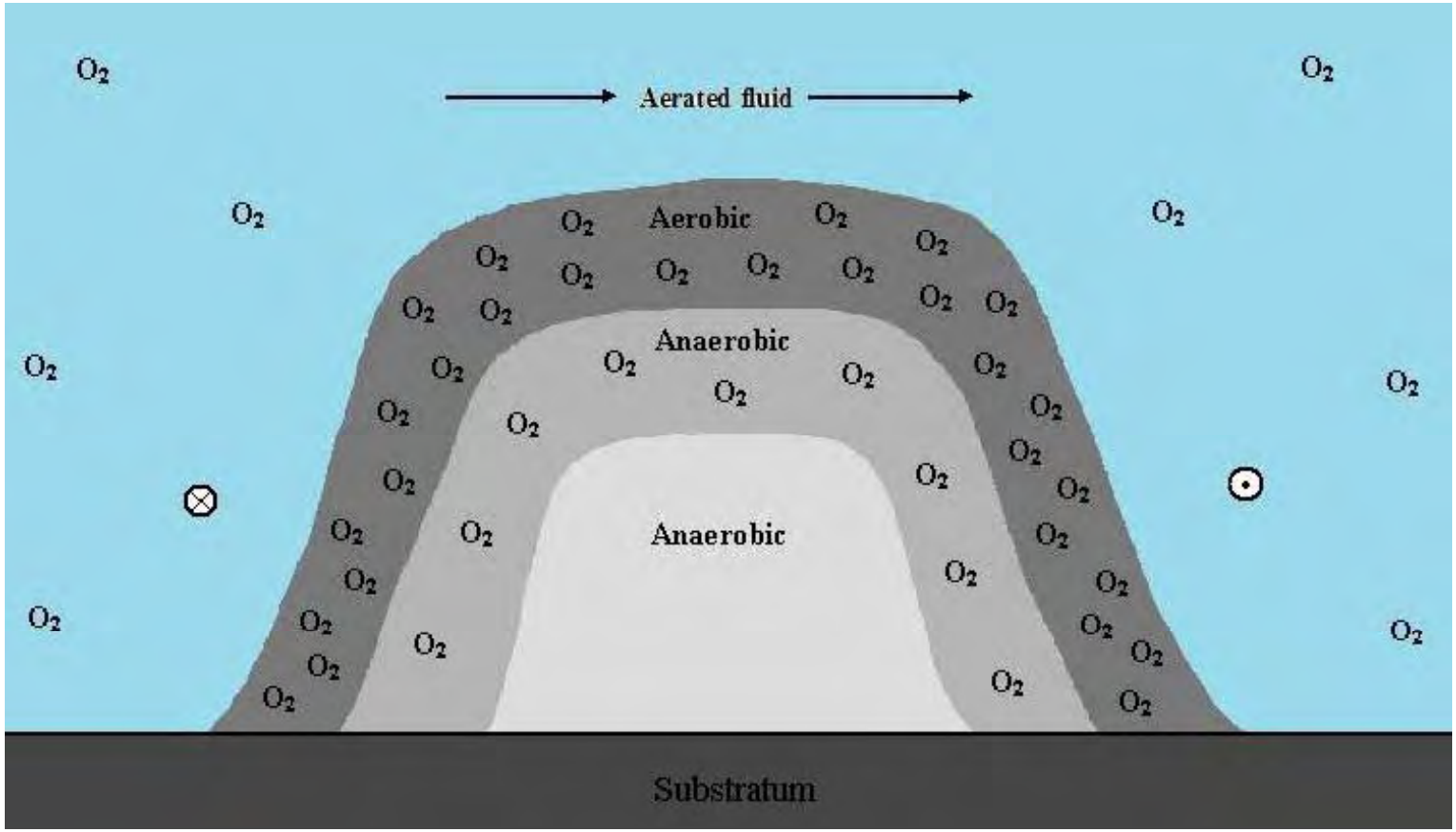


Reaction-diffusion phenomena

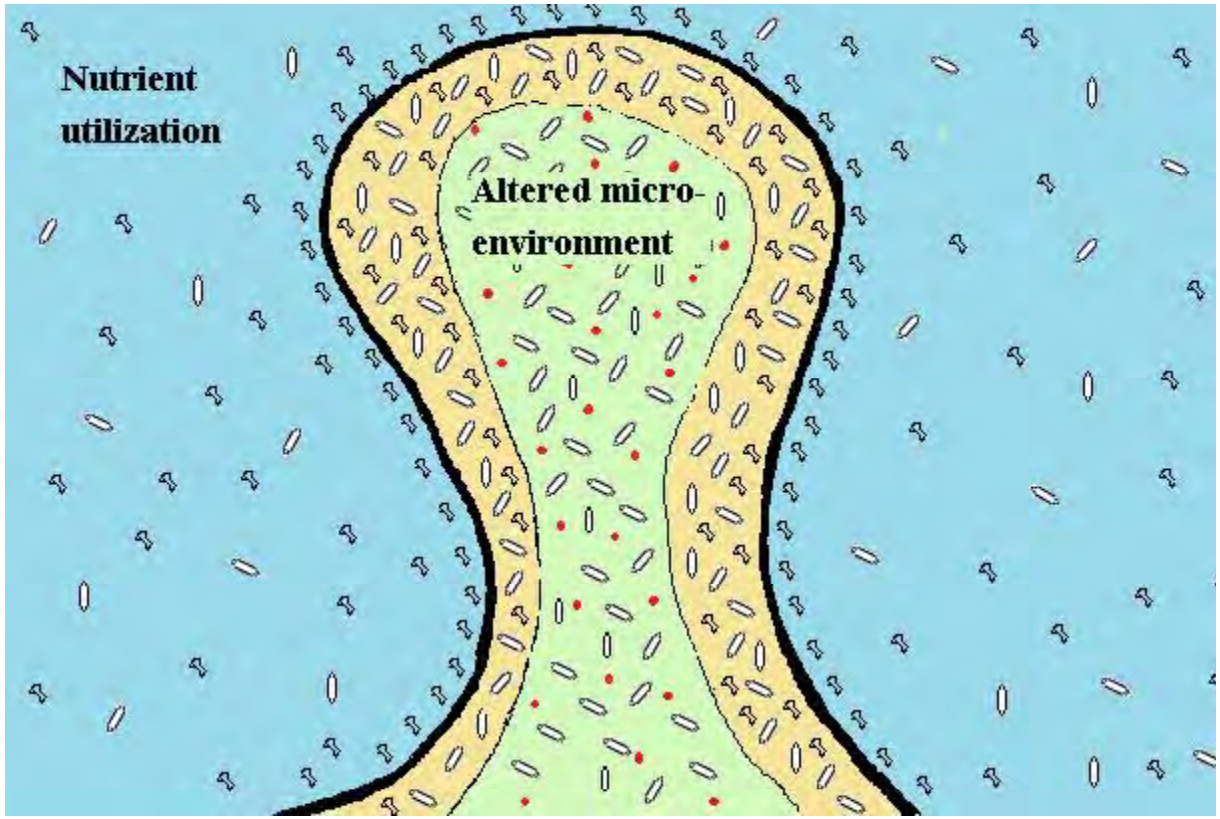
- Allow for the co-existence of diverse species
- Regulate the distribution of specific bacterial species based on metabolic processes
- Activities of cooperative species associations can affect gradients in different areas within the biofilm



Microenvironments



Nutrient Gradients



Implications

- The exact structure is probably unique feature of environment in which it is formed
  - Snap-shot in time
- Matrix provides protection from environmental attack and abuse
- Differences in the response of biofilm architecture to environmental conditions reflect differences in composition of EPS matrix (EPS, cells, protein, cellular detritus, etc.)
- The composition and structure of matrix plays an important role in bacterial survival.