AN INTRODUCTION TO BIOFILMS IN THE DAIRY INDUSTRY

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Speakers of the Day

Geoff Bayman

Justin Peterson

Speakers of the Day
Geoff Bayman

- Joined NutroChem in October 2019 as the National Sales Director
- Actively selling speciality chemicals within the food & beverage industry since 1990
- Focused on providing best practice hygiene outcomes for all our customers

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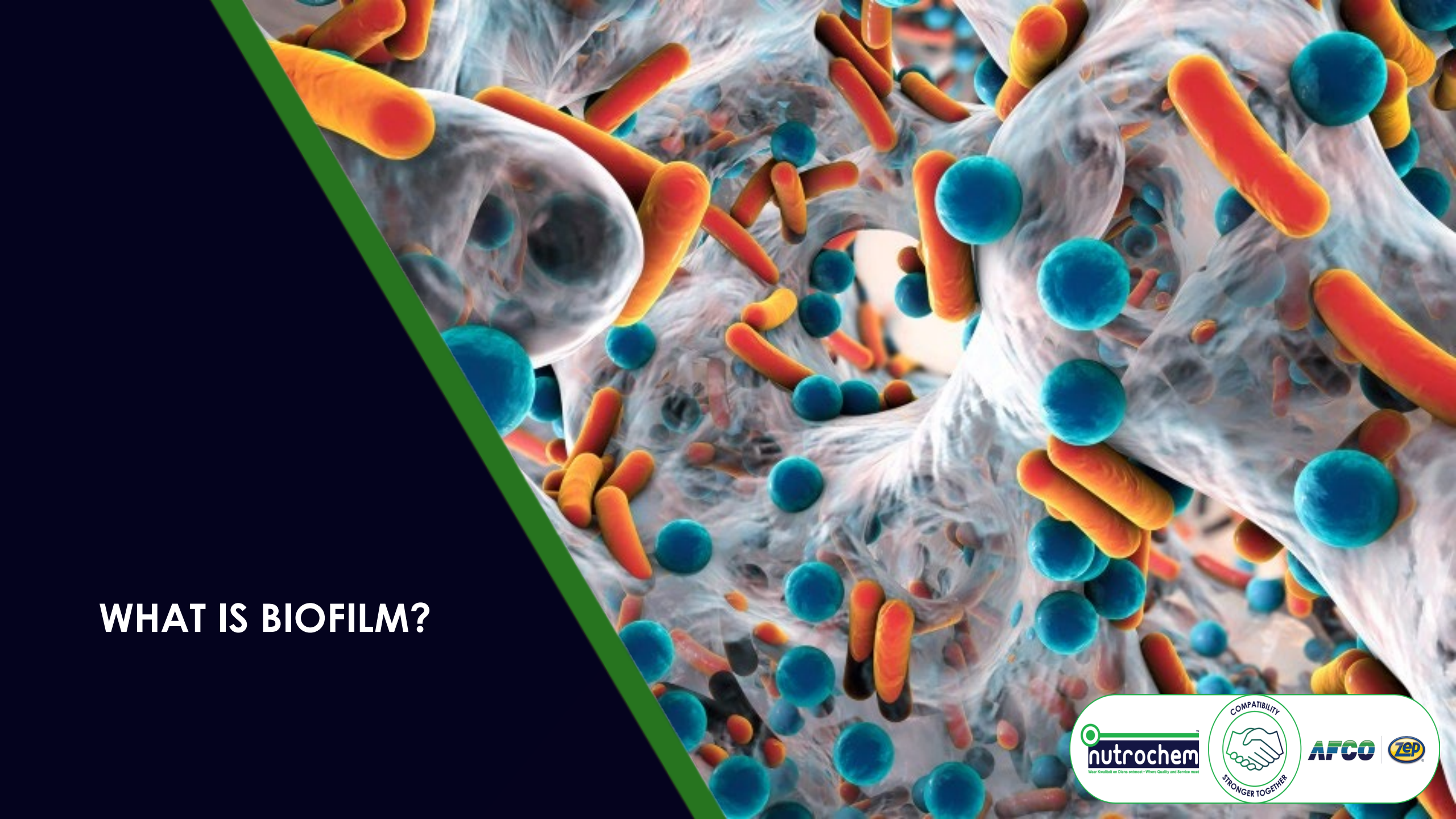
Speakers of the Day
Justin Peterson

- Director of Research and Development - AFCO | Zep's North American Food and Beverage Division.
- Joined AFCO in 2005
- Focus on Research & Development in the Quality Control laboratories of AFCO and Zep, developing new technologies.
- Food and Beverage Safety and Hygiene consultations, training, and educational presentations.

WHAT IS BIOFILM?

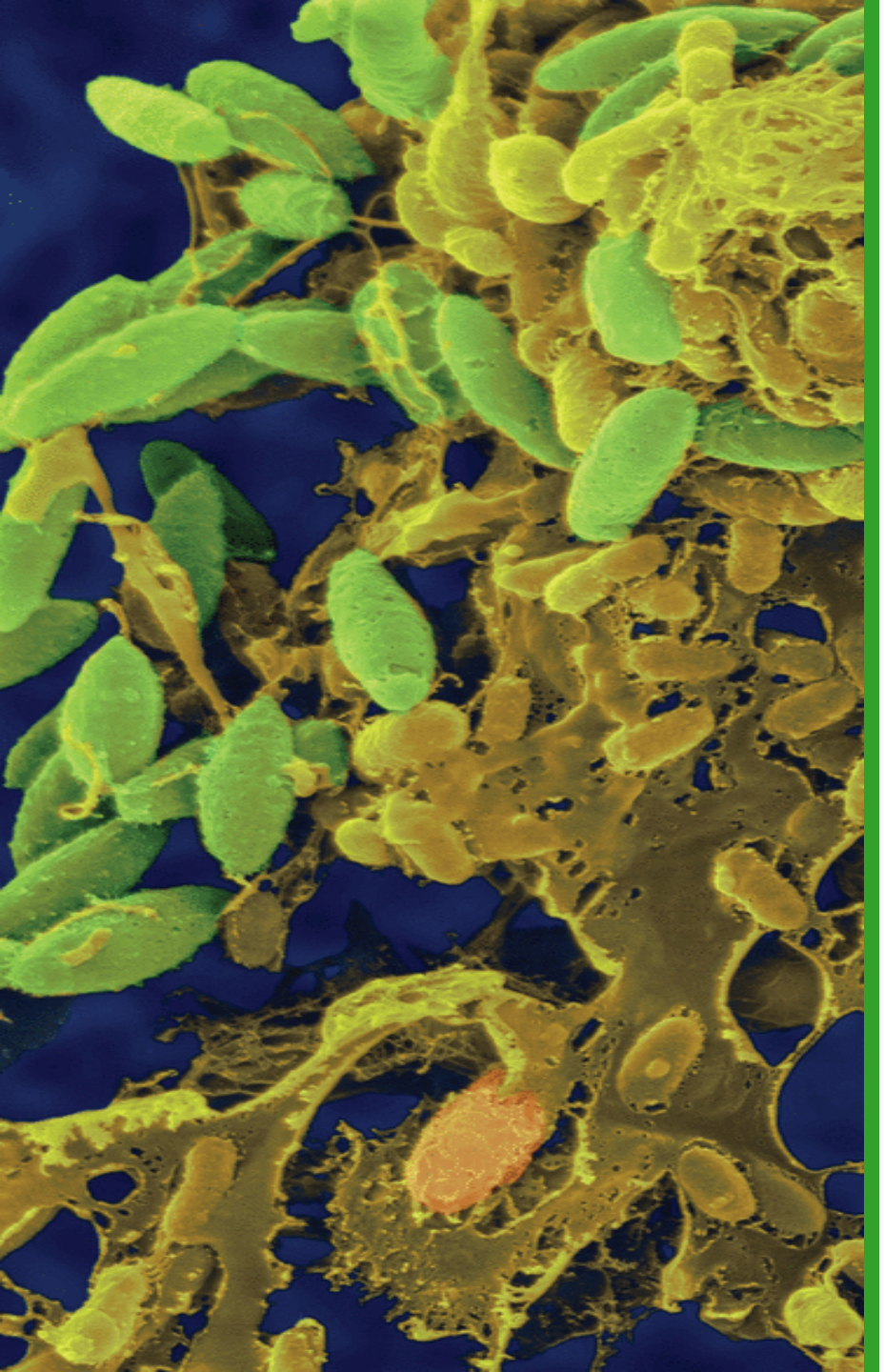


WHAT IS BIOFILM?

- A biofilm is defined as an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix.
- More simply - a biofilm can be thought of as microscopic cities in which microorganisms can live and thrive.
- Biofilms form because some bacteria excrete sticky substances, predominately polysaccharides, that adhere to environmental surfaces like stainless steel, forming the matrix of the city.

WHAT IS BIOFILM?

- A natural habitat for microorganisms
- Formed by microorganisms
- It consists of populations of microorganisms, proteins, fats, sugars (carbohydrates), byproducts, acids, mineral scales and polysaccharides, etc.
- Can be formed within 8 – 24 hours
- It develops most rapidly in flowing and wet systems where adequate nutrients and water are available



WHAT IS BIOFILM?

- Numerous species of bacteria can form biofilms including Pseudomonas and Listeria
- Non-biofilm forming bacteria, moulds & viruses can all become encapsulated in biofilms - they don't need to be able to build the city in order to live there
- Microorganisms within the biofilm are much more protected from cleaners and disinfectants than freely dispersed microbes
- Biofilms can be seen with the human eye if they become large enough, but many are invisible without the aid of a microscope

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FORMATION OF BIOFILMS

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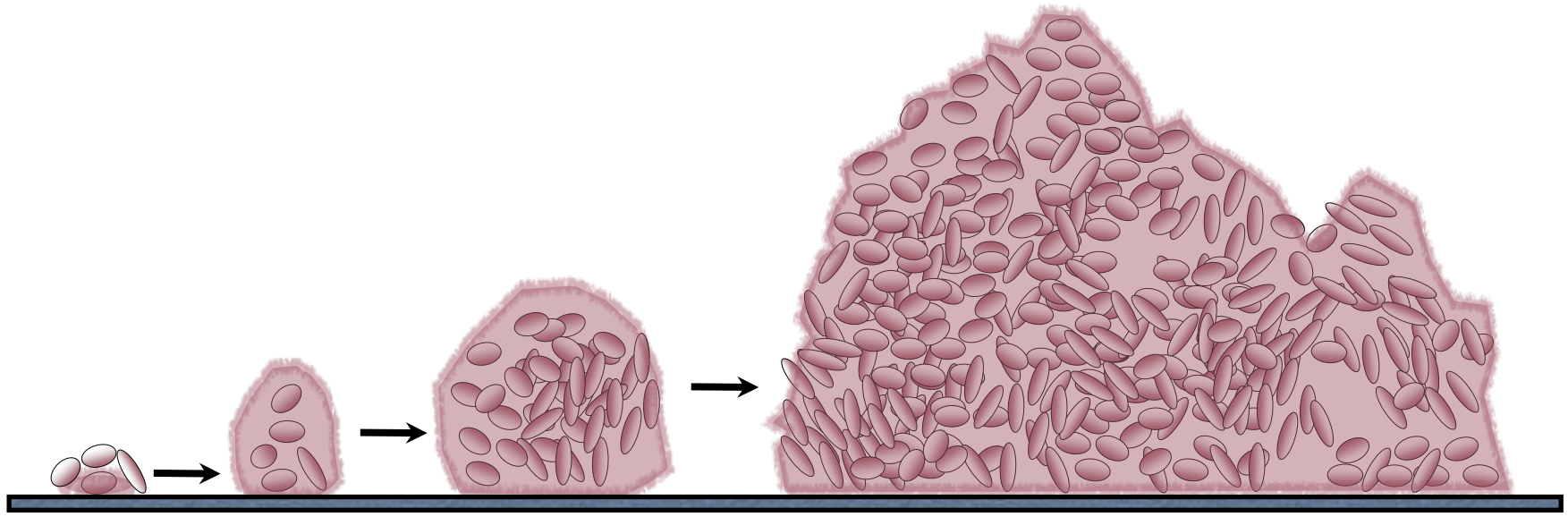
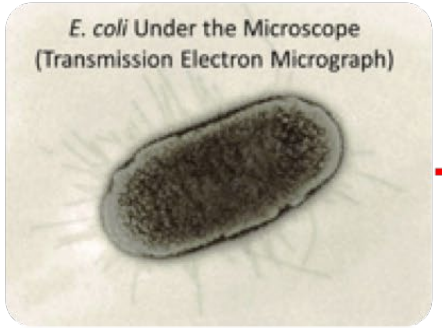
FORMATION OF BIOFILM

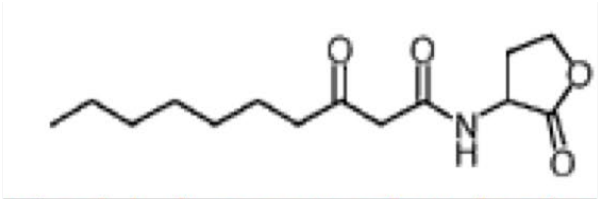
- Bacteria can attach to surfaces such as metal, glass, and plastic by weak polar forces similar to static electricity. Many bacteria also have the capability of attaching themselves to surfaces by means of finger-like structures if conditions are favorable for bacterial growth.
- Once a bacteria capable of forming a biofilm lands on a surface where nutrients are available, it attaches itself and begins secreting the sticky gel-like substance that holds the biofilm together. This gel acts like an adhesive, capturing nutrients and holding the bacterial cells in close proximity.



FORMATION OF BIOFILM

- One of the categories of compounds responsible for triggering the formation of the biofilm is N-acylated-L-homoserine lactones (AHL's)
- Once bacteria begin to release these signaling molecules, it causes changes in gene expression resulting in the formation of pili for bacterial conjugation rather than flagella for motivity as well as other changes leading to the formation biofilm.

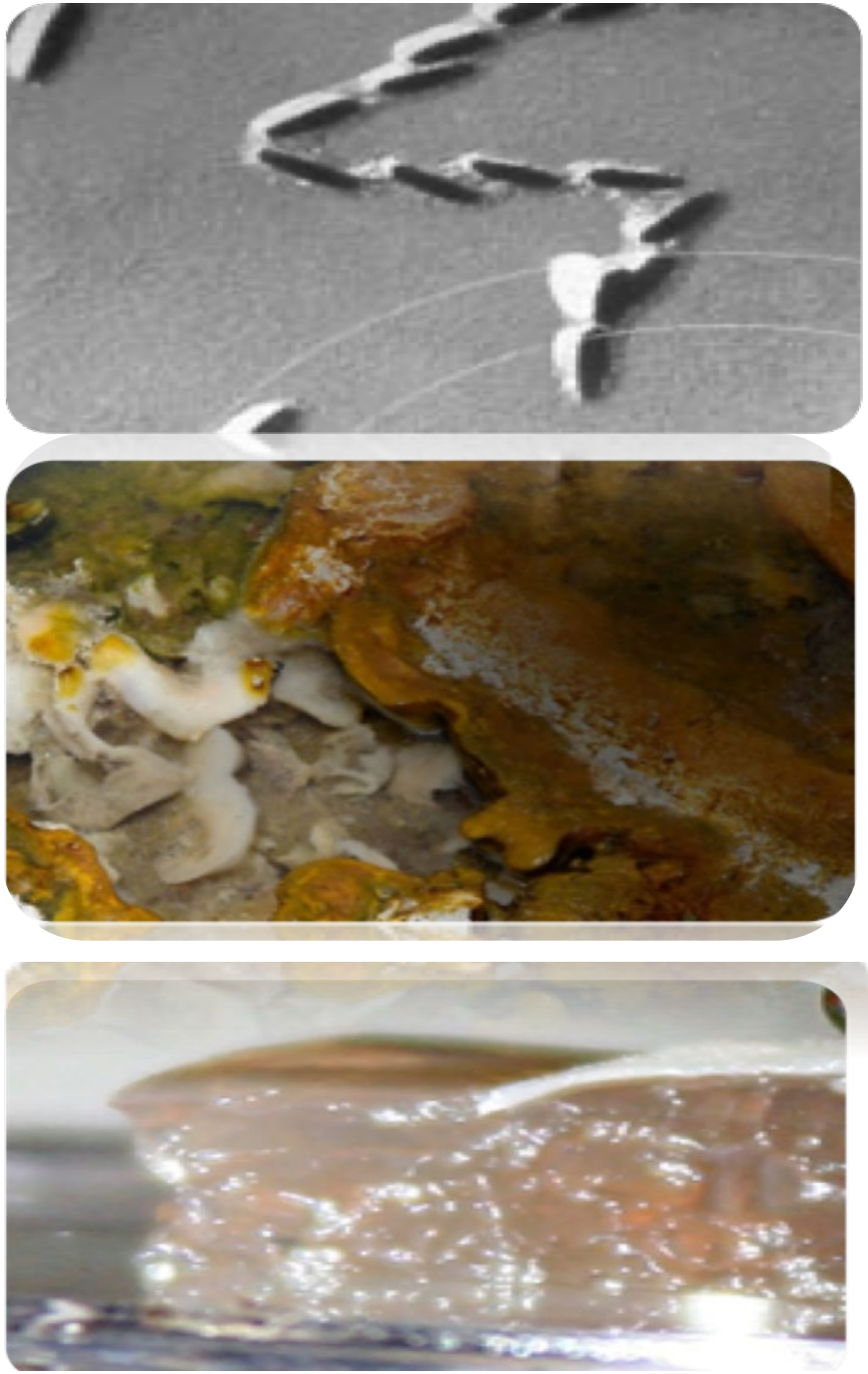




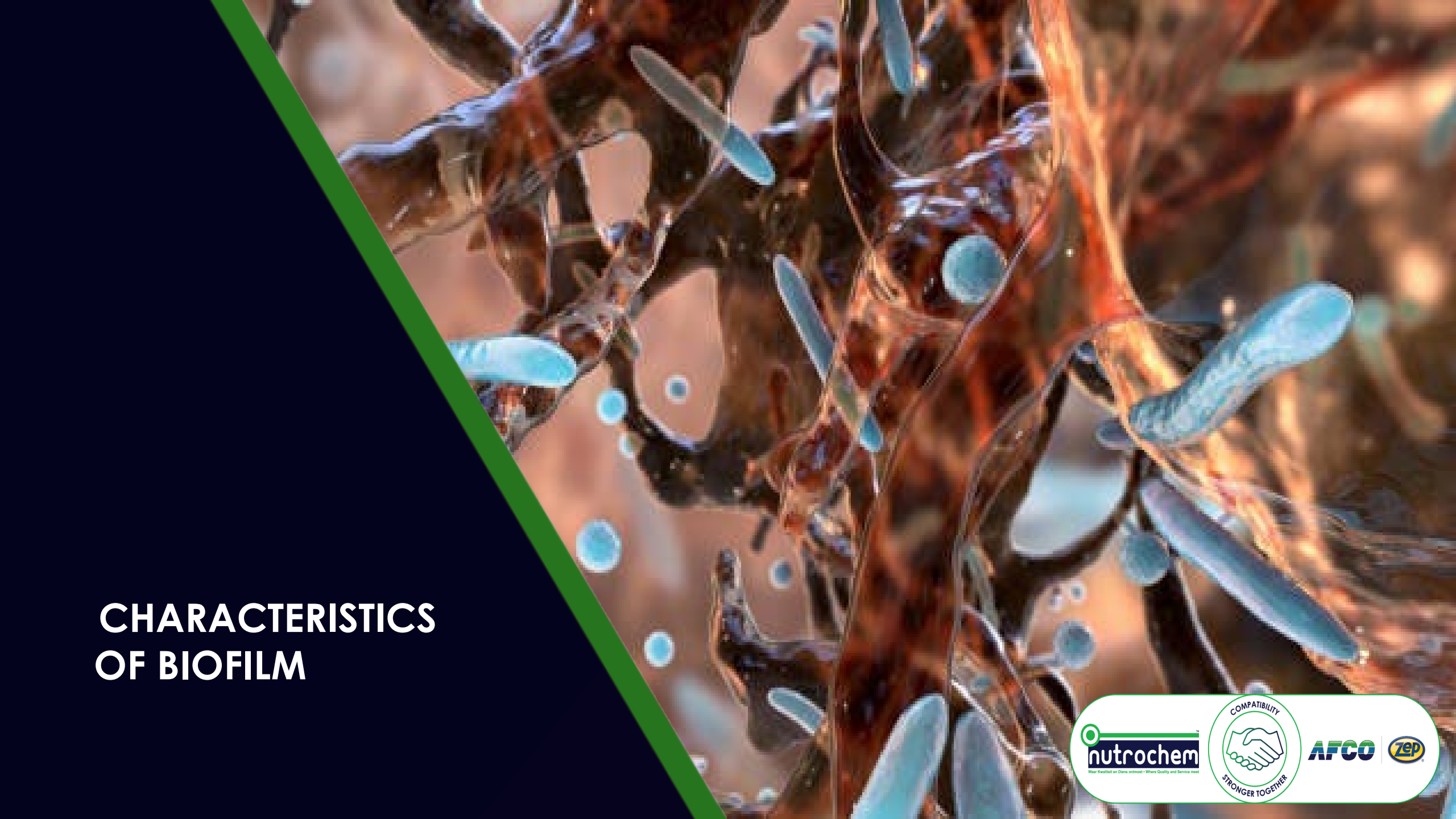
N-acylated-L-homoserine lactones (AHL's)

FORMATION OF BIOFILM

- In the early stages, a biofilm consists of one microbial cell layer attached to a surface
- As microbial cells grow and multiply the biofilm becomes numerous thick layers
- Biofilm layers can range from very thin (invisible) to few inches thick
- During the multiplication and growth, bacteria capture nutrients (protein, carbohydrates, minerals, etc.) from the surrounding environment

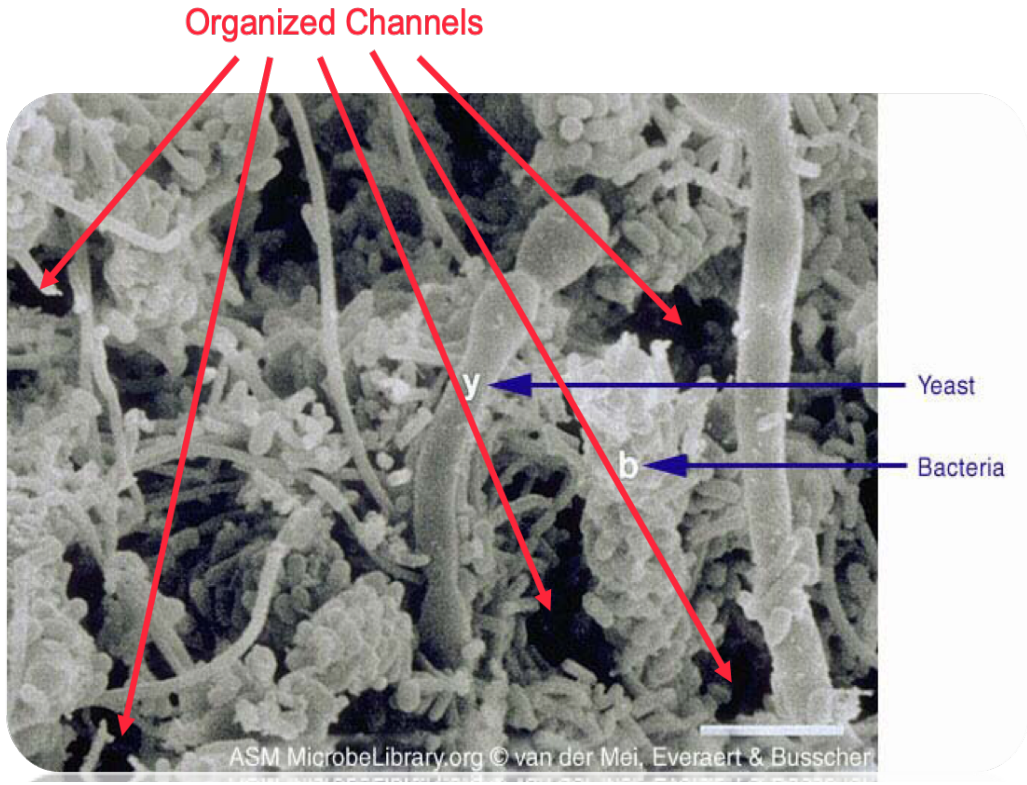


CHARACTERISTICS OF BIOFILM



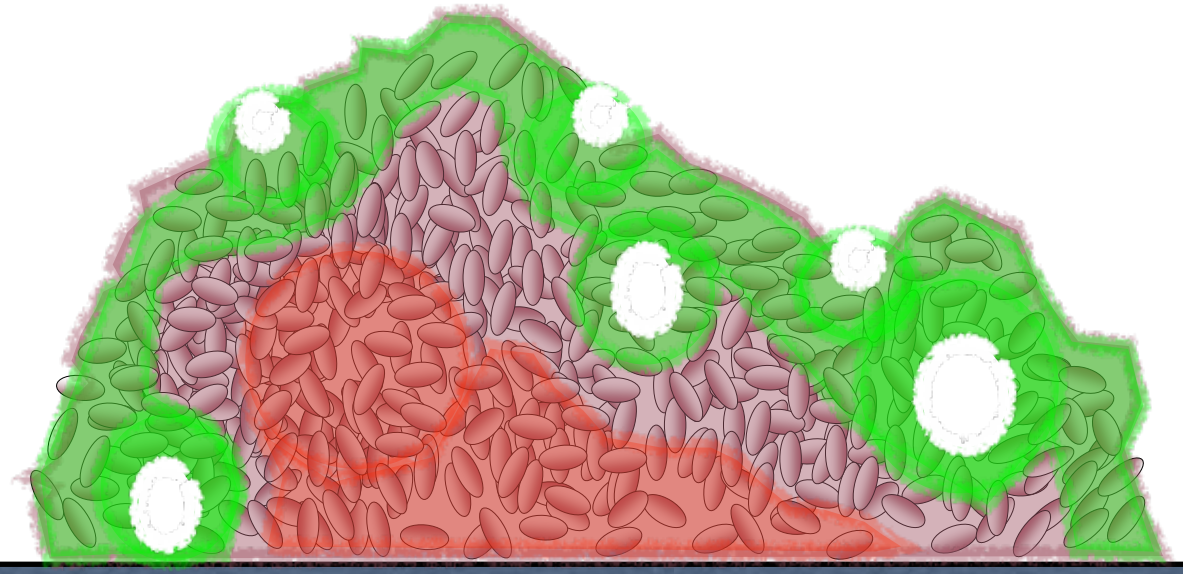
FURTHER CHARACTERISTICS

- The biofilm structure is connected by channels that allow for the flow of water, nutrients, oxygen an d waste
- The biofilm matrix produced by the microorganisms in a biofilm helps protect the microbes within from cleaners and antimicrobial agents



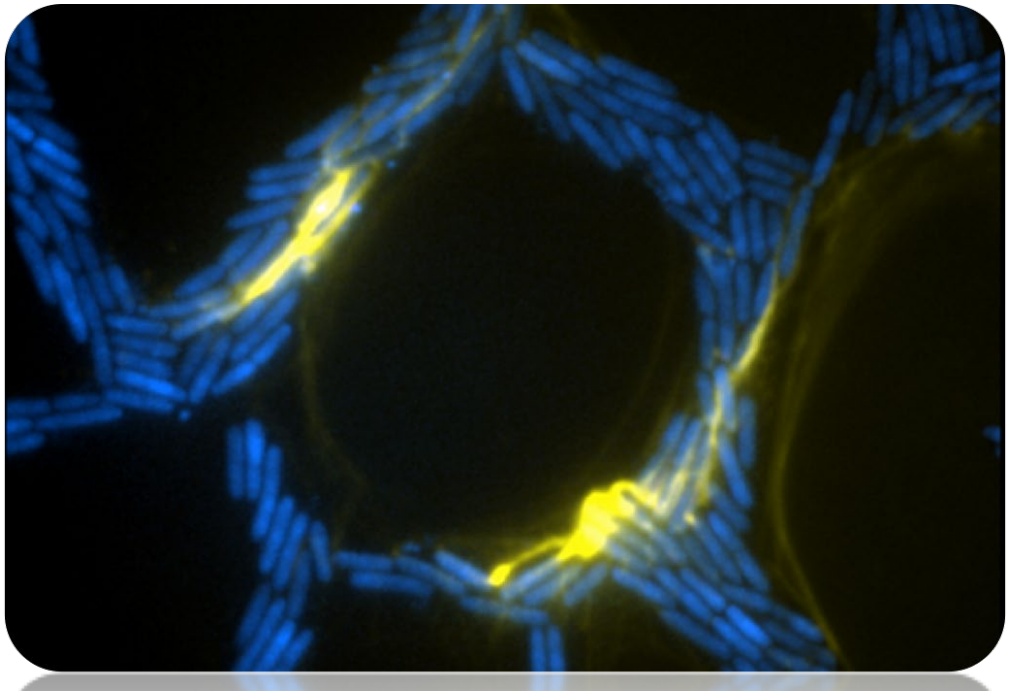
FURTHER CHARACTERISTICS

- Additionally, there are “micro-environments” within the biofilm itself where dissolved oxygen and nutrient concentrations differ. Oxygen starved areas of the biofilm will favor anaerobic microorganisms or may yield slower or halted growth. These areas are typically deeper within the biofilm where the diffusion of oxygen and nutrients is minimal. Nearer the surface of the biofilm or near the channels, aerobic respiration and or increased growth will typically occur.



FURTHER CHARACTERISTICS

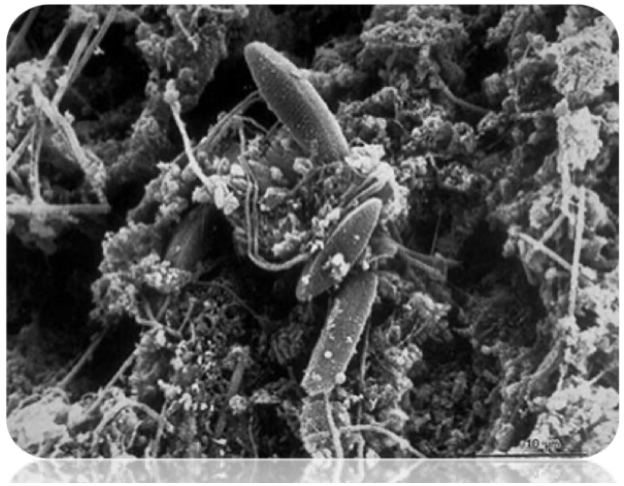
- Extracellular DNA (yellow) helps regulate movement and prevents channels from growing in as bacterial biofilms expand

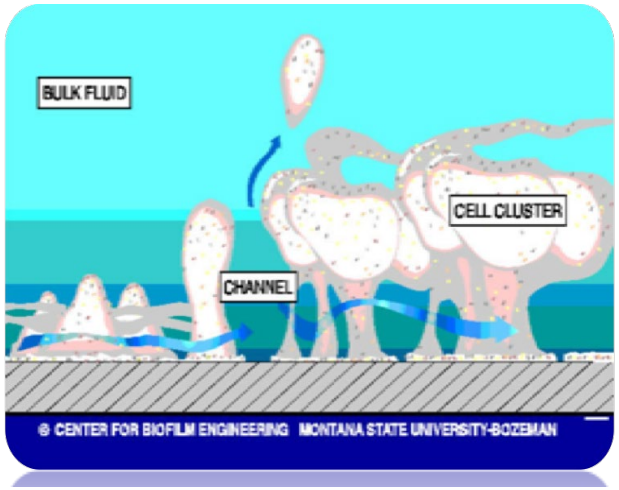


Gloag et al., see *PNAS*, doi:10.1073/pnas.1218898110

FURTHER CHARACTERISTICS

- Two types of microorganisms in a biofilm based on their position in relation to the biofilm:
- Planktonic: floating on the surface of the biofilm (easy to kill)
- Sessile: embedded in the matrix, anchored to the surface by polymeric sugars or polysaccharides (difficult to reach by disinfectants and to kill)
- Control of planktonic organisms does not necessarily mean control of biofilm

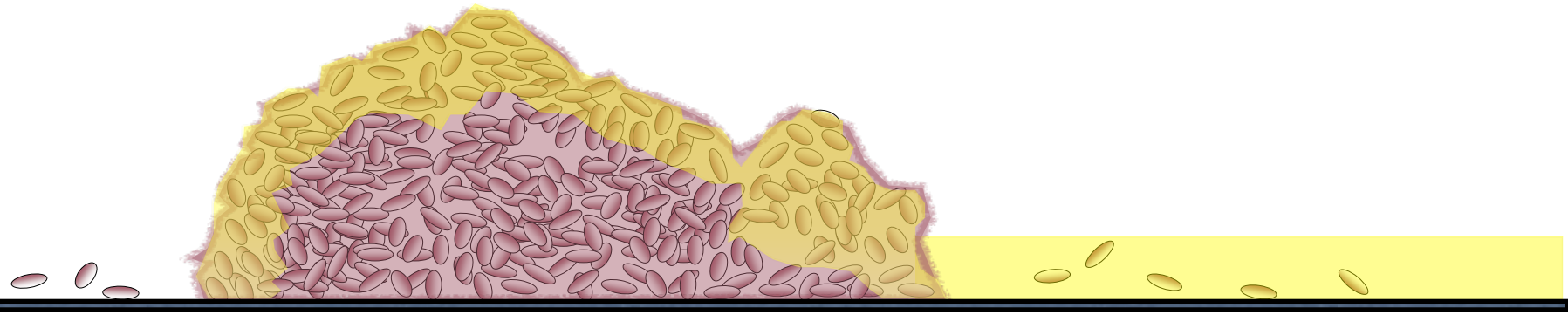




FURTHER CHARACTERISTICS

- ***Increased Chemical Resistance of Biofilms***

- Biofilms are more resistant to cleaners, disinfectants and disinfectants than free floating or individual cells. Part of this resistance results simply because the compounds need to penetrate much deeper than just the surface to eradicate all the cells and much of the active ingredients are used up reacting with the microbes and the matrix of the biofilm near the surface.



SPREADING OF MICROBES

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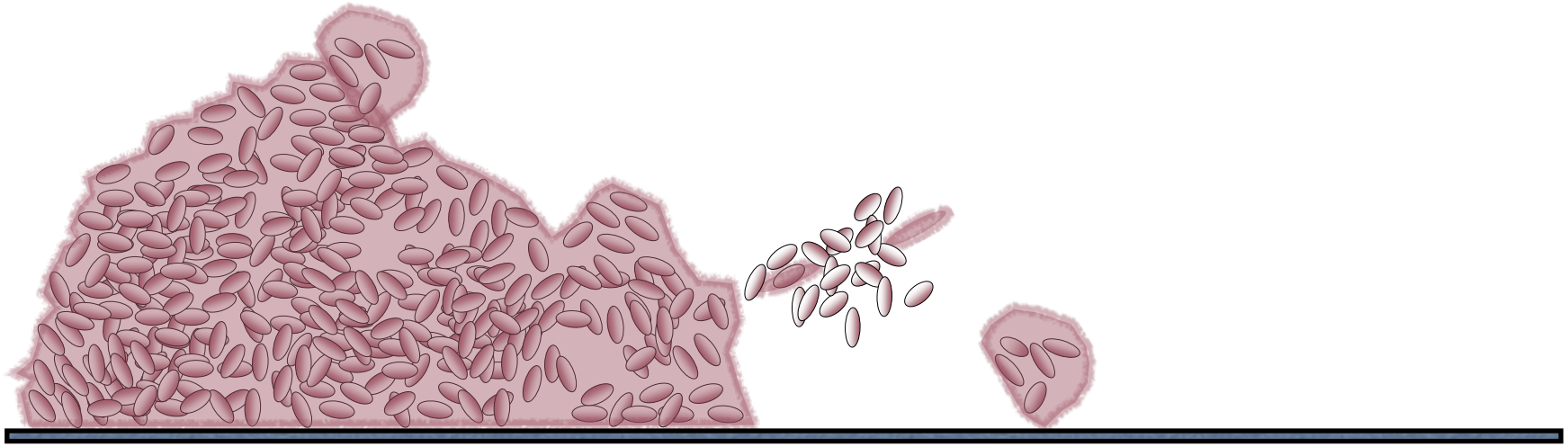
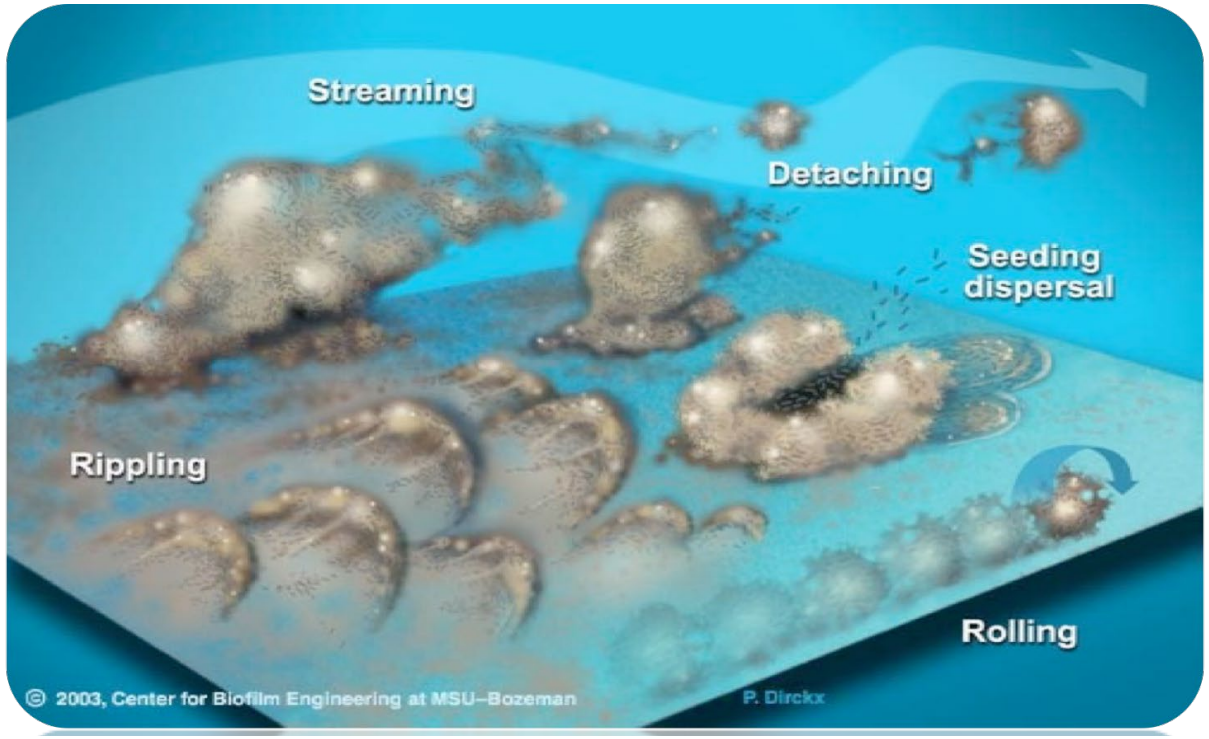
THE SPREADING OF MICROBES

- Biofilms, when exposed to outside forces or at certain points in their life cycle, often break apart and spread in one of several ways. The illustration below demonstrates how biofilms might spread when exposed to flowing liquids (water, milk or other beverages) being moved inside pipelines for example.



THE SPREADING OF MICROBES

- This movement of microorganisms from biofilms can contaminate foods and beverages



BIOFILM MITIGATION STRATEGY

BIOFILM MITIGATION STRATEGY

- One of the best strategies in combatting biofilms is the prevention of their formation in the first place. If we properly clean and disinfect environmental surfaces, bacteria are much less likely to attach themselves and begin producing biofilms to begin with.

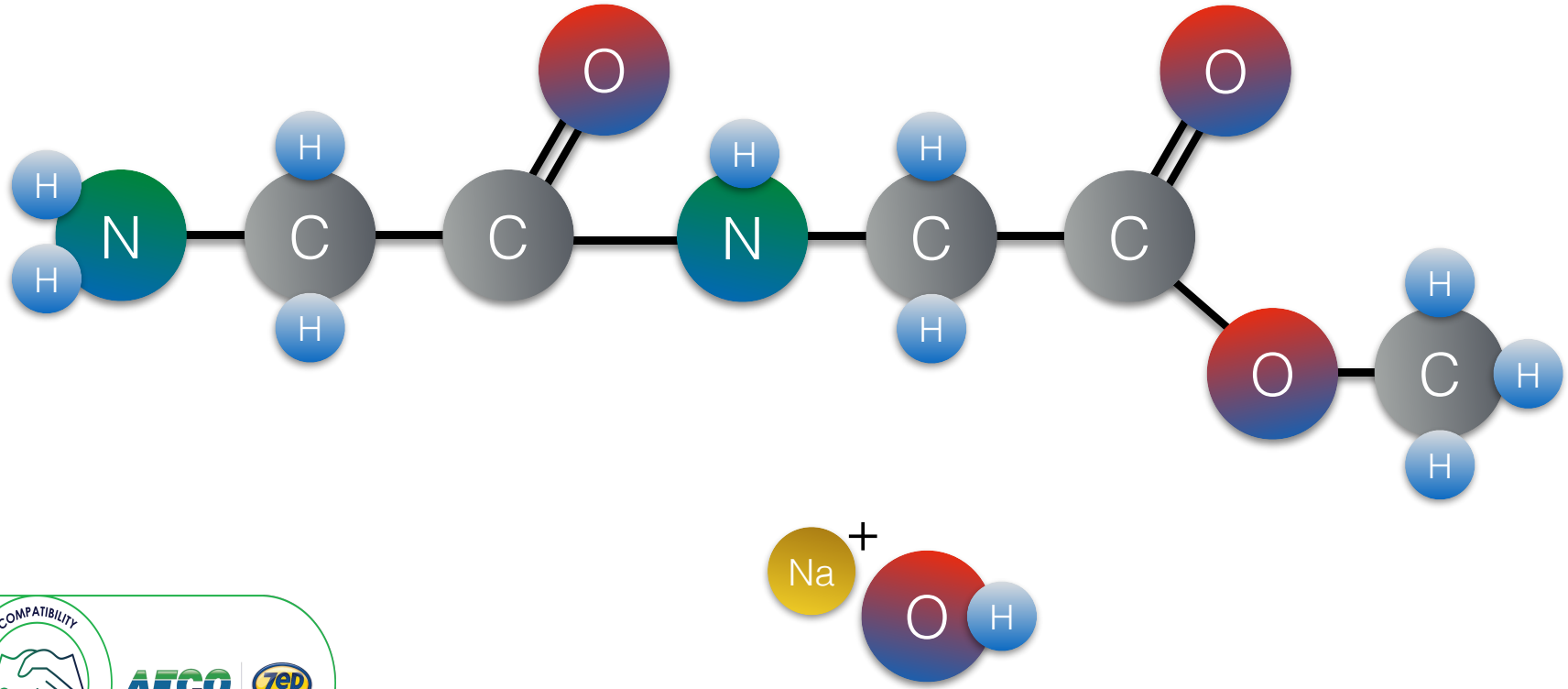
- Getting back to the basics of plant hygiene is one of the best defenses against biofilms.
  - Good First Rinse & Dry Pick-up
  - 100% Coverage of cleaning compounds
  - Sufficient CIP velocity to produce turbulent flow
  - Correct Concentration, Contact Time & Temperature
  - 100% Flood application of antimicrobial agents with a registered product (oxidizing chemistries are a good option)
  - Rotational use of Acid Cleaners and or Disinfectants

THE SPREADING OF MICROBES

- Chlorinated alkaline cleaners and disinfectants are a good option for mitigation as they hydrolyze proteins and neutralize organic acids in the biofilm
- Active alkalinity as sodium hydroxide levels of at least 0.5%
- Increased CIP circulation time with oxidizing cleaners and antimicrobial agents
- Strong oxidizing agents help penetrate and remove the polysaccharide matrix of biofilms and include.
    - Sodium hypochlorite
    - Chlorine dioxide
    - Peroxyacetic acid
    - Enzymatic detergents designed to breakdown biofilms

ENZYMES VS MORE CONVENTIONAL PRODUCTS

- Hydroxides and oxidizers are consumed in their reaction with soils as seen below in the reaction of a protein with sodium hydroxide.



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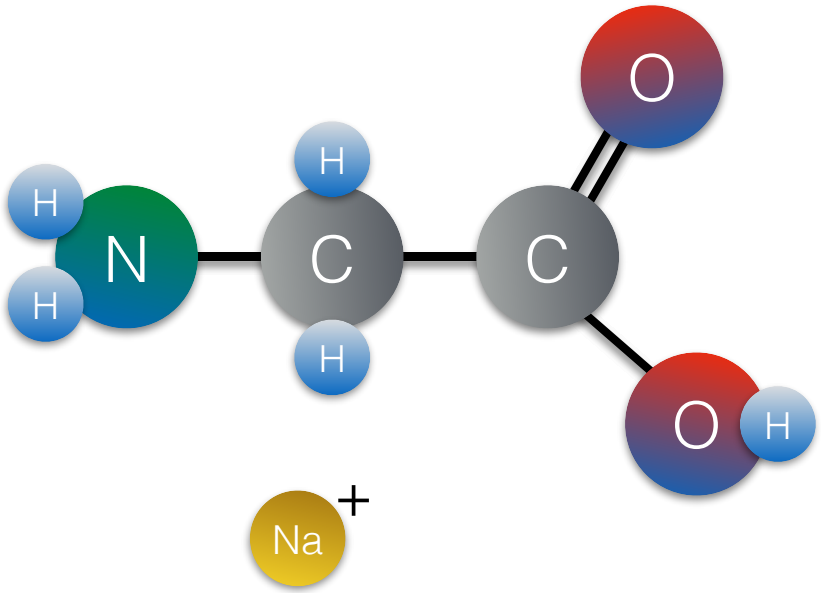
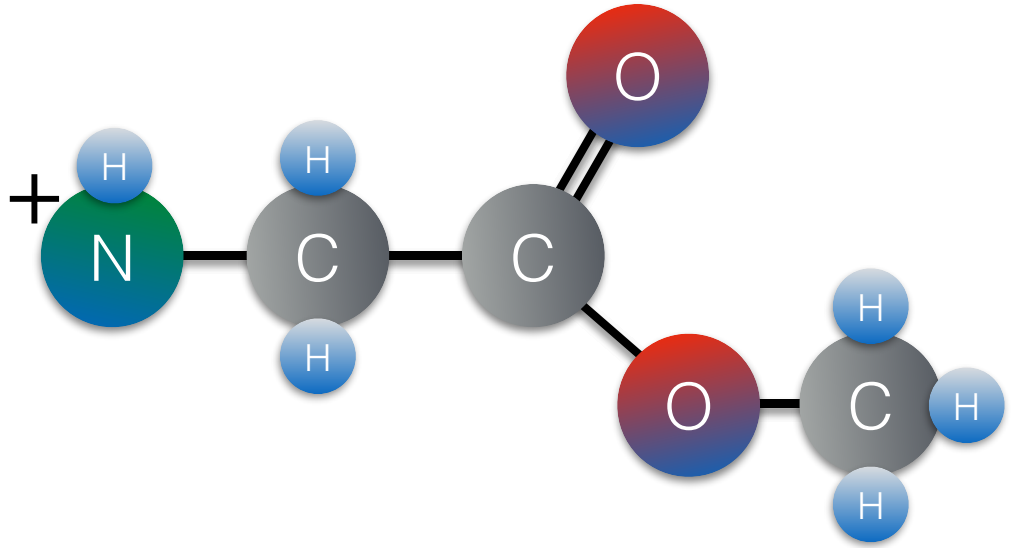
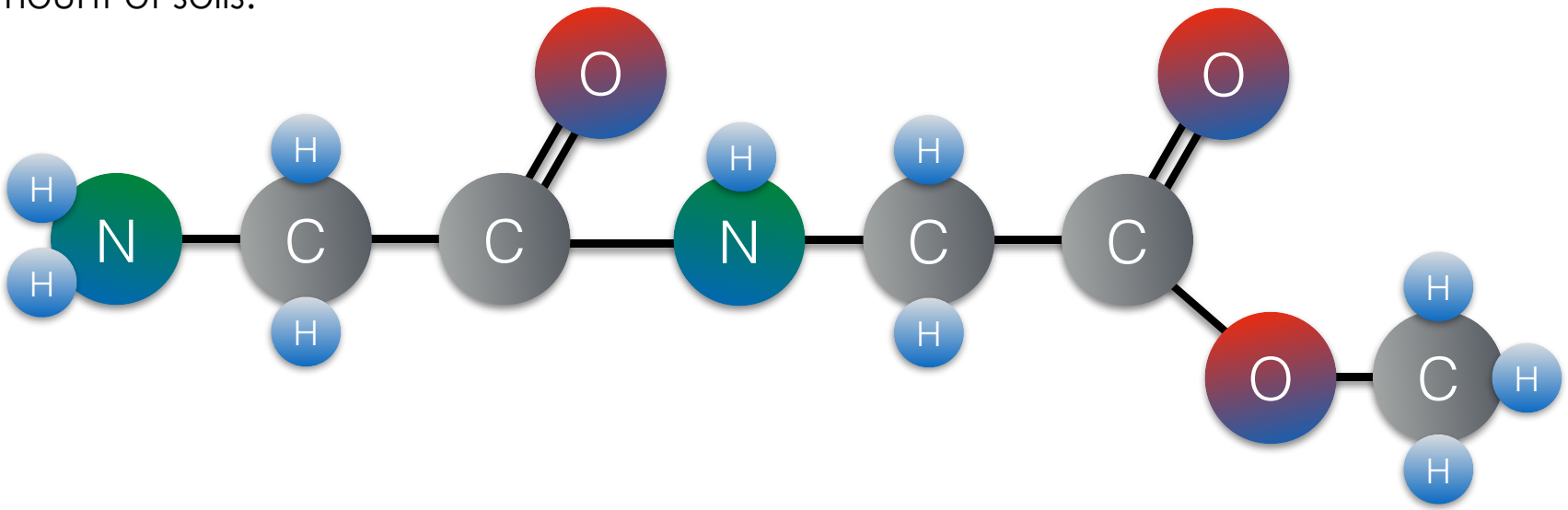




ENZYMES VS MORE CONVENTIONAL PRODUCTS

- Enzymes on the other hand catalyze the breakdown of larger molecules into smaller ones but are not consumed in the process so a small concertation of enzymes can react with a large amount of soils.



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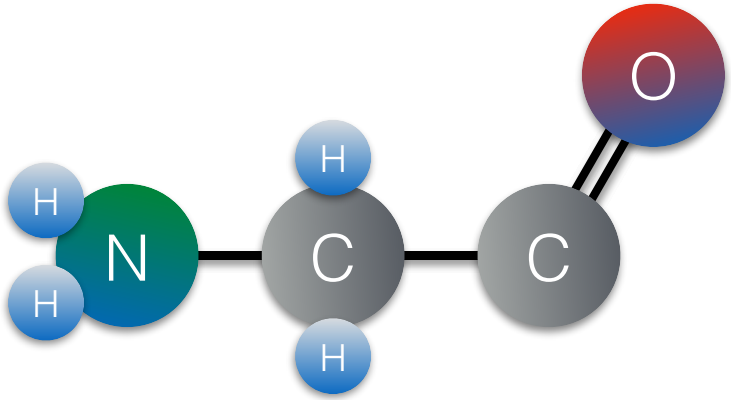
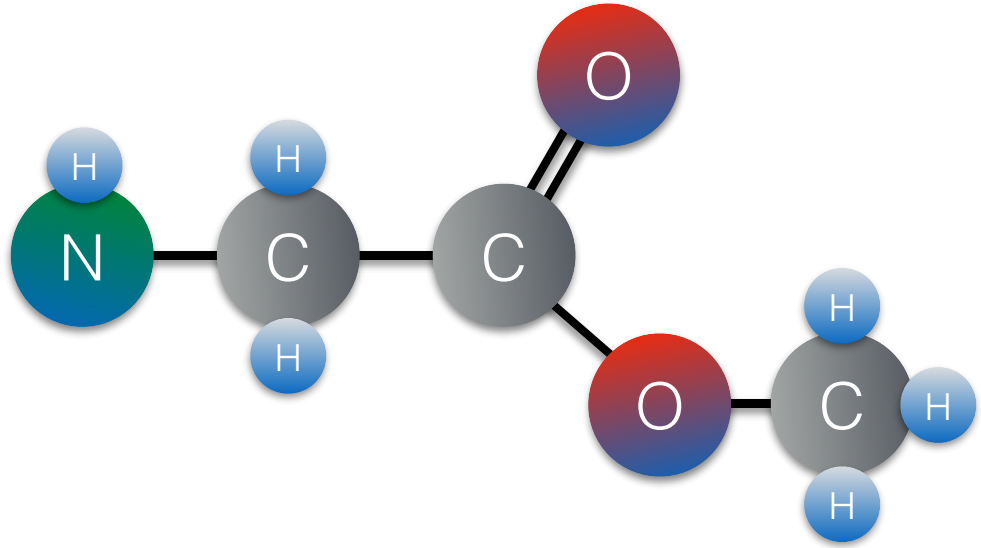
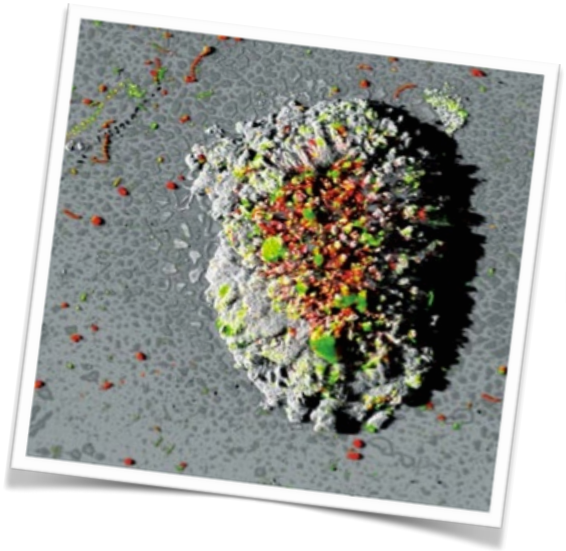




BIOFILM MITIGATION STRATEGY

- Rotational Use of Acid Cleaners and Antimicrobial Agents
- Hard water deposits can also stimulate the growth of biofilms - providing an initial structure on which the biofilm can form.



Biofilm growing on a hard water deposit

- Keeping our environmental surfaces free of hard water buildup by rotationally cleaning or sanitizing with acids will further aid in the prevention of biofilm formation.

BIOFILM MITIGATION STRATEGY

- Even if plant hygiene is perfect, however, biofilms may still form during the production day as soils and microbes that may be present in the milk we are processing are deposited on equipment.

- Once biofilms are formed, special cleaning may be needed.

VERIFICATION THROUGH COLOUR CHANGING TECHNOLOGY

Clean Soiled

If a surface is area is not clean, colour change technology will show the contaminated area

BIOFILM IDENTIFICATION

BIOFILM IDENTIFICATION

- These identification tools can also be used to determine if biofilm removal efforts have been successfully implemented

Original color at the beginning PURPLE

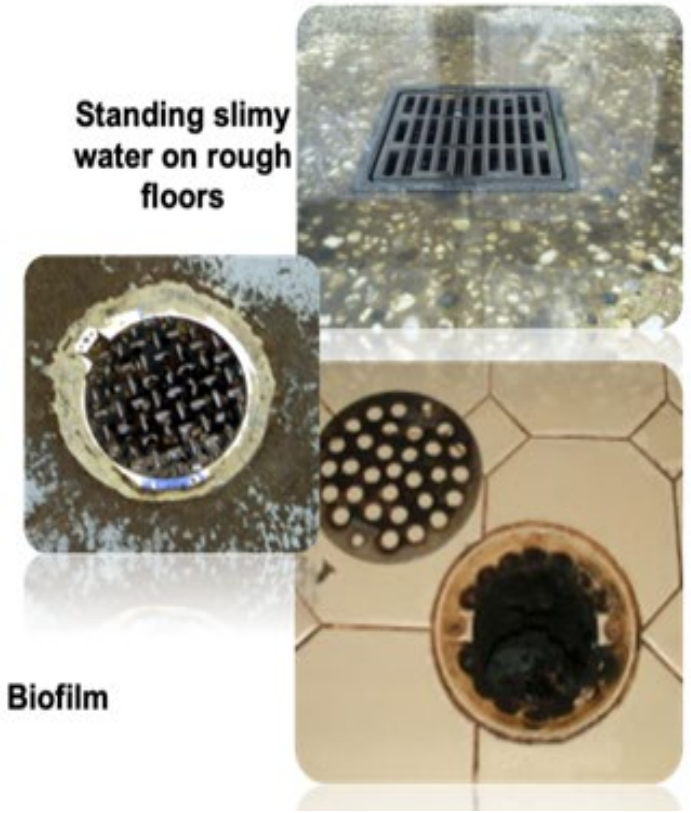
Heavy soiling at the start of the process YELLOW

Organic soiling still present GREEN

PURPLE - verified that the system is clean

BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:
  - Rough Wet Floor
  - Floor Drains
  - Standing Water

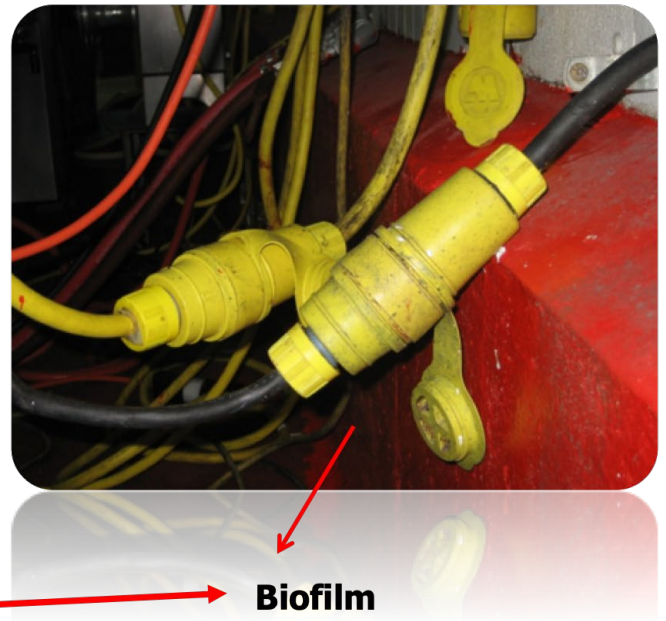


BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:

BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:
  - Underside of processing equipment, operation hoses, electric wires and plugs



BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:
  - Cooling units drip pans and the draining pipes
  - Foaming, sanitizing and rinsing hoses

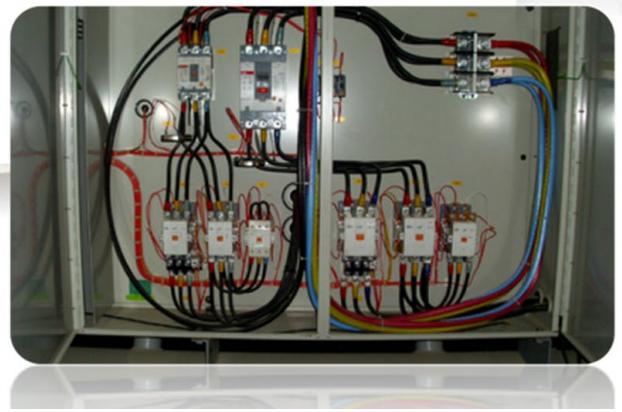
Slimy water and Biofilm in and on the surfaces of the Cooling Unit Drip Pan

BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:
  - Dirty electric boxes
  - Start and Stop Buttons
  - Cabinet door rubber gaskets

Electric Boxes

soiled Start and Stop Buttons



BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:

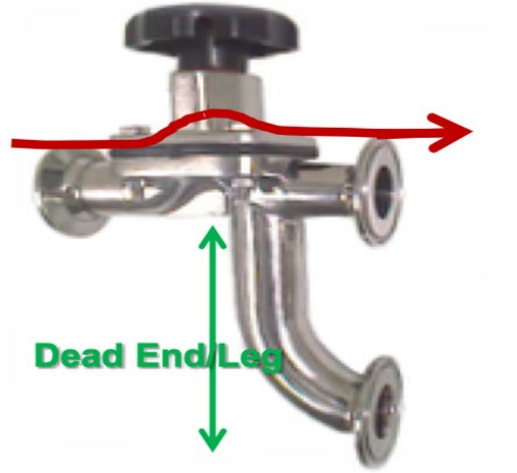
- Difficult to reach areas
- Areas that are not cleaned on a regular basis

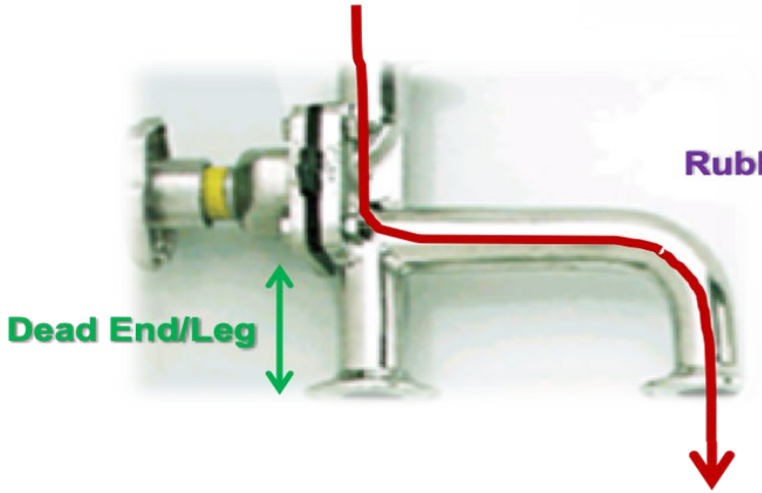
BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

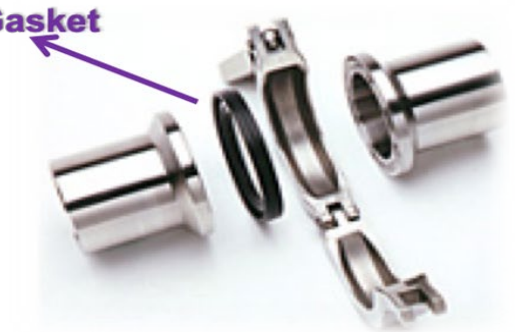
- Common Locations of Biofilms:
  - Dirty unclean maintenance tools can have biofilm
  - All tools should be cleaned and sanitized before and after use
  - After repairing, processing equipment should be re-cleaned and re-sanitized

BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

- Common Locations of Biofilms:
  - Potable water distribution systems
  - Product transfer piping and dead ends/legs
  - Product holding tanks
  - Rubber gaskets in CIP systems at pipes connection points
  - Cooling towers and heat exchangers







BIOFILM IDENTIFICATION - REAL WORLD EXAMPLES

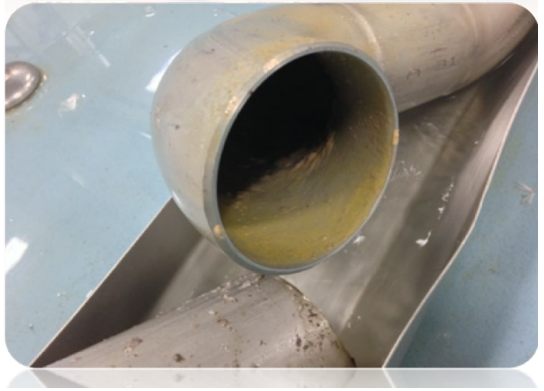
- Common Locations of Biofilms:

Biofilm in Exhaust Fan

Biofilm in Drain Trench

Biofilm on Bottom of Tanks

Biofilm in CIP System Pipe



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Questions & Answers