Biofilm growth, structure and function

OEST 740

011508

Biofilm basics

- Biofilms are now believed to be the primary mode of existence for bacteria in aqueous environments.
  - 1,000 – 10,000x greater populations than planktonic
- Defined as a consortium of microorganisms encased in a complex 3-D gelatinous matrix of extracellular material secreted by the inhabiting organisms.
- The establishment, maintenance and existence of biofilm communities are highly complex, socially organized processes.

Biofilm biology

- Investigates the consequences of the close association of microorganisms at interfaces.
  - Physiological adaptation to the proximity of other cells and surfaces.
- Biofilms are highly diverse in nature
- Common principals
  - Attachment
  - Coadhesion
  - Regulation of biofilm phenotype
  - Biofilm architecture

Interfaces

- Solid:Liquid
  - Most common type of biofilm
- Gas:Solid (often exposed to liquids)
  - Lichen, trickling filters, myxobacterial swarms
- Gas:Liquid
  - Neuston, penicillin produced by fungal fermentation
- Liquid:Liquid
  - Hydrocarbon oxidizing biofilm at oil:water interfaces
- Solid:Solid (periodically exposed to liquid)
  - Endolithotrophic communities

Biofilm impact

- The development of biofilms has important impacts
  - Bacterial properties
    - Virulence
    - Survival
    - Diversity
  - Settlement and metamorphosis of higher organisms
  - The physical and chemical structure of the surfaces on which they are established

Biofilm Impact

- Thus, they are potentially relevant to a wide variety of disciplines that aim to study processes in aqueous environments
  - Oceanography
  - Ecology
  - Biology
  - Engineering
  - Medical
  - Dental

Biofilm Composition

Component	% Total	Description	Origin
Water	Up to 95%	Characteristics determined by dissolved solutes	
Microbial cells	2-5 %		
Polysaccharides	1-2 %	Neutral and polyanionic ; homo and heteropolysaccharides	Extracellular
Proteins	<1-2%	Enzymes	Extracellular and cell lysis
DNA and RNA	<1-2%		Cell lysis
Ions	?	Bound or free	

Exopolysaccharides

- EPS present in biofilms is thought to closely resemble the corresponding polymers synthesized by planktonic cells.
- Quantity of EPS depend on availability
  - Carbon (intra and extra-cellular)
  - Carbon:Limiting nutrient
  - Vary in proportions of various components
- Some are neutral macromolecules, but majority are polyanioninic
  - Uronic acids (D-glucuronic>D-galacuronic = D-mannuronic)
  - Ketal-linked pyruvate

Exopolysaccharides

- Very few are polycationic
  - *S. epidermidis*
- In nature, exist in ordered configurations of low temperature, saline conditions
  - Very long, thin molecular chains
  - Molecular mass 0.5-2.0 x 10^6 Da
- Association of structure
  - Electrostatic and hydrogen bonding are dominate forces
- Provide bacteria with backbone structure, measure of homeostasis, primitive circulatory system and a large measure of protection

Formation

- Biofilm formation begins with a transition of bacteria from the planktonic (free swimming) form to its genetically distinct attached form.
- The genetic transition occurs across the life cycle of the biofilm and is comprised of seven distinct steps
  - Conditioning
  - Contact
  - Adsorption
  - Growth
  - Production of extracellular products
  - Attachment
  - Re-entrainment

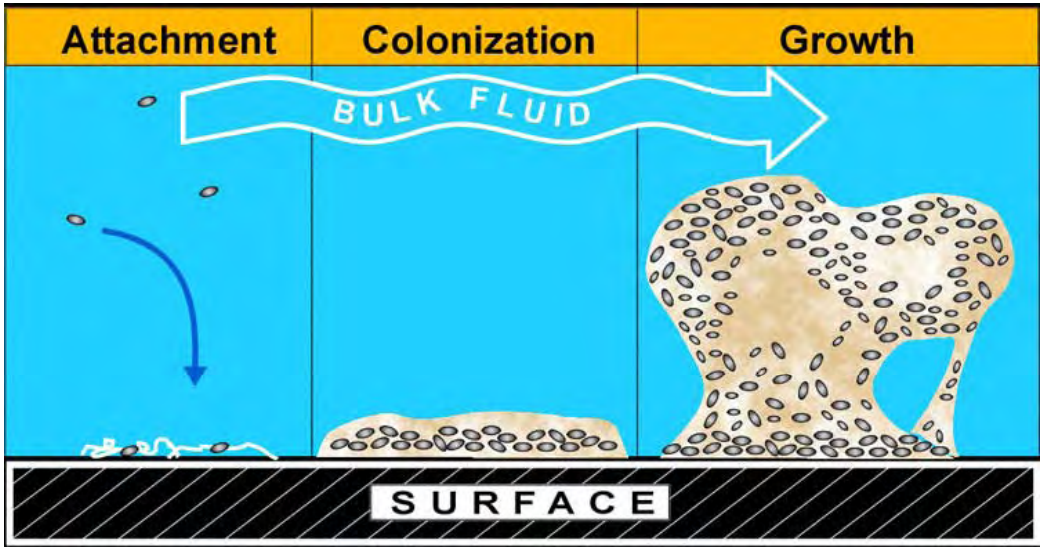


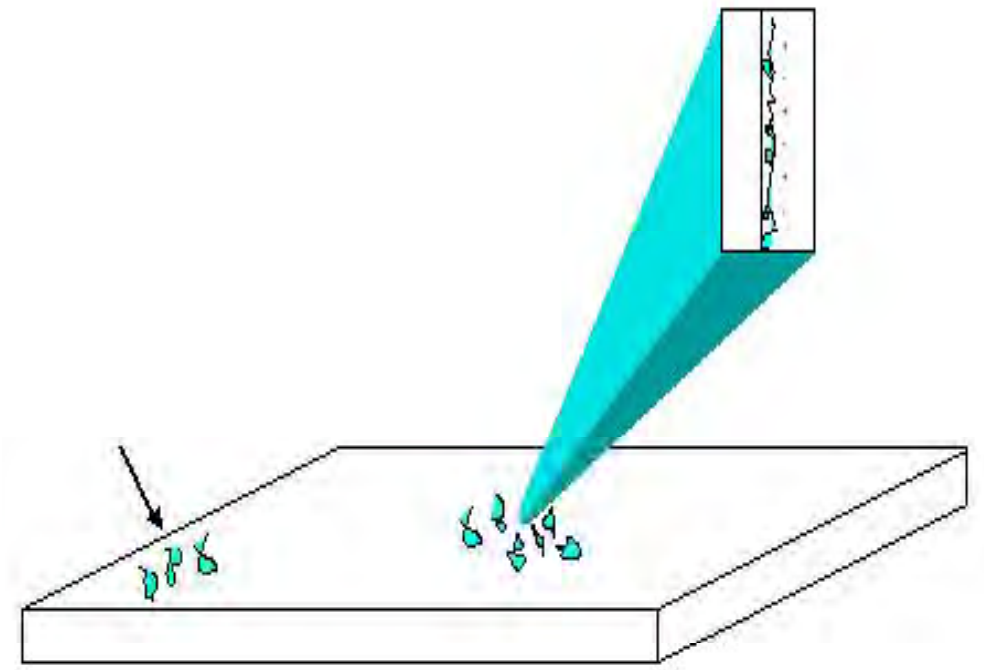
Photo: CEB : MSU-Bozeman

Step 1: Conditioning

- A clean surface is immediately covered with a conditioning film of organic molecules and macromolecules.
- Transport of molecules and small particles is rapid and as a result adsorption of conditioning film occurs instantaneously
- The presence of the conditioning film alters the characteristics of the substratum.

Effect

- Substrate hydrophobicity decreases
- Substrate obtains a negative charge
- Substrate potentials increase or decrease
- Critical surface tensions increase or decrease



Step 2: Contact

- Bacteria in fluid contact the substrate via mass transport mechanisms
- Strongly influenced by mixing in the bulk fluid
  - Related to flow regime
- Laminar flow transport
  - Sedimentation
  - Motility
  - Molecular diffusion
- Turbulent flow transport (larger particles)
  - Convection
  - Diffusive transport
- Bacteria penetrate the viscous sublayer (~1 cm) via eddy diffusion
- Bacteria actively migrate through the diffusive sublayer using pili (~1 mm)

Step 3: Absorption

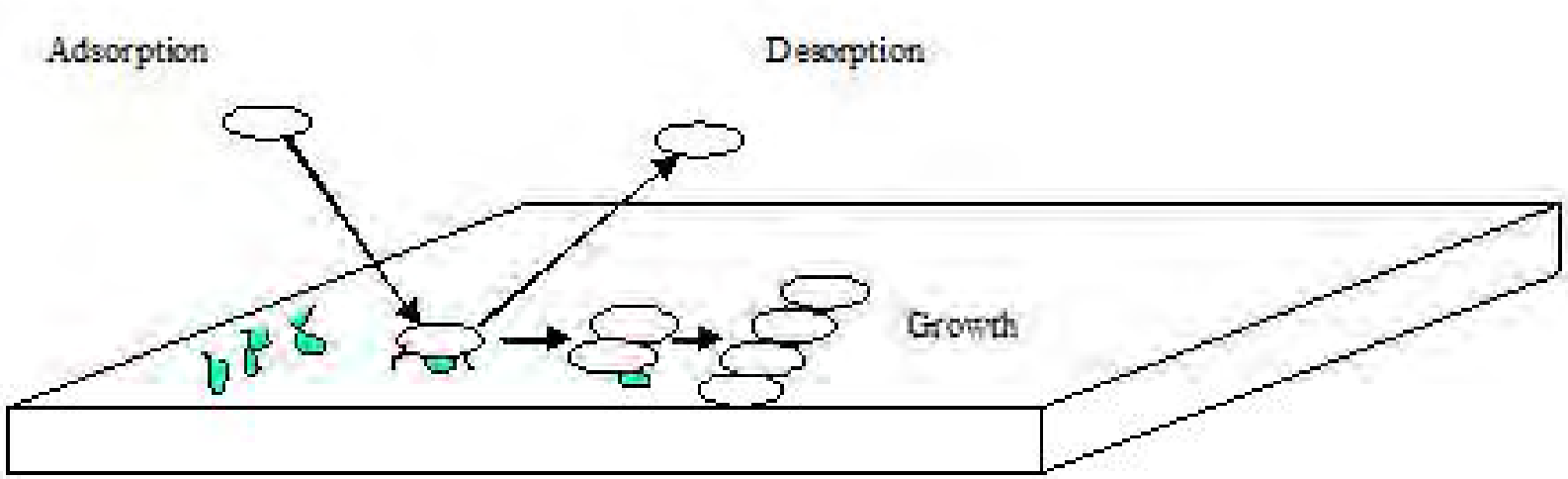
- At the substrate the cells absorb reversibly or irreversibly
  - Primary (early) colonization
  - mediated through specific or non-specific physiochemical interactions with components of conditioning film
  - Adsorption – accumulation of cells directly on surface of substrate
  - Desorption – re-entrainment of cells into the bulk fluid
    - Complex process

Adsorption

- Initial adsorption occurs through long-range (100s nm), weak interactions with low specificity
  - Electrostatic or van der Waals forces
- Irreversible adsorption is short-range (5nm), highly specific interaction
  - Dipole, ionic, hydrogen bonding, hydrophobic interactions, etc.
  - Can take place by secretion of EPS or fibrillar structures

Step 4: Growth

- The number of irreversibly adsorbed cells increase due to replication
  - Limited by physiological processes
  - Concentration of rate-limiting nutrient important

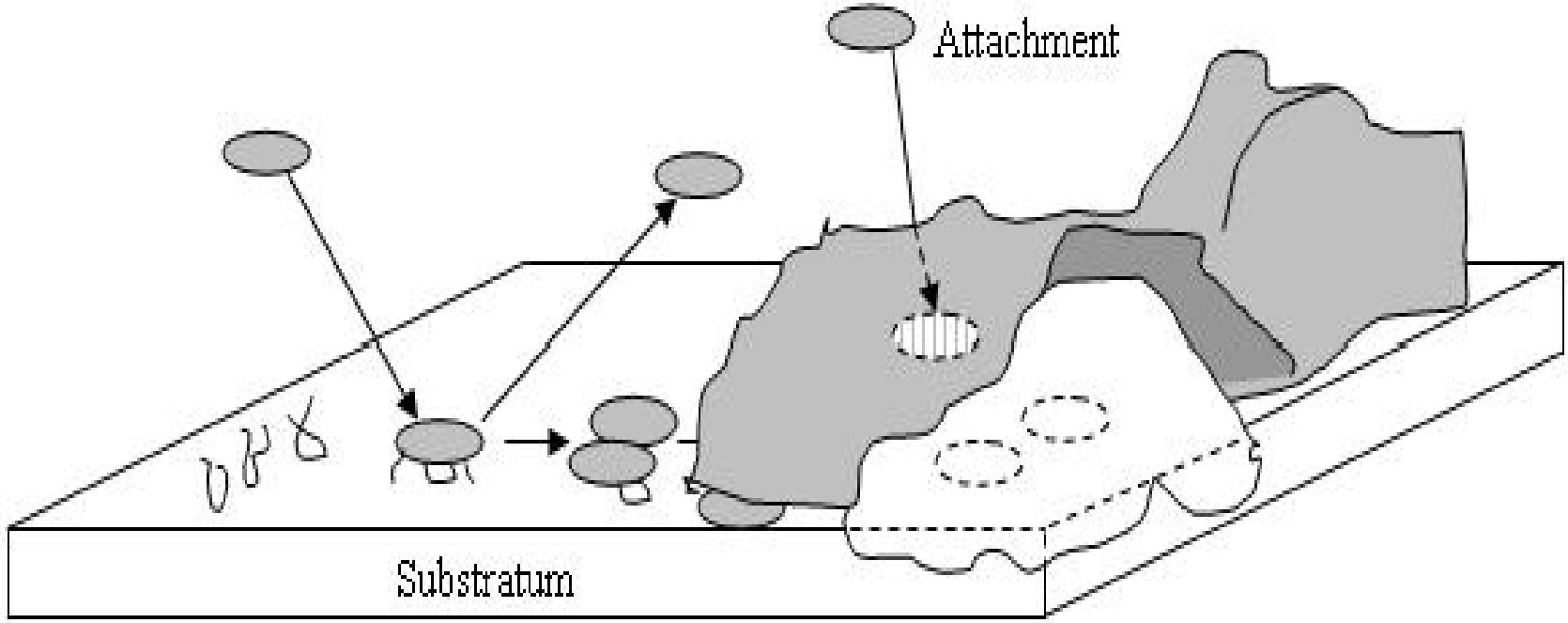


Step 5: Production of extra-cellular products

- Affixed cells transition from planktonic form to attached form
  - Processes controlled by gene encoding for the production of products

Step 6: Attachment

- Secondary (late) colonizing cells from bulk fluid attach to the existing biofilm
  - Can result in species displacement



Attachment and Coaggregation

- Coaggregation – is the attachment of distinct bacteria via specific molecules
  - Single cells in bulk fluid specifically recognize and adhere to genetically distinct cells in developing biofilm
  - Prior coaggregation in suspension followed by subsequent adhesion to existing biofilm
- Multi-species biofilms are a functional consortium that often posses a combined metabolic activity that is greater than the individual component species

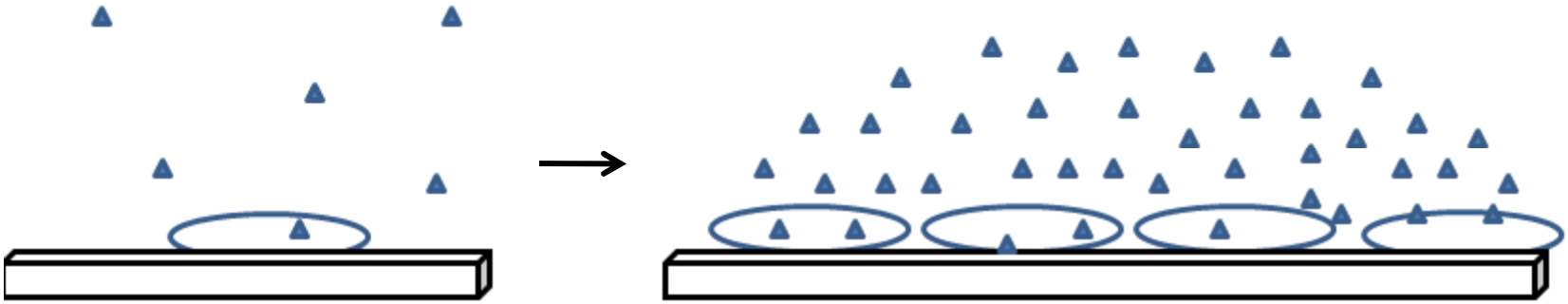
Step 7: Re-entrainment

- Cells detach from the surface and return to the bulk fluid and planktonic form of growth
- Detachment can occur
  - Erosion
  - Sloughing
  - Human intervention
  - Predatory grazing
  - Abrasion
  - Starvation
- Detachment can be an active or passive process leading to further survival or colonization

Biofilm degradation

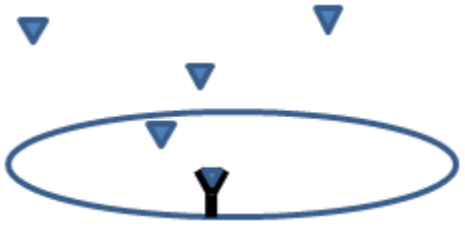
- Microorganisms require energy to “maintain” existing structures and processes
- Important process during starvation survival
- Can occur from endogenous decay and death
  - Endogenous decay – the depletion of intracellular constituents that occurs when cells have insufficient exogenous supplies
    - i.e. Starvation
  - Death – the permanent loss of a cell’s reproductive and metabolic activity

Cell-cell signaling

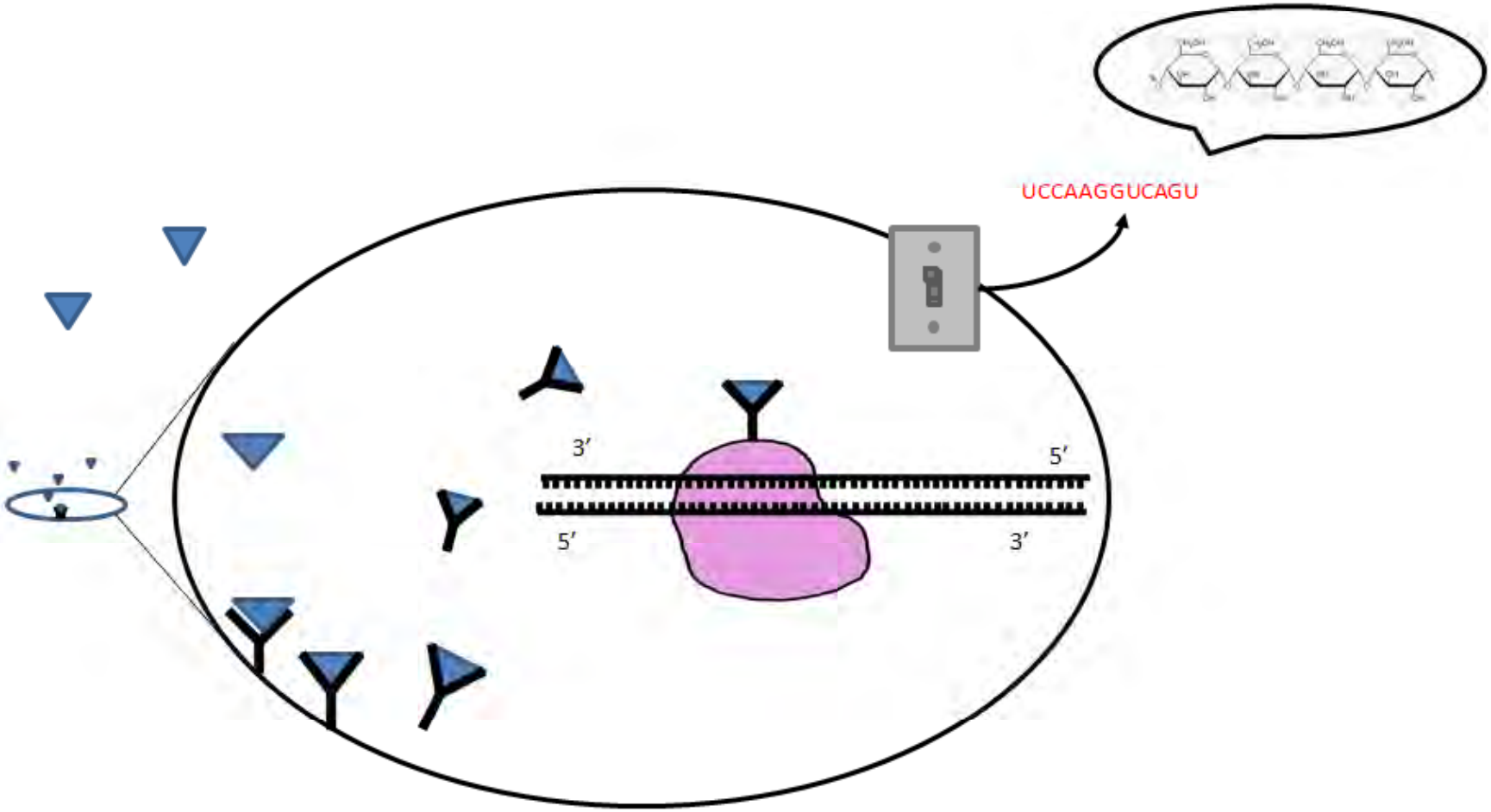


"Quorum" – the minimal number of people who must be present for a decision to be binding

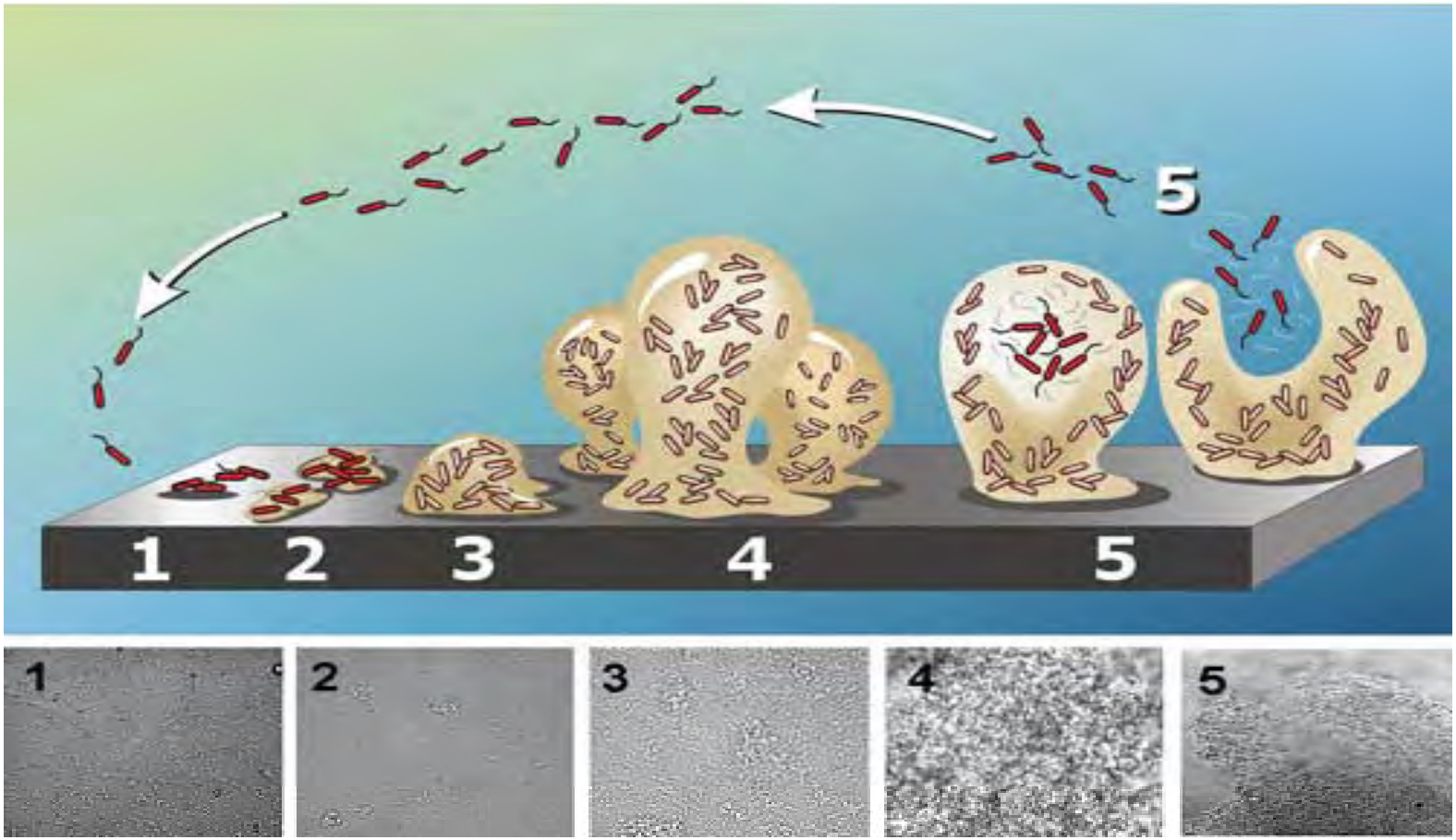
Autoinduction



Quorum Sensing – Genetic Regulation



Biofilm Formation



Biofilm Communities

- Multispecies communities
- Described in terms of
  - Organisms
  - Structure
  - Interactions
  - Coordination

Biofilm Advantages

- Enhanced scavenging of nutrient from bulk liquid and surface
- Physiological alterations
  - Enhanced growth rates
  - Higher DNA synthesis and RNA turnover rates
  - Enhanced resistance
  - Enhanced virulence
  - Greater local diversity
- Physical protection and stabilization
- High densities – provide framework for coordinated and socialized behavior

Questions ?