

**DIRECTORATE OF ADVANCED STUDIES
EVENT CATALOGUE
2021**

9TH SEMINAR OF DAS EVENTS CALENDAR – 2021

BIOFILMS-A THREAT TO FOOD INDUSTRY



9th Seminar (Online through ZOOM) of DAS Events Calendar - 2021

CONTROL OF BIOFILM IN FOOD INDUSTRY

Wednesday, 5th May, 2021 at 02:00 pm
ZOOM Meeting ID: 955 408 3170 - Passcode: 67890

Speaker:

Dr. Asma Suhail
Associate Professor
Institute of Food & Nutritional Sciences

Organizer:

**Directorate of Advanced Studies
PMAS-AAUR**

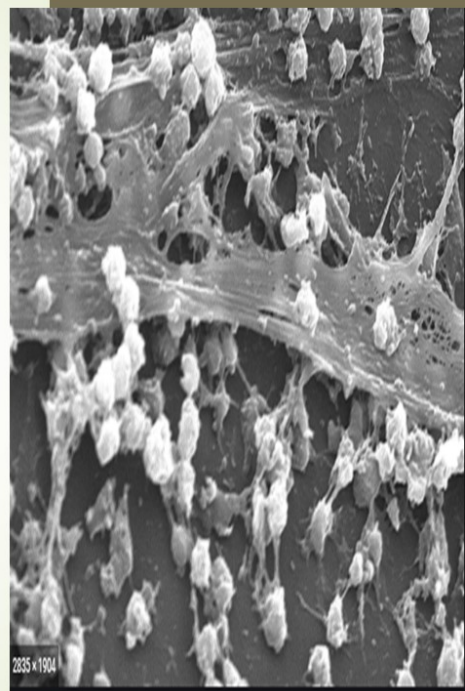
ACTIVITIES



**INTRODUCTION-
BIOFILM**

**BIOFILM- IMPACT ON
FOOD INDUSTRY**

**BIOFILM-CONTROL
MEASURES**



BIOFILMS?

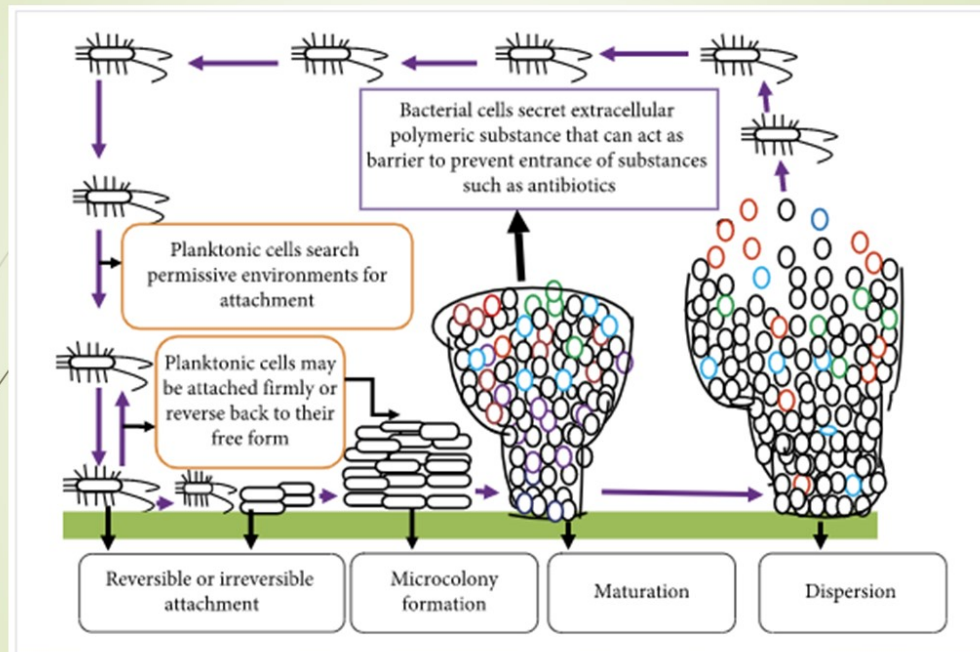
- Biofilms are microbial cells that have aggregated together on a surface
- Biofilms can be made up of one type of cell or a multitude of cell genera
- Biofilms are held together by sugary molecular strands known as EPS (Extracellular Polymeric Substance)
- Source of constant contamination

BIOFILM FORMATION

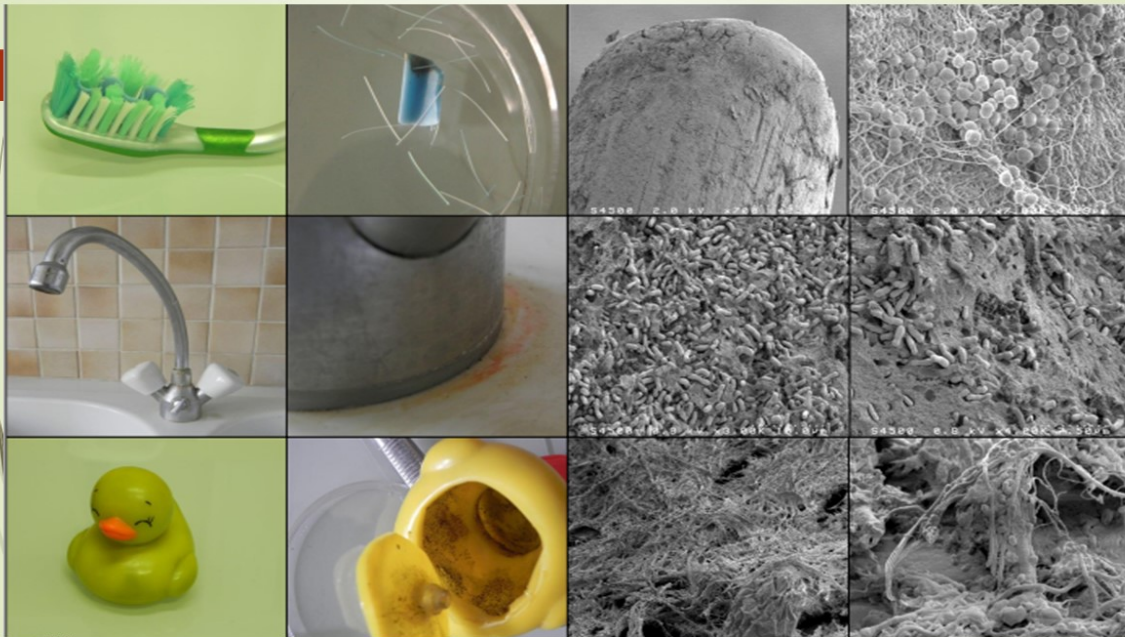


Parsek et al. (2003); Meyer, (2015)

3



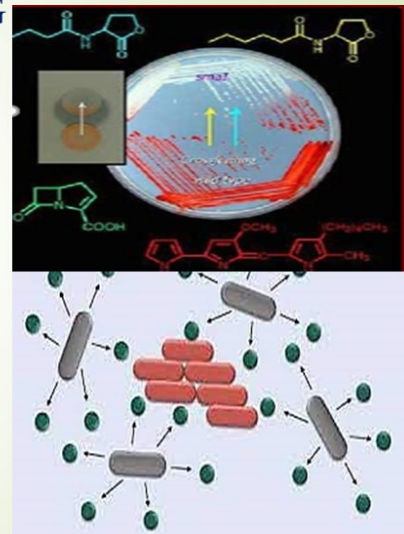
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5

MICROBIAL COMMUNICATION QUORUM SENSING

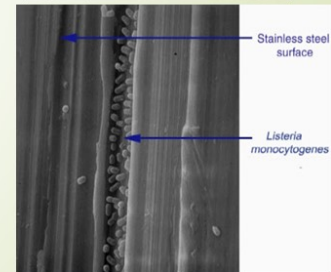
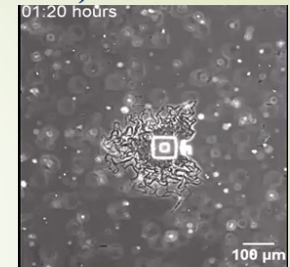
- ❖ Quorum sensing is a mechanism bacteria use to communicate
- ❖ Cell-to-cell molecular signaling, known as quorum sensing, is a system of **stimulus** and **response**
- ❖ In this process bacteria produce and detect signal molecules and thereby coordinate their behavior in a cell-density-dependent manner



6

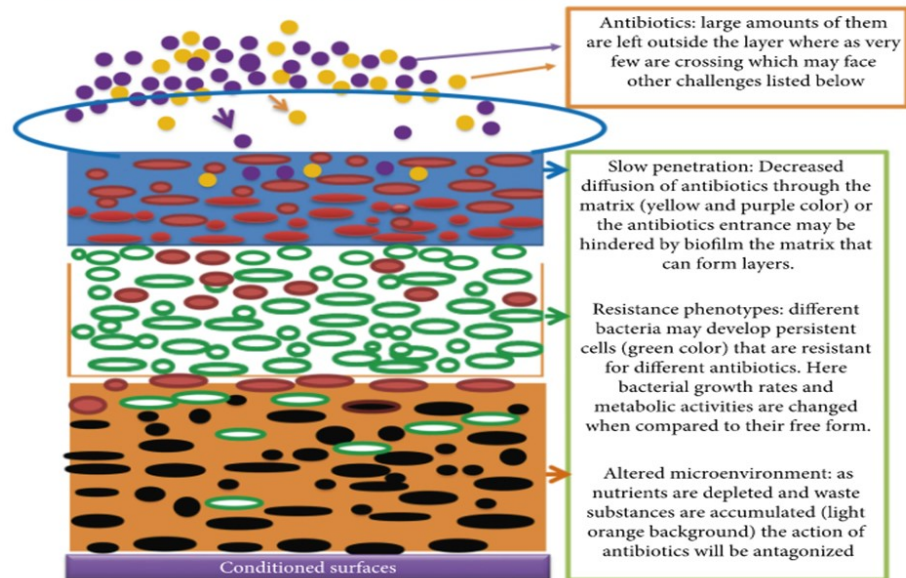
BIOFILMS-PROTECTION FOR MICROBES (CHALLENGE FOR COLLECTIVE RESISTANCE)

- Biofilms cause 60% of foodborne illness
- **Physical resistance** -Against dessication
- **Chemical protection** -Microorganisms are 10 to 1000 times more resistant to antimicrobial agents or biocides, disinfectants (low diffusion of antimicrobial substances)
- **Mechanical resistance** (against liquid streams in pipelines)
- Biofilm formation requires moisture, nutrients and a surface like natural material or stainless steel, polyethylene, polypropylene, wood, glass, rubber, more.



7

RESISTANCE TO ANTIBIOTICS



Kostakioti et al., 2013

8

The growth and resistance to sodium hypochlorite of *Listeria monocytogenes* in a steady-state multispecies biofilm

D.E. Norwood¹ and A. Gilmour^{1,2}

¹Department of Food Science (Food Microbiology), The Queen's University of Belfast and ²Department of Agriculture for Northern Ireland, Belfast, Northern Ireland, UK

7343/08/99: received 11 August 1999, revised 29 October 1999 and accepted 11 November 1999

D. E. NORWOOD AND A. GILMOUR. 2000. A constant-depth film fermenter (CDFF) was used to culture a steady-state multispecies biofilm consisting of one strain each of *Listeria monocytogenes*, *Pseudomonas fragi* and *Staphylococcus xylosum*. These bacteria were initially grown together in a conventional chemostat to achieve a steady state before being inoculated into the CDFF over an 18-h period. A dilute tryptone soya broth (TSB) medium was supplied to the CDFF and the biofilm allowed to develop over a 28-d period. This mature biofilm was then subjected to increasing levels of sodium hypochlorite solution to measure any antimicrobial effect. The three organisms were seen to reach a steady state after 6 d in the chemostat before being transferred to the CDFF where the mature multispecies biofilm reached steady state at 17 d. *Listeria monocytogenes* in both planktonic and biofilm growth stabilized at 1.8 and 1.5%, respectively, of the total plate counts, while *Ps. fragi* and *Staph. xylosum* were the predominant organisms in the biofilm at 59% and 39.5%, respectively, of the total microbial population. Steady-state biofilms in the CDFF were exposed to increasing strengths of sodium hypochlorite; 200, 500 and 1000 p.p.m. free chlorine, but a substantial two-log cycle drop in bacterial numbers was only achieved at 1000 p.p.m. free chlorine. In planktonic culture all three organisms were completely eliminated when exposed to 10 p.p.m. free chlorine for a 30-s period.

MICROBIAL RESISTANCE TO CHLORINE

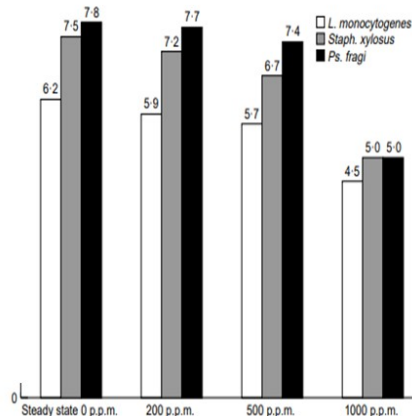


Fig. 4 The survival, after 20 min sodium hypochlorite treatments, of *Listeria monocytogenes*, *Staphylococcus xylosum* and *Pseudomonas fragi* within the steady-state biofilms. $n = 18$

BIOFLM AND FOOD INDUSTRY

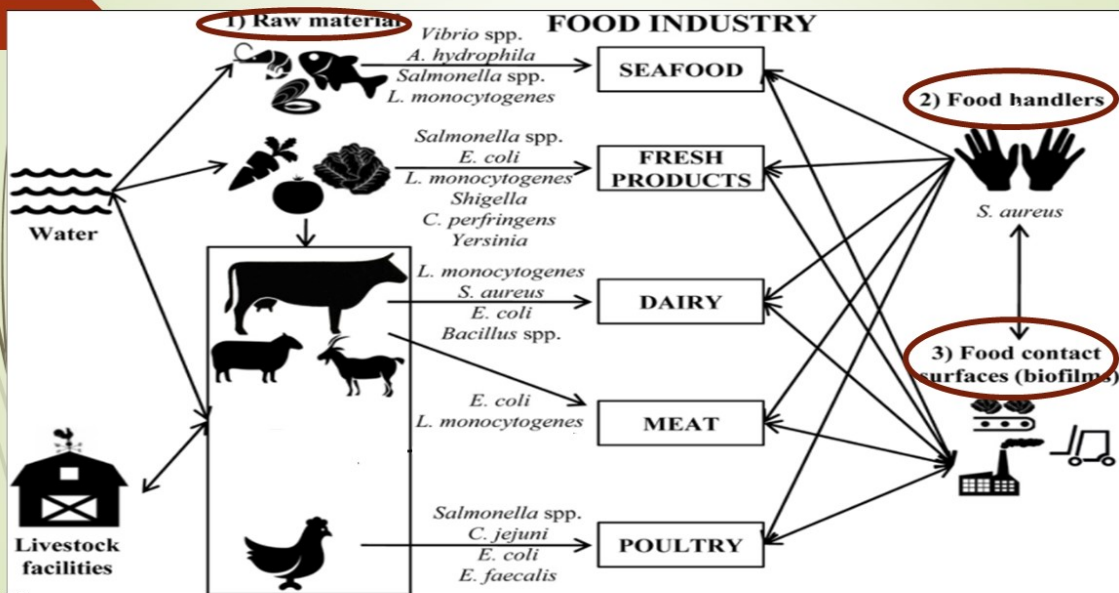
BIOFILM FORMATION IMPACT ON FOOD INDUSTRY

- Continuous recontamination of foods Foodborne illnesses/ pathogenicity
- Faulty Operations
 - Decreasing heat transfer
 - Blocking tubes
 - Plugging filters
 - Surface damaging (metal corrosion)
 - Off-flavours, off-notes, organoleptic issues
 - Defective batches
 - Shelf-life reduction
 - Market returns and complaints



11

MAIN SOURCES OF CONTAMINATION IN THE FOOD INDUSTRY



12

Diana et al. 2016

PROBLEMS IN THE FOOD INDUSTRY

DAIRY INDUSTRY

Dairy Industry processes include structures like

- Raw milk tanks, pipelines, butter centrifuges, cheese tanks, pasteurizers, packing tools, conveyer belts, trolley wheels etc.
- Biofilm in milking equipment (stainless steel, polystyrene)
- Psychotrophic----*Pseudomonas* spp
- Thermophilic ---*Geobacillus stearothermophilus*

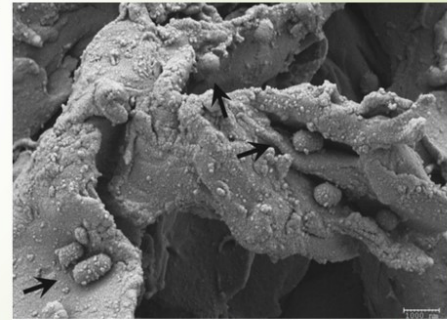
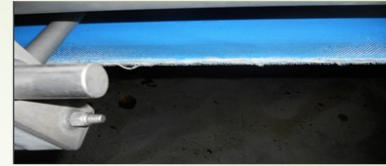


Figure 4. Scanning electron microscopy image of scratches on the surface of the bottom cover of milk meter 3-left (scale: 1,000 nm). Arrows indicate the presence of bacteria associated with these scratches in the plastic material.

13

Lattore et al., 2010

PROBLEMS IN THE FOOD INDUSTRY

FISH INDUSTRY, POULTRY INDUSTRY

FISH INDUSTRY

- Fresh fish products may suffer from biofilm formation by pathogenic species resulting in significant health and economic issue
 - *Aeromonas hydrophila*
 - *L. monocytogenes*
 - *Salmonella*
 - *Vibrio* spp.

POULTRY INDUSTRY

- Dust, surfaces, feces, poultry feed, and transportation of live poultry between production and processing units are known to be the important risk factors in *Salmonella* contamination
- Approximately 50% of the strains isolated on poultry farms were able to produce biofilms
- Pathogens forming biofilms in poultry and poultry processing include
 - *Salmonella* spp.
 - *Campylobacter* spp.

14

PROBLEMS IN THE FOOD INDUSTRY

MEAT INDUSTRY



International Journal of Food Microbiology
Volume 328, 2 September 2020, 108668

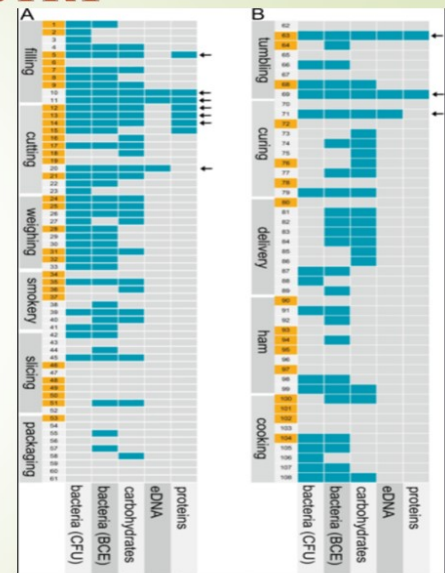


Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms

Eva M. Wagner ^a, Nadja Prascer ^b, Sarah Thalgueter ^a, Katharina Fischel ^b, Nicole Rammer ^b, Lucie Pospíšilová ^c, Merima Alispahic ^d, Martin Wagner ^{a, b}, Kathrin Rychli ^{b, d, e}

Overall, we identified ten biofilm hotspots, among them seven of which were sampled during operation and three after cleaning and disinfection. Five biofilms were detected on food contact surfaces (cutters and associated equipment and a screw conveyor) and five on non-food contact surfaces (drains and water hoses) resulting in 9.3 % of the sites being classified as biofilm positive. From these biofilm positive samples, we cultivated bacteria of 29 different genera. The most prevalent bacteria belonged to the genera *Brochothrix* (present in 80 % of biofilms), *Pseudomonas* and *Psychrobacter* (isolated from 70 % biofilms). From each biofilm we isolated bacteria from four to twelve different genera, indicating the presence of multi-species biofilms.

15



PROBLEMS IN THE FOOD INDUSTRY

READY TO EAT (RTE) INDUSTRY

- RTE foods can be considered as a relatively high-risk food
 - Refrigerated RTE products
 - smoked fish
 - sliced materials
 - Unpackaged or repackaged RTE foods
- *L. monocytogenes* *E. coli* O157:H7 strain could be a concern in a wide variety of Ready to Eat food products since it can form biofilms on food contact surfaces during food processing.

16

Pathogen	Survivability	Resistant factors	Food industries	Health issues	Biofilms forming surfaces
<i>Bacillus cereus</i>	4–50°C	-Heat -Chemicals -Radiations	Dairy Food & beverage plants	Diarrhea	Stainless tanks, pipes
<i>Escherichia coli</i>	30- 37 °C		Post harvest industries		Stainless steel, Teflon, glass, polystyrene, polypropylene, PVC
<i>Salmonella enterica</i>	Low moisture		Poultry , meat industry	gastroenteritis	Stainless steel
<i>Staphylococcus aureus</i>	enterotoxins 10 - 46°C	heat			
<i>Listeria monocytogenes</i>	High moisture		Seafood, dairy products, meat, ready-to-eat products, fruits, soft cheeses, ice cream, unpasteurized milk, candied apples, frozen vegetables, and poultry	Gastroenteritis Listeriosis	polypropylene, steel, rubber or glass surfaces

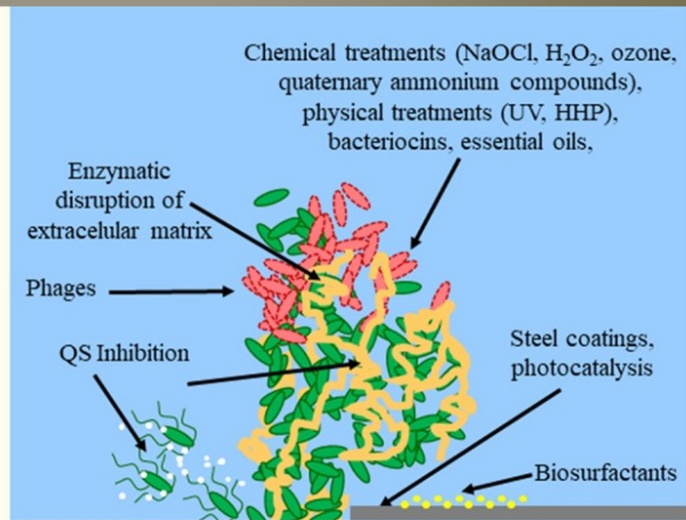
17

PREVENTION OF BIOFILMS

- Chemical treatments
- Enzymatic disruption
- Nanotechnology agents
- Quorum sensing inhibitors
- Bacteriophages
- Biosurfactants
- Bacteriocins
- Essential Oils
- Physical treatments

Galie et al., 2018)

18



METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- CHEMICAL TREATMENTS

- **Chlorine-based sanitizers (NaOCl)** are the most widely used in the food industry, but resistance to chlorine treatments has arisen in some microbes.
- **Quaternary ammonium compounds (as Metaquats)** are widely used as sanitizers in food industry, including biofilms removal. These positively charged water soluble compounds disrupt the bacterial cell membrane, causing bacterial lysis
- Other less common sanitizers, such as **salicylate-based polyanhydride esters**, interfere with the biofilm formation in *S. enterica* at the air-liquid interface.

19

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- ENZYMATIC DISRUPTION

- Enzymes are considered green counter measures against biofilm formation since they are biodegradable and have low toxicity
- Degradation of biofilm structural component allows the increased penetration of antibiotics which enhances the antibiotics efficiency

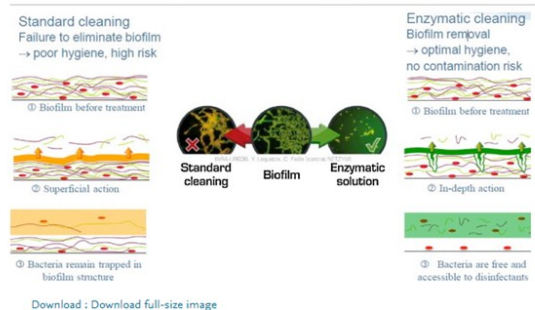
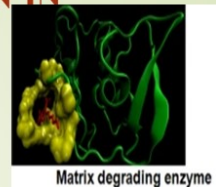


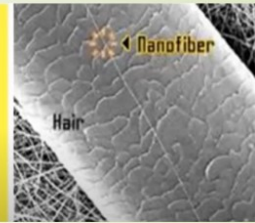
Fig. 1. Comparison of effectiveness between the traditional CIP and enzyme cleaning technique.

Xiaobo et al., 2014

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- NANOTECHNOLOGY AGENTS

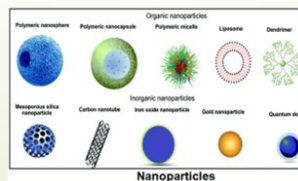
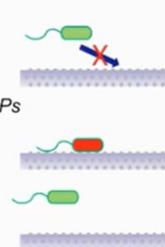
- **Silver compounds** - due to their potent antimicrobial effects, have been used to prevent microbial infections
- **Metal oxide NPs –**
 - Iron oxide (Fe_3O_4)
 - Titanium oxide (TiO_2)
 - Zinc oxide (ZnO)
 - Copper oxide (CuO) and
 - Magnesium oxide (MgO)

"Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications."



Nanotechnology for Biofouling Control

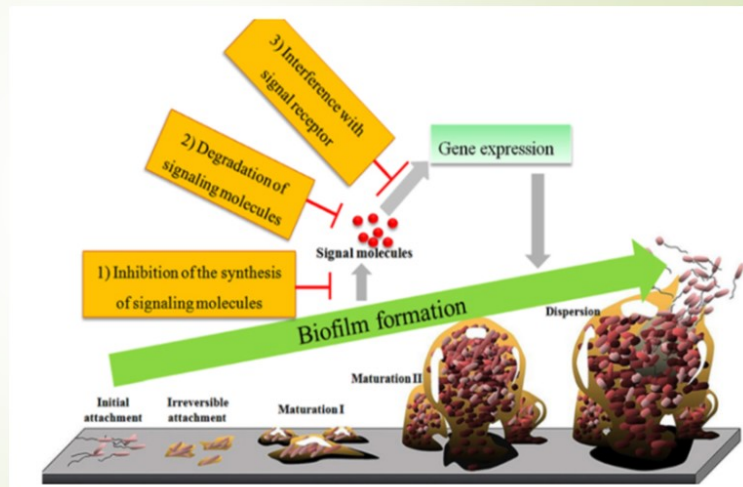
- **Discourage adhesion**
 - Nano-patterned topology
 - Surface chemistry
 - Interrupt quorum sensing
- **Incorporate antimicrobial NPs**
 - Nano silver
 - Carbon nanotubes
 - Porous nano-carriers
- **Photocatalytic ROS**
 - Semiconductors, e.g., TiO_2
 - Fullerene derivatives



21

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- QUORUM SENSING INTERRUPTION

- QS regulators can be knocked down by using certain antimicrobials
- QS-interrupting metals (Nickel, Cadmium) inhibit DNA and RNA polymerases (thus inhibit QS at the transcription level)

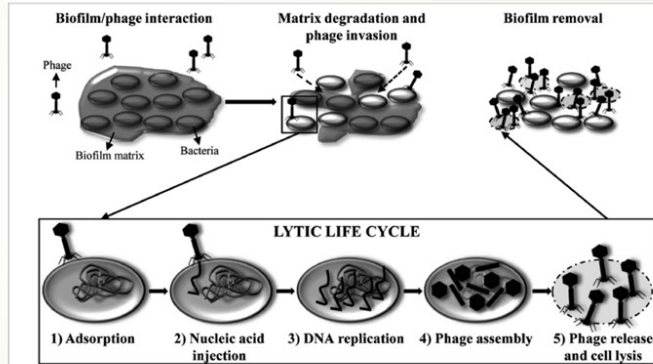


22

Zhang et al., 2015

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- BACTERIOPHAGES

- Phage therapy is currently an attractive alternative to antibiotics
- The primary limitation of phage treatments is their ability to access and target bacterial cells inside the biofilm.
- Some phages possess **exopolysaccharide depolymerases**, an excellent solution for this diffusion problem. The presence of these enzymes enhances the phage invasion and dispersion process through the biofilm under treatment
- To aid in the disruption of biofilms, the phage T7 was modified to express the enzyme, dispersin B (*dspB*), which is known to assist in the degradation of biofilms

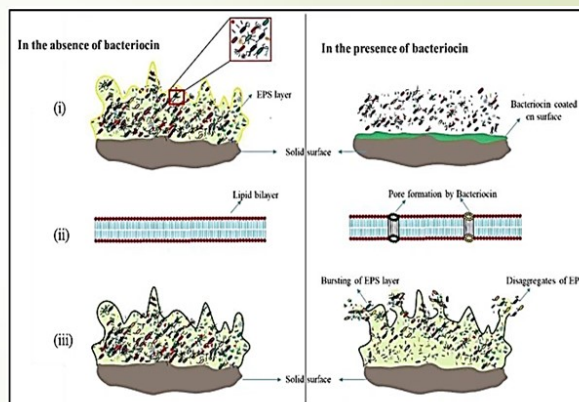


METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- BIOSURFACTANTS

- Biosurfactants are natural compounds, usually of microbial origin, able to modify the hydrophobic characteristics of the bacterial surface
- Hydrophobic microorganisms cause the damage of surfaces by biofilm formation
- Biosurfactants alters the adhesion properties and binding capacities to any given surface. These molecules insert themselves into the microbial cell membranes, or chelating cations.
- Their effect alters the membrane permeability, eventually disrupting it and causing cell swelling and death
- Example: lichenysin, a cyclic non-ribosomal lipopeptide produced by *B. licheniformis*.

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- BACTERIOCINS

- Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain.
- Nisin (FDA approved bacteriocin), isolated from *Lactococcus lactis* was able to prevent adhesion and biofilm formation by *L. monocytogenes*
- Other bacteriocins have been extensively investigated for preventing bacterial colonization, especially those produced by GRAS (Generally Recognized as Safe) microorganisms, such as lactic acid bacteria.



Duraisamy et al., 2020

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- ESSENTIAL OILS

- Several compounds derived from plants demonstrated anti biofilm properties.
- Plant-based essential oils are primarily a species-specific complex mixture of monoterpenoids (such as borneol, camphor, carvacrol, eucalyptol, limonene, pinene, thujone), sesquiterpenoids (such as caryophyllene, humulene) and flavonoids (such as cinnamaldehyde and other phenolic acids)



METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- HIGH HYDROSTATIC PRESSURE

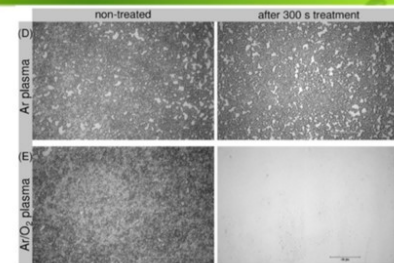
- High hydrostatic pressure (HHP, 300–900 MPa) can destroy or inactivate vegetative bacterial cells.
- However, this technology is not effective in the case of endospores (such as those in the case of *B. cereus*), unless a pretreatment is carried out at lower pressures (300–400 MPa) in order to allow germination of existing spores

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- NON-THERMAL PLASMA

- Non-Thermal Plasma Non-thermal plasma (NTP) is a novel and emerging antimicrobial tool
- Partially ionized gas works at low temperature and produced at atmospheric pressure by mixing UV light with oxygen, nitrogen, ozone, and water and helium, under an electrical discharge.
- These act on the unsaturated fatty acids of the lipid bilayer of the cell membrane, preventing the transport of biomolecules across it.
- Decontamination process easier and less expensive.
- However, its use is still restricted to some laboratory applications, due to its high cost



Figure 2. Plasma-dependent removal of 7-day old *Candida albicans* biofilms grown on polystyrene wafer.

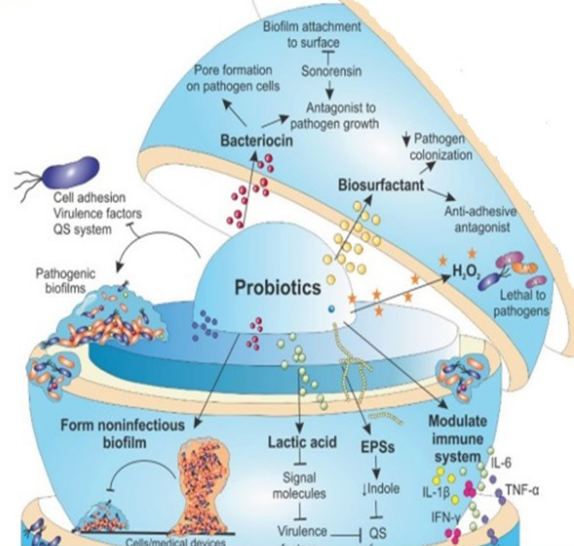


Atmospheric Pressure Plasma: A High-Performance Tool for the Efficient Removal of Biofilms.

METHODS FOR CONTROLLING BIOFILM FORMATION IN THE FOOD INDUSTRY- PROBIOTICS

29

- Probiotics can target microbial biofilms
- Produce antagonistic substances like biosurfactants, bacteriocins, organic acids, hydrogen peroxide that can hinder the activity of pathogenic bacteria
- Prevent quorum sensing
- Generate unfavourable environmental conditions to pathogens (pH alterations, surface and nutrient competitions)



Barzegari et al., 2020

30

SUMMARY POINTS

- BIOFILMS ARE MICROBIAL CELLS THAT HAVE AGGREGATED TOGETHER ON A SURFACE, HELD BY EXTRACELLULAR POLYMERIC SUBSTANCE (EPS)
- BIOFILM IN MIXED CULTURE IS MORE THAN IN SINGLE CULTURE, INDICATING THAT INTERSPECIES INTERACTION WOULD CONTRIBUTE TO INCREASING THE CO-CULTURED BIOFILM FORMATION.
- BIOFILMS AS IMMOBILIZED CELLS IS IMPORTANT AND CHALLENGING APPROACH IN FOOD INDUSTRY
- CONTROL MEASURES (CHEMICAL, PHYSICAL TREATMENTS, ENZYMATIC DISRUPTION, NANOTECHNOLOGY AGENTS, QUORUM SENSING INHIBITORS, BACTERIOPHAGES, BIOSURFACTANTS, BACTERIOCINS, PROBIOTICS ETC)

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32

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