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Biofilm in dairy industry: Detection and common process for control biofilms

Suchita Bhosale, Priyanka Brahmane, Ashwini Kubade and RJ Desale

Abstract
Milk obtained from the udder of a healthy milch animal is almost in sterile condition but gets contaminated during milking, transportation, storage and processing and also due to the entry of microbes through many other sources. Insufficient sanitization and cleaning causes contaminants to accumulate in milk processing equipment which subsequently form biofilm that further become significant source of contamination of dairy products. Biofilm is aggregations of microbial cells interconnected by extracellular polymeric substances which accelerate growth on different material surfaces adversely affect the dairy industry. Biofilm formation possesses profound implications and throws a major challenge to the dairy sector where they act as the principal reservoir of microbial contamination. These lead to financial crisis by impairment of raw material and its products. It is emphasized that god manufacturing practice, good hygienic practice and hazard analysis and critical control point should be implement in dairy industry to prevent the contamination of dairy products. There are different methods to detect the biofilm forming colonies. Different common processes involved in the control of biofilm forming colonies. i.e. Physical Control, Cleaning and disinfection, Ultrasonication, Steel Coatings, High Hydrostatic Pressure, Non-thermal Plasma, Chemical Control, Mechanical Control. Some of positive aspects of biofilm also reviewed in this article.

Keywords: biofilm, contamination, dairy industry, extracellular polymeric substances (EPSs) etc, transmission electron microscope (TEM) scanning electron microscope (SEM), confocal laser scanning microscopy (CLSM), Congo red agar (CRA)

Introduction
According to the changing scenario of global market, the dairy industry is considered to be one of the major food industry in world which manufactures a wide range of perishable (e.g. butter, yoghurt and cheese) and semi perishable (milk powder, casein) milk products. To maintain the quality and safety of these products, microbiological guidelines are an essential requirement. Milk obtained from the udder of a healthy milch animal is almost in sterile condition but gets contaminated during milking, transportation, storage and processing and also due to the entry of microbes through many other sources. Insufficient sanitization and cleaning causes contaminants to accumulate in milk processing equipment which subsequently form biofilm that further become significant source of contamination of dairy products. Biofilm is aggregations of microbial cells interconnected by extracellular polymeric substances which accelerate growth on different material surfaces adversely affect the dairy industry. This polymicrobial community contains altered phenotype which differs them from planktonic microbes physiologically. It affects the quality and safety of raw materials and their products.

What is a biofilm?
The existence of biofilm has been explored for several years in the food industry. The first documentation was done roughly 75 years back in 1943.
Biofilms are three dimensional aggregations of microorganisms attached to surfaces. Bacteria in the biofilm join together and form a protective matrix around each other. It is estimated that up to 90% of microbial populations exist as biofilms, rather than as discrete organisms (planktonic cells) floating around in the environment.
"Biofilms are sessile microbial communities where microbes live together in association with each other on biotic or abiotic substrates which are bounded by extracellular polysaccharides, proteins, lipids and DNA." In other words, simply, biofilms represent an important mode of bacterial life colonizing most of the surfaces in nature. (Singh *et al.* 2019) [7]

History of biofilm

Year	Investigator	Contribution
17th century	Antony van leeuwenhook	First examined microorganisms from his own teeth surfaces

(Patel, 2014) [6]

Why microorganisms prefer to exist in biofilm?

Microorganisms residing in biofilms get many advantages as compared to freely swimming planktonic stage, and that's the reason for them to prefer biofilm mode of living. Some of these potential advantages are:

- Microorganisms in biofilms exhibit elevated antimicrobial tolerance and also get protected from environmental stresses such as extreme pH, oxygen, osmotic shock, heat, freezing, UV radiation, predators, and so on.
- Extracellular polymeric matrix formed from the secreted exopolysaccharides (EPS) increases the binding of water resulting in decreased chance of dehydration (desiccation) of the bacterial cells, which is a common stress condition experienced by planktonic cells.
- The adherent nature of microbial cells in biofilms allows rapid exchange of nutrients, metabolites, and genetic material.

Extracellular polymeric substances (EPSs)

Extracellular polymeric substances consists primarily of polysaccharides. EPSs provide the matrix of structure for the biofilm. They are highly hydrated (98% water) and tenaciously bound to the underlying surface. Has "water channels" that allow transport of essential nutrients and oxygen to the cells growing within the biofilm. Biofilm acts as filters to entrap particles of various kinds including minerals and host components such as fibrin, RBCs and platelets. EPSs may associate with metal ions, divalent cations other macromolecules (such as proteins, DNA, lipids and even humic substances)

Biofilm locations

- Dairy processing plants
- Food and beverage plant products line
- Water system
- Pharmaceuticals manufacturing processes
- Raw materials suppliers processes
- Cleaning chemicals
- Steam lines
- Cosmetics and nutraceuticals plants
- Heat exchangers

Why study of biofilm in dairy industry is important?? (Genesis)

- Biofilm formation in dairy industry is always noted as threat which affects the product safety and thereby resulting in food borne illness. So, it is considered as an emergent public health concern throughout the world.
- Consumer demand for higher quality products with respect to their shelf life and safety.
- Current trends towards lower processing runs, automation, complexity of equipment and increased awareness of the problems caused by pathogens such as Listeria *monocytogenes* makes biofilm a concern.
- *Bacillus subtilis*, and *Bacillus cereus* will often cause sweet coagulation and bitter taste in milk and cream and the gas producing *Clostridium tyrobutyricum* may cause spoiled texture and late-blowing in semi-hard cheeses.
- Spore forming thermoduric and thermophilic bacteria are commonly found in high numbers in milk powder after 16-20 hours production time due to biofilms formed on the large internal surface in evaporators and spray dryers.
- To determine density of the biofilms formed by bacteria isolated from dairy equipment.

Biofilm Forming Microbes of Dairy Industry:

Microorganisms occurring in the food industry could be a source of secondary contamination in food products. The other important biofilm forming genera of dairy industry are *Bacillus*, *Pseudomonas*, *Listeria*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Salmonella* Typhimurium and *Coronobacter sakazakii* etc.

Biofilm Forming Microbes of Dairy Industry

Biofilm forming genera in Dairy Industry	Why they form biofilm?
1. *Bacillus*	Present in raw and even pasteurized milk, Example – *B. subtilis* (It requires mainly carbon and energy to make the biofilm and use a number of sugars, organic acids and different organic compounds for this task).
2. *Pseudomonas*	Example –*P. fluorescens* (It is well-known for this cause because of its high heat resistance and short generation time and these characteristics make it a successful biofilm former)
3. *Listeria*	Example- *Listeria monocytogenes* (It is mainly affected by temperature, strain origin and nutrient level and also has the property of attachment to surfaces passively and its biofilms are primarily comprised of teichoic acids which can grow on polypropylene, steel, rubber and/or glass surfaces)
4. *Staphylococcus*	Example- *Staphylococcus epidermidis* (In the process of staphylococcal biofilm formation, the accumulation and development of a mature stage depend mainly on the polysaccharide intercellular adhesions (PIA) that promote bacterial accumulation, especially polysaccharide poly-N-succinylb- 1-6 glucosamine (PNAG))
5. *Streptococcus*	Example- *Streptococcus. Thermophilus* (Affects mainly cheese and pasteurized milk – In the heating chamber of the section where temperature remains within

	30 to 730C lies, the maximum degree of biofilm formation occurs. As a result the defects in milk and cheese quality like acidic flavour and undesirable texture are spotted.)
6. *Lactobacillus*	Example-*Lactobacillus* like *L. rhamnosus* (Biofilm formation by *Lactobacillus* spp. Is relatively beneficial because of its property of colonization and longer mucosal permanence of the host as these help in avoiding pathogenic bacterial colonization)
7. *E. coli*	Example- *E. coli* (The autoinducer- 2 (AI-2) of *E. coli* O157:H7 act as supplementary force for biofilm production as AI-2 signals regulate chemotaxis, flagellar synthesis and motility of genes. The *E. coli* O157:H7 yields exopolysaccharides (EPS) which helps in cell attachment and formation of 3D structures of biofilms.)

(Singh *et al*., 2019) [7].

Methods of Biofilm Detection

A) Direct Obseravation

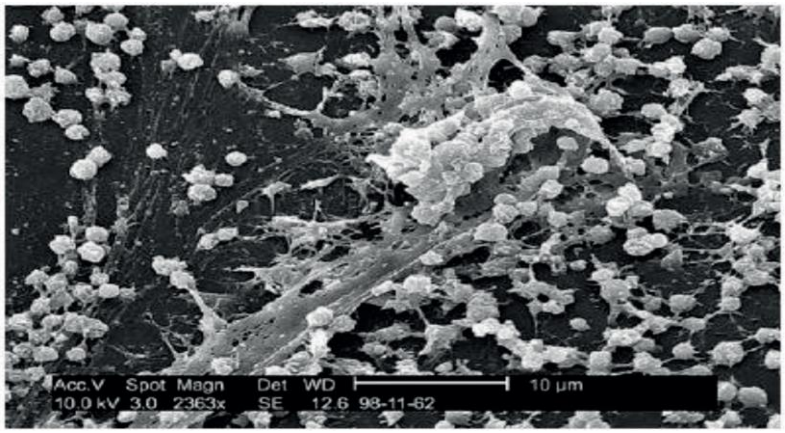
1. Light microscope

Microorganisms - *Candida albicans*, *E. coli*, *Pseudomonas*, and *Staphylococcus epidermidis* adhered on acrylic sheets of polymethacrylate films, glass cover slips, and polystyrene petri dishes have been observed.

Dyes used -Epifluorescence and fluorescent to enhance image clarity of microorganisms. The observation with the light microscope enables researchers to compare morphologies of sessile form and planktonic form of microorganism required by making smear and centrifuging of sample.

2. Transmission electron microscope (TEM)

Images of cells and cell structures such as protein and nucleic acid are obtained by electrons at high magnification and resolution. Monitoring of components of cell can be done directly in TEM by negative staining. Due to photons and electrons penetrating cells poorly, thin section of cell cut is stabilized and stained by certain chemicals with the treatment of osmic acid, permanganate, uranium, lanthanum, or lead salts. These stains contain high atomic weight. Due to stains having high atomic weight, contrast is accelerated by electron dispersion from sample.



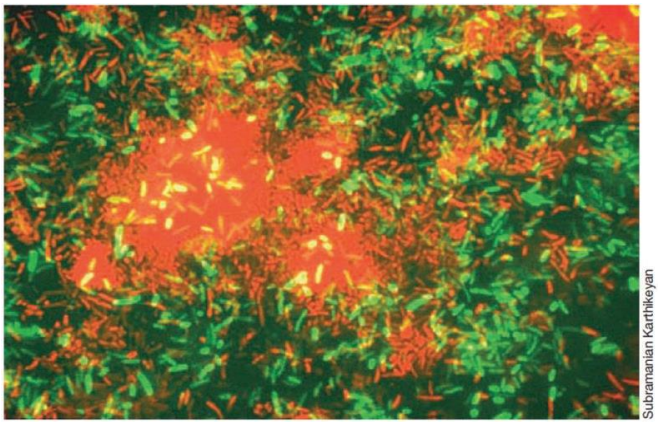
The SEM image of S. aureus embedded in biofilm colonized on intravenous catheter

3) Scanning electron microscope (SEM)

To visualize 3D images of cell sample is coated with heavy metals such as gold. Electrons released from metal coating of sample are caught by SEM for image production. The procedure of SEM is similar to TEM except for some additional chemicals (gold), lacking infiltration, embedment in resin, polymerization, and thin section staining with lead citrate and uranyl acetate.

4) Confocal laser scanning microscopy (CLSM)

Biofilms formed on flow cells of which surface are transparent can be observed by confocal laser screening microscopy (CLSM). Three-dimensional (3D) morphology and physiology of biofilms can be screened by CLSM [2]. Thick samples such as biofilms and microorganisms localized in the depth such as biofilm-embedded microorganisms need to be observed by CLSM.



Bacterial community embedded in a biofilm matrix visualized by CLSM. Each bacterium observed with a distinct color located at different depths of biofilm

5) Bio Finder

Traditional method can take a long time to detect biofilms on food-contact surfaces using traditional microbiological culture methods, but for the food industry it is very important to reduce the time required to confirm microbial contamination to know the hygienic state of the environment and be able to take fast corrective measures. This is why using BioFinder is a good solution, because it visually detects the presence of biofilms just seconds or minutes after application. The product is designed to react with catalase, an enzyme present in almost every living cell and universally found in biofilms. When biofilms are present, it produces a highly visible color change and a layer of many small bubbles. BioFinder can also be used to check critical inspection points just before disinfection and to validate correct hygiene procedures at production sites.

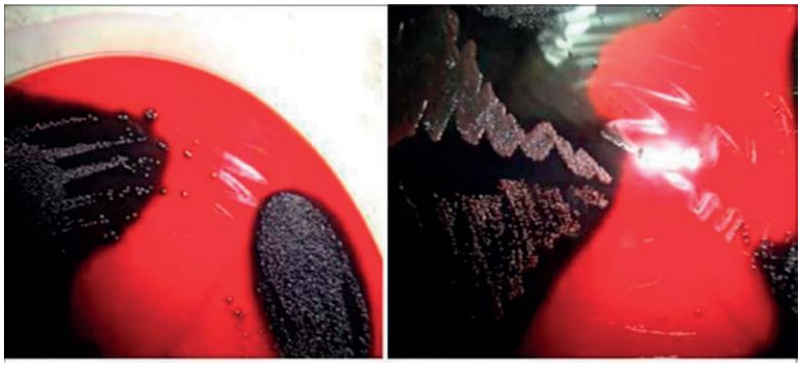
B) Indirect observation

1. Roll plate method

Roll plate method is applied for the detection of possible microbial colonization having a potential to develop indwelling device-associated infection on the outer surface of cylindrical materials such as catheters and vascular grafts. Microorganism colonize on external surface of catheter is detected by roll plate method, instead of microorganism colonize on intraluminal site of catheter. Material is touched and rolled on the surface of medium.

2. Congo red agar (CRA) method

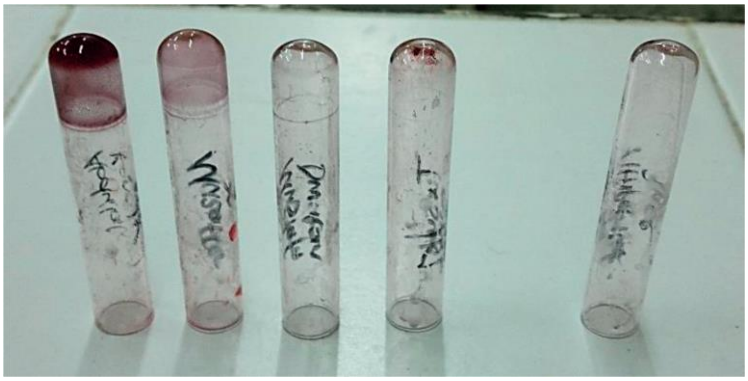
Congo red agar (CRA) method that is a qualitative assay for detection of biofilm producer microorganism, as a result of color change of colonies inoculated on CRA medium, is described by Freeman et al. The CRA medium is constructed by mixing 0.8 g of Congo red and 36 g of sucrose to 37 g/L of Brain heart infusion (BHI) agar. After incubation period that was 24 h at 37 °C, morphology of colonies that undergone to different colors is differentiated as biofilm producers or not. Black colonies with a dry crystalline consistency indicate biofilm producers, whereas colonies retained pink are non-biofilm producers.



CRA method applied on CRA medium. Black crystalline colonies of biofilm producer cell and pinkish-red Colonies of biofilm nonproducer cell.

3. Tube method (TM)

Tube method (TM) that is a qualitative assay for detection of biofilm producer microorganism, as a result of the occurrence of visible film, is described by. Isolates are inoculated in polystyrene test tube which contained TSB and incubated at 24 h at 37 °C. The sessile isolates of which biofilms formed on the walls of polystyrene test tube are stained with safranine for 1 h, after planktonic cells are discharged by rinsing twice with phosphate-buffered saline (PBS). Then, safranine-stained polystyrene test tube is rinsed twice with PBS to discharge stain. After air drying of test tube process, the occurrence of visible film lined the walls, and the bottom of the tube indicates biofilm production



Tube method: The first two polystyrene test tubes from the left indicate biofilm production. Other test tubes rather than the first two polystyrene test tubes from the left indicate lacking of biofilm production.

4. ATP bioluminescence

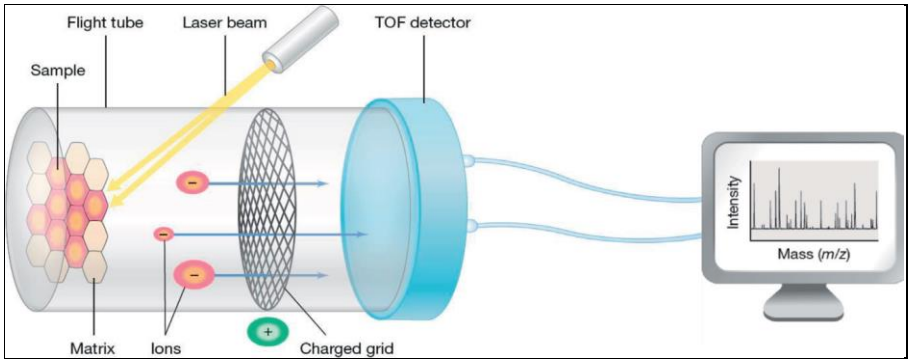
ATP bioluminescence, based on using the enzyme luciferase, which emits light in the presence of ATP that is then measured quantitatively with an instrument called a luminometer. Although results can be available within 20 s, this method is not very suitable for sampling biofilms, especially when they are mature, because ATP transference inside the biofilm is very low, and the resulting low ATP numbers can indicate a low microbial load when in fact there is a mature biofilm composed of a high concentration of microorganisms.

5. Mass spectrometry

EPS can be detected and characterized by mass spectrometry (MS) Large biologic molecules can be also detected and characterized in complex biologic structures such as EPS by MS. Chemicals involved in biofilm process are examined in detail by MS. Electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) are the types of MS. In time-offlight (TOF) mass spectrometer, mass is analyzed by ions desorbed in vacuum chamber. These two technics are combined and called MALDI-TOF.

Sample is ionized and vaporized by laser. Ions generated from sample by laser pass through the column of MALDI-TOF device toward TOF detector by an electric field. Depending on the mass/charge ratio of molecule, measurements are done by TOF. If this ratio is smaller, ions move faster.

Bacteria are identified, expression of bacterial proteins such as surface proteins and exoenzymes like β-lactamase in response to antimicrobials can be monitored, and growth of bacteria is measured by applications of MALDI.



MALDI-TOF mass spectrometry device

Common Process for Biofilms Control

1. Physical Control

- **Cleaning and disinfection**

In the dairy industry the classical operations of cleaning and disinfection are essential parts of milk production. The efficiency with which these operations are performed greatly affects the final product quality. Generally, disinfectants- do not penetrate the biofilm matrix left on a surface after an ineffective cleaning procedure, and thus do not destroy all the biofilm living cells. Therefore, cleaning is the first step and of utmost importance to improve the sanitation of the processing equipment It is important to effectively remove food debris and other residues that may contain microorganisms or promote microbial growth.

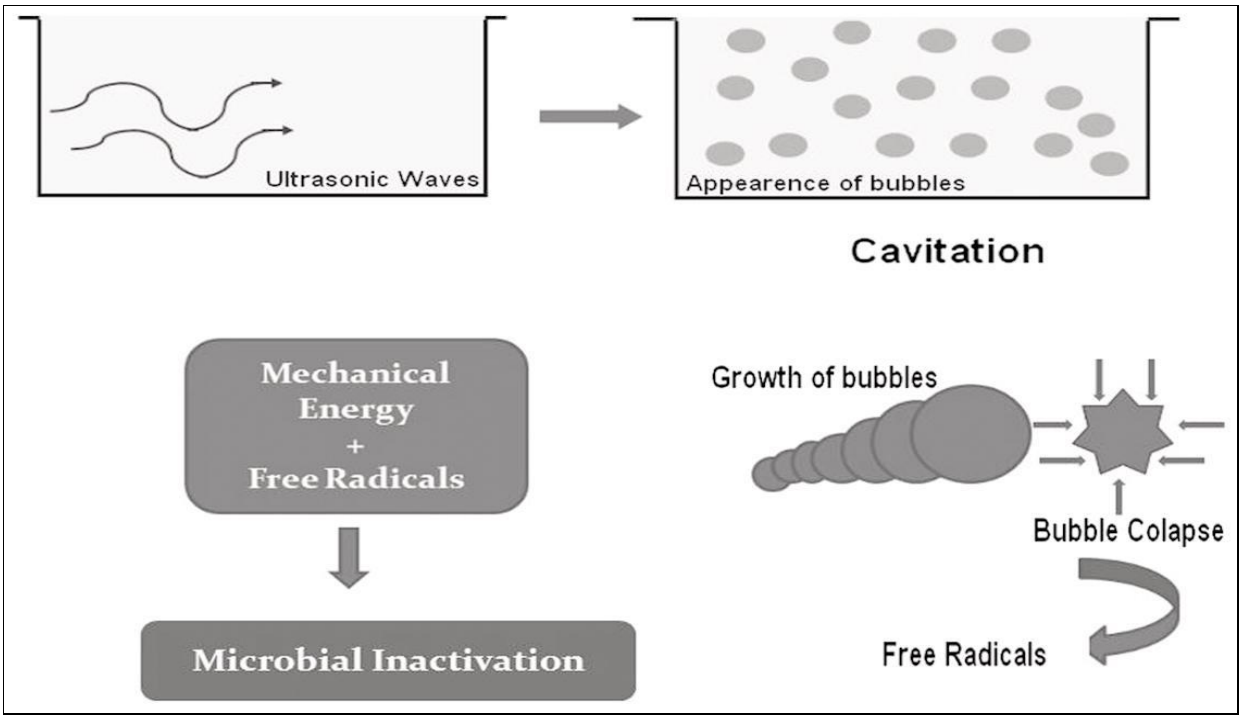
The use of high temperature can reduce the need for the application of physical forces such as water turbulence and scrubbing

Chemical products commonly used for cleaning are surfactants or alkali products, used to suspend and dissolve food residues by decreasing surface tension, emulsifying fats, and denaturing proteins. Cleaning-in-place (CIP) procedures are usually employed in milk processing lines. An independent quality control system to monitor the cleaning results for a dairy plant can be integrated in the Hazard Analysis Critical Control Points (HACCP) program. Evaluation of biofilm sanitation should be part of the HACCP development plan in order to control those biofilms prevalent in the processing areas. Moreover, impairing the formation of biofilms can be achieved through a better knowledge of the mechanisms that contribute to their formation, development and maintenance. (Menon, 2016) [5]

- **Ultrasonication**

Combined effect of power ultrasound and heat (thermosonication) has proved to be more efficient method of microbial in cativation than either of the two methods alone. Microbial inactivation of ultrasound treatment accounts for generation of acoustic cavitations, resulting in increased permeability of membranes, selectivity loss, cell membrane thinning, confined heating, singlet electron transfer in cooling phase, and hydroxyl radical formation. High-frequency ultrasound method, patented as sonoxide, has more than 600 applications and provided best results in inhibiting bacterial and algal growth in industrial waters. Ultrasonic-treated cells were found to lack internal content when viewed under transmission electron microscopy, but disintegration was not affirmed to be main reason of cell death.

Ultrasonication has achieved the FDA requirement of a 5-log reduction in microbial population. Exploitation of ultrasound as means of inhibiting and killing micro-organisms came from the observation that sonar used for anti-submarine warfare resulted in killing of fishes. Ultrasound frequency of 20 kHz and power of 12.8 W was used on 50 cm^3 water contaminated with *Streptococcus mutans* for a period of 15 min and 97% microbial reduction was achieved. Ultrasonic power of around 100 W was found to be optimal for maximum microbial inactivation and ultrasonication has been found to be effective method for microbial inactivation in *Escherichia coli*, *Listeria monocytogenes*, and other pathogens. Efficiency of ultrasonic treatment as antimicrobial tool depends on the physical (size, hydrophobicity) and biological (gram-status, growth phase) characteristics of the micro-organisms. It has been demonstrated that micro-organisms with “soft” and thicker capsule are extremely resistant to ultrasonic treatment.



Cavitation phenomenon and microbial inactivation by ultrasonic waves

- **Steel Coatings**

One promising approach focuses on nanotechnology agents. The unique properties of nanoparticles (NPs) distinguish them from their bulk chemical counterparts.

Mechanism - One such property is their large surface area to volume ratio, which creates a higher number of functional sites and can enhance the influence of NPs on a given microorganism. Since the antibacterial properties of some NPs are mediated mainly by direct contact with the bacterial cell wall and do not require penetration, most bacterial antibiotic resistance mechanisms are not relevant when dealing with NPs. This favorable property has stimulated extensive research on the antibacterial effects of diverse NPs types, such as carbon-based materials (fullerenes and carbon nanotubes), dendrimers that provide cavities for other molecules, nanocomposites, natural NPs and metal-based NPs, including silver, gold, metal oxides. Due to their potent antimicrobial effects, silver compounds have been used since ancient times to prevent microbial infections associated for example to water consumption. Currently, silver NPs are the most widely studied. However, metal oxide NPs are more commonly used within industry. They include iron oxide (Fe3O4), titanium oxide (TiO2), zinc oxide (ZnO), copper oxide (CuO) and magnesium oxide (MgO). These NPs show antimicrobial properties and can be applied in diverse industrial environments.

Example - is the coating of a stainless steel surface with the modified plastic Ni-P-polytetrafluoroethylene. This compound was able to reduce biofilm formation by *Geobacillus stearothermophilus* and *Bacillus licheniformis* by two orders of magnitude, in comparison with the control stainless steel surface. It was also effective in preventing milk deposition along the same surface.

In line with this latter strategy, functionalized surfaces with polymers including lysozyme in their composition showed antibacterial and antibiofilm properties of great industrial interest: they were able to kill 95, 92, and 94% of E. coli, S. aureus, and *C. albicans* biofilms, respectively. (Galle *et al.* 2018) [1]

- **High Hydrostatic Pressure**

High hydrostatic pressure (HHP, 300–900 MPa) is able to 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. Anyway, some non germinating spores could remain in the food matrix after HHP treatments, and therefore, at industrial level, HHP is usually combined with thermal treatments (50_C to 100_C), or in some cases with essential oil components. One important advantage of HHP treatments is that they do not alter the organoleptic and nutritional properties of the food matrixes (taste, vitamins, etc.), a great adavantage with respect to high temperature methods. (Galle *et.al*. 2018) [1]

- **Non-thermal Plasma**

Non-thermal plasma is a partially ionized gas with low temperature and interesting antimicrobial properties. It is produced at atmospheric pressure by mixing UV light with oxigen, nitrogen, ozone, and water and helium, under an electrical discharge. It is able to destroy bacterial biofilms of Gram-negative (*Pseudomonas* spp., *S. enterica*) or Gram-positive (*Bacillus* spp.) species in just 10 min, However, its use is still restricted to some laboratory applications, due to its high cost. (Galle *et.al*, 2018) [1]

2. Chemical Control

Biofilms can be control by the use of biocides, antibiotics, and ion coatings. Studies show that use of amino glycosides in combination with iron-chelating compounds is important in the disruption of *Pseudomonas aeruginosa* biofilms. Application of sodium citrate can also inhibit biofilm formation of *Staphylococci* species *in vitro*. Antimicrobial agent such as N-alkylpyridinium bromide attaches to a poly (4-vinyl-N- hexylpyridine) is capable of inactivating about 99% of *E. coli*, *S. epidermidis*, and *P. aeruginosa* bacteria. Peroxyacetic acid (PAA) is a sanitizer with high oxidizing potential is effective against bacteria, fungi and spores in the

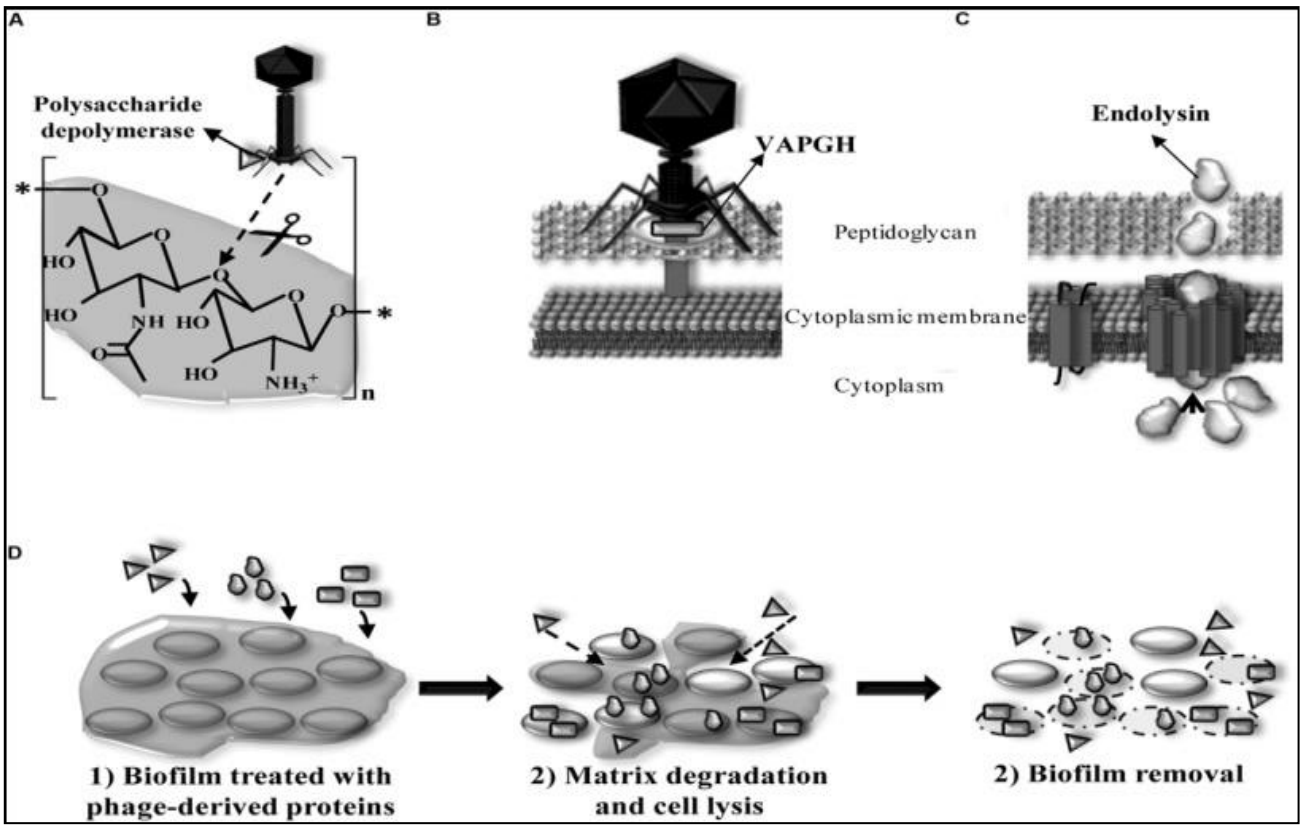
dairy industry because it is not inactivated by catalase or peroxides. Studies show that use of ozone in Europe was effective in disinfecting drinking water and it is also a better oxidizer than chlorine and hence effective in inactivating Pseudomonas fluorescents on glass slides. (Kabwanga *et al*., 2018) [2]

3. Mechanical Control

The mechanical biofilm control methods aim at the disturblization of bacteria from surface attachment, surface charge and hydrophobicity through the application of compounds that can prevent the biofilm formation and their spread on surface. The use of smooth surfaces equipment is more preferred as they are less susceptible to biofilm adhesion. Modification of the surface charge of polymers also enables the prevention of biofilm. Positively-charged polycationic chains enable the molecule to stretch out and generate bactericidal activity. (Kabwanga *et al*., 2018) [2]

4. Bacteriophages Control of Biofilms

Bacteriophages are a numerous group of viruses which are easily manipulated, and they have various functions in biotechnology, bacterial control, and therapeutics. Bacteriophages are ubiquitous in nature that infects bacteria naturally and may provide a natural, highly specific, non-toxic, feasible approach for controlling biofilm formation. They may either coexist with their host by inserting themselves into the bacterial genome (lysogenic bacteriophages) or destroy them. Phage T4 and E27 help in the control of E. coli and Pseudomonas aeruginosa biofilms. *Enterobacter agglomerans* type of biofilms can be destroyed through cell lysed by bacteriophage. Many studies show that phages alone disrupt *Staphylococcus epidermidis* growing biofilm colonies on silicon catheters. Phages are also effective in the removal of biofilms in their early stages of development about 5 days old biofilms of *P. fluorescens*. A bacteriophage such as *L. monocytogenes* phage ATCC 23074-B1 helps to inactivate *L. monocytogenes*. (Kabwanga *et al*., 2018) [2]



5. Enzymatic Control of Biofilm

Biofilm in the dairy industry are formed by; *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Streptococcus thermophiles* are the most common microorganism that form biofilms. Enzymes like α-amylase, β-glucanase and protease were proved effective in the cleaning of adhered industrial biofilm formed during paper pulp production. Exopolysaccharide degrading enzymes more so the colanic acid degrading enzymes derived from a Streptomyces isolate was reported for the removal and prevention of biofilm formation. Biofilms control with proteases such as Proteinase K and Trypsin, ensuresass the destruction of biofilm formation and biofilm removal and can disrupt biofilms formed by *S. aureus*. Synergistic action of enzymes in combination with surfactants and phenolic antimicrobials are important in the control of biofilms although the application of enzymes in biofilm control is still limited.

Enzymes like lipases and proteases are often selected as complementary cleaning agents when simple chemicals such as alkaline and acid are not enough for cleaning and recovering the membrane capacity. However, most of the studies using enzyme cleaners focus on the removal of protein fouling, but did not aren't effective on biofilms. (Kabwanga *et al*., 2018) [2]

Novel safe approaches for the control of biofilm formations

1. Polysaccharides	Can inhibit the biofilm formation of bacteria, possibly by modifying the physical properties of both abiotic and biotic surfaces. It was shown that *E. coli* exopolysaccharides can alter the abiotic surface properties such as increase the hydrophobicity of glass surfaces and also can prevent cell-to cell auto aggregation via adhesions of bacteria
2. Enzymes	Serine proteases were efficiently reducing *Bacillus* biofilms whereas polysaccharides remove more efficiently *Pseudomonas fluorescens* than serine proteases. Polysaccharide polymerases and esterase can also control biofilm formations.
3. Nisin- produced by some strains of *Lactococcus lactis* and has been employed as an antibiofilm agent	Nisin has a mode of action that results in the formation of pores in the cell membrane of the bacteria. Pore formation leads to cell lysis and death. The bactericidal activity of nisin has been shown to target other Gram positive bacteria closely related to *Lactococcus lactis* and some Gram positive pathogens, such as *Listeria monocytogenes*. Nisin is effective against planktonic cells of multi-drug resistant staphylococci
4. Citric acid- alternative disinfectant in controlling biofilm formation in the dairy industry.	The prevention and removal of biofilm formation of *S. aureus* strains isolated from raw milk by citric acid treatments (2% and 10%) for 20 min were assessed for comparison with peracetic acid treatment (0.3%) on both on microtitration plate and stainless steel coupons. The prevention and removal of biofilm formation ratios and the numbers of prevented or removed *S. aureus* strains were observed to be higher by using citric acid treatments compared with peracetic acid treatment on both surfaces. Moreover, the prevention and removal of biofilm formation were substantially higher when the concentration of citric acid treatment increased from 2% to 10% and the stainless coupons were used.
5. Gallic acid- phenolic products found in plants such as tea leaves, fruits and flowers	It has been shown that gallic acid has strong antimicrobial activity against several bacterial strain.also reported antibiofilm activity of gallic acid for the prevention and removal of *E.coli*, *P. aeruginosa*, *S. aureus* and *L. monocytogenes* biofilms. The researchers found that gallic acid can prevent and remove these pathogens by promoting reductions in biofilm activity >70% of all tested microorganisms.
6. Malic acid	The antimicrobial action of malic acid is to lower the pH value or cause the significant damage to the cytoplasm of bacteria also found that malic acid was also effective in food industry for complete inhibition of *Salmonella* Typhimurium biofilm in carrot and other food contact surfaces.

(Meltem, 2015) [4]

Positive aspects of biofilm

1. Water and wastewater treatment

Natural biofilm forming ability of microbes in developing water-treatment systems like trickling filters for removal of biological pollutants. Biological filters are employed for reducing the concentration of biodegradable organic carbon entering the water distribution systems.

2. Fungal-rhozobial biofilm

Fungal-rhozobial biofilm may be used as inoculum to improve nodulation and nitrogen fixation in Rhizobiumlegume symbiosis. Higher nodulation and enhanced N2-fixation was observed in *Penicillium-Rhozobium* biofilm treated plants.

3. Oil degradation/recovery

Biofilms are used for microbially enhanced oil recovery (MEOR). Biofilm of *Clostridium acetobutylicum* was employed to enhance oil recovery from fields in Arkansas, USA.

4. Removal of heavy metals

Phototrophic biofilms have important role in the detoxification of waste water polluted with heavy metals. Mucilage sheaths of cyanobacteria, *Microcystis aeruginosa* and *Aphanothece halophytica* are known to have high affinity to heavy metal ions including copper, lead, and zinc. These types of applications are based on biosorption or bioaccumulation of metal ions by microbial biomass.

Conclusions

Biofilm formation possesses profound implications and throws a major challenge to the dairy sector where they act as the principal reservoir of microbial contamination. These lead to financial crisis by impairment of raw material and its products. Therefore, choosing of a profound, prominent and efficient measure is in an urge in order to safeguard the whole sector from further deficiency and mitigating the present problem. In particular, biofilms formed on milk-processing equipment and other food contact surfaces act as a persistent source of contamination threatening the microbiological quality and safety of milk products, and may result in food-borne disease and economic losses.

Cross-contamination has been shown to be a risk that causes 25% of food poisoning outbreaks, many of which originate in contaminated surfaces, implying a direct relation with poor surface hygiene. It is important to detect biofilms of microorganisms, determine antibiofilm activity of agents against biofilm. Biofilm formation can be reduced by various natural ingridients. It is emphasized that god manufacturing practice, good hygienic practice and hazard analysis and critical control point should be implement in dairy industry to prevent the contamination of dairy products.

References

1. Galié, Serena, Coral García-Gutiérrez, Elisa M Miguélez, Claudio J Villar, Felipe Lomb. Biofilms in the Food Industry: Health Aspects and Control Methods. Frontiers in Microbiology 2018;9:1-18.
2. Kabwanga Ismail T, Atila Yetisemiyen, Shakira Nankya. Dairy Industrial Hygiene: A Review on Biofilm Challenges and Control. International J of Research – Granthaalayah 2018;6(2):268-273.
3. Kirmusaoglu Sahra. The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents. Intech Open 2019, 1-17.
4. Meltem Yesilicimen Akbas. Bacterial biofilms and their new control strategies in food industry. The battle against Microbial Pathogens: Basic Science, Technology Advances and Educational Programs 2015,383-394.
5. Menon Vrinda K. Biofilm and Food Industry. International J. of Advanced Res. In Biological Science 2016;3(4):137-142.
6. Patel Ina, Vaibhavi Patel, Asha Thakkar, Vijay Kothari.

Microbial Biofilms: Microbes in Social Mode. International J of Agriculture and Food Research 2014;3(2):34-49.
7. Singh Parul, Basak G, Barkha Sharma, Udit Jain, Raghavendra Mishra, Vaishali. Biofilm: An Alarming Niche in Dairy Industry International J of Livestock Research 2019;9(4):10-24.