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Advances in Industrial Biofilm Control with Nanotechnology - A Review

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Abstract

Biofilm is an assemblage of microbial cells that are irreversibly associated with a surface and enclosed in a matrix of primary polysaccharide material. It is not removed by gentle rinsing. In industrial systems, biofilms frequently grow on cooling water tubes and heat exchanger channels. They cause increased pressure drop and reduced heat transfer efficiency which ultimately lead to an increase in costs of production and maintenance, as well as to public health concerns and environmental impacts. Nanotechnology which involves the science of manipulating materials on an atomic, molecular and macromolecular scale has proven to be an advance in the control of industrial biofilms due to the resistance of industrial biofilms to biocides. This method has been successful due to the unique interaction of nanoparticles with biological systems making these biofilms susceptible to these nanoparticles therefore destroying them indefinitely. The increase in costs of operation in industries have brought about recent advancements in industrial biofilm control which has identified nanotechnological approaches as potential tools in eradicating industrial biofilms. The inability of nanoparticles to produce disinfectant by-products is an advantage of nanotechnological approaches over the use of biocides to tackle industrial biofilms. Further studies on nanotechnological methods of controlling biofilms are being developed and so far have proved very effective in the control of industrial biofilms.

Keywords

Biofilm, Control, Industry, Microorganisms, Nanotechnology

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1. Introduction

Biofilms are complex microbial ecosystems formed by one or more microorganisms in an extracellular matrix. A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances (EPS). Biofilms exhibit high level of microbial and life organization where microbes form and live in a well structured, coordinated and functional ecosystem on surfaces of material including living things. The biofilm production is a successful strategy for microbial survival and infection establishment [1]. Bacteria, yeasts and molds can form biofilms on various surfaces. The presence of more than one bacterial species in a biofilm matrix facilitates biofilm's attachment and survival to a surface. This mixed species microbial community increases the biofilm's resistance to antimicrobial substances and enhances colonization of new environments through their extracellular matrix [1]. The extracellular matrix is mainly composed of polysaccharides, such as cellulose, proteins or exogenous DNA. This matrix can be fixed to hard surfaces (food industry equipment, transport, dispensing and storage surfaces, soil, etc.) or to biological structures (vegetables, meat, bones, fruits, etc.) [2].

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Microorganisms especially bacteria are very capable of adjusting their nutritional requirements for survival in their environments. In the last decades a substantial effort has been put forth to improve our understanding on microbial biofilms, defined as complex and well-organized biological communities embedded in a self-produced extracellular polymeric matrix that can develop into moist surfaces either biotic or abiotic [3, 4].

Biofilms are ubiquitous in organic life. Nearly every species of microorganism have mechanisms by which they can adhere to surfaces and to each other. Biofilms will form on virtually every non-shedding surface in non-sterile aqueous or humid environments. Biofilms can grow in the most extreme environments: from, for example, the extremely hot, briny waters of hot springs ranging from very acidic to very alkaline, to frozen glaciers. Bacteria often form biofilms on surfaces of wide variety of infrastructure, such as plumbing, oil refineries, water treatment plants, paper mills, heat exchangers and medical implants. Biofilms are important components of food chains in rivers and streams and are grazed by the aquatic invertebrates upon which many fish feed. Biofilms are found on the surface of and inside plants. They can either contribute to crop disease or, as in the case of nitrogen-fixing Rhizobium on roots, exist symbiotically with the plant. The presence of biofilms is common in industrial systems such as the food industry and represents a concern because bacteria can adhere to almost any type of surface, such as plastic, metal, glass, soil particles, wood and food products [5].

In industrial systems, biofilms frequently grow on cooling water tubes and heat exchanger channels. They cause increased pressure drop and reduced heat transfer efficiency, which ultimately lead to an increase in the costs of production and maintenance, as well as to public health concerns and environmental impacts [6]. Biofilm formation in recirculating water systems such as cooling towers lead to many undesired conditions in terms of public health concern, operational damage and economic loss. Circulating cooling systems are key enablers of any industrial process. In such systems, microbial clusters or layers are predominant due to reasons such as the presence of nutrients and water, suitable temperatures, high surface-volume ratio [7].

Food-borne diseases associated with bacterial biofilms on food matrixes or factory equipment may arise via intoxications or infections. Toxins, for example, can be secreted by biofilm found within food processing plants. From there, they can contaminate a food matrix, causing individual or multiple (in the case of an outbreak) intoxications. In either case, the presence of biofilms in a food factory puts human health at risk. The amount of risk is dependent on the bacterial species forming this tridimensional living structure. The main locations for biofilm development depend on the factory type, but may include water, milk and other liquid pipelines, pasteurizer plates, reverse osmosis membranes, tables, employee gloves, animal carcasses, contact surfaces, storage silos for raw materials and additives, dispensing tubing, packing material, etc. [8].

Bacteria in biofilms confer survival advantages to its members since they are protected from environmental stress such as ultraviolet light, dehydration or treatment with antimicrobial and sanitizing agents, which makes their elimination a huge challenge. Biofilm layers are potential niches for pathogenic organisms, especially *Legionella pneumophila* [9] and can lead to problems such as decrease in heat transfer rate and significant energy losses due to increased heat transfer resistance, increase in frictional resistance and blockage in pipes, local corrosion of metallic substrata (microbiologically induced corrosion [MIC]) [7]. As a result of adverse effects of biofouling, serious economic losses and hygiene problems including those associated with *Legionella* may occur [10]. Serious expenses required to eliminate biofouling in systems, increase the capacity of the equipment and premature replacement of corroded equipment before the schedule. Costs for biofouling prevention are estimated to reach billion dollars for individual countries or industries per year.

Biocides (chemical agents with antiseptic, disinfectant or preservative actions), with their broad spectrum of usage, appear to be a good way to control or prevent undesirable biofilm formation. The applications of these chemical require a considerable economic effort and expensive infrastructures [11], but they are also responsible for the production of harmful disinfection by-products (DBP). Chemicals like free chlorine, chloramines and ozone can react with various natural water constituents thus forming DBPs, many of which are toxic and/or carcinogenic [12, 11]. For these reasons more efficient cleaning procedures are needed in order to reduce the consumption of chemicals and energy, as well as to minimize the health and environmental risks of these chemical disinfectants.

Recent advances in the micro-nanotechnology field have gained significant interest in its environmental and biological applications. In fact nano and microtechnology presents a unique alternative to biofilm control and elimination. Nano and microparticles are excellent adsorbents, catalysts and sensors due to their large surface to volume ratio (optimized for loading and carrying antimicrobial agents, for example), are highly reactive and present unique interactions with biological systems [12, 13]. These particles are also non-toxic, economical to produce and stable, once made [13]. Antimicrobials can be loaded into these particles by physical

encapsulation, adsorption or chemical conjugation. This can present several advantages such as significant improvement of the agents' activity, in contrast to the free product, and release of the antimicrobial in a sustained and controlled manner [14, 15].

1.1. Biofilm

Microorganisms are capable of growing in each a free form (planktonic) or as biofilms hooked up to solid surfaces. Biofilms are defined as the assemblage of microbial communities that commit to find organic material as their nutrient source; it can get adhered to the surface and go on to continue with their growth and maturation stage by using suitable methods of adherence [16]. A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material. It is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix [17]. Biofilms can be a bacterial type or a fungal type. The biofilm comprising of a single type of microbial cells is "pure" biofilm and the one in which different microbial cells reside in the biofilm matrix is "mixed" biofilm. Biofilms might form on a large variety of inert solid surfaces, as well as living tissues [18].

Noncellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix. Biofilm-associated organisms also differ from their planktonic (freely suspended) counterparts with respect to the genes that are transcribed. Biofilms may form on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems [17]. Biofilms protect their constituent cells in various ways, which makes industrial contamination difficult to treat. As self-organized communities, biofilms have evolved to feature differentiated cell phenotypes performing complimentary functions. The associated cooperative behavior of bacterial cells, mediated by cell-cell communications and other factors, enables an increased metabolic diversity and efficiency as well as an enhanced resistance to environmental stress and antimicrobial agents [19].

1.2. Definition and Scope of Nanotechnology

Nanotechnology has been defined as the creation of functional materials, devices and systems through control of matter on the nanometer scale (1–100nm), and exploitation of novel phenomena and properties (physical, chemical and biological) at that length scale. Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions approximately 1 and 100 nanometers, their unique phenomena enable novel applications. Encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. Nano is derived from Greek word for dwarf. In simple terms, it is engineering at atomic or molecular level. The prefix 'nano' means ten to the power of minus nine (10^{-9}), and s usually combined with a noun to form words, such as nanometer, nanotechnology, nanorobot. Nanotechnology involves the following;

a. A Research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of approximately 1 to 100-nanometer range.

b. Creating and using structures, devices, and systems that have novel properties and functions because of their small and/or intermediate size.

c. Ability to control or manipulate on the atomic scale.

1.3 Biofilm Formation

Biofilms formation involves:

1) Initial attachment of planktonic cells to the surface.

2) Production of extracellular polymeric matrix (EPS).

3) Microcolony formation and secretion of chemical signals.

4) Maturation of biofilm architecture and

5) Dispersion of cells [20].

The first prior step surely involves the adherence to the solid support and the van der Waals force plays the vital role in the adherence. Before the adherence, the surface conditioning is executed it simply means the exposure of solid surface to the watery medium, coated with the polymers from the medium, providing the chemical modifications that upgrades the rate and extent of attachment [21]. This initial, reversible microbial adherence is mostly dependent on bacterial cell surface characteristics and on the nature of the material surface. However it is mainly due to the physicochemical interactions that bacteria firmly adhere to the biomaterial surface during the adhesion process [22].

In the second step, the microbial cells get affixed to the solid substrate by forming the stronger adhesive compound exopolymeric material. The third stage is the glycocalyx or slime formation stage during which the micro colonies begin to mature. Once the biofilm get mature biofilm the cells use energy frequently in the production of exopolysaccharides, which act as a nutrient source. Fourth step is the maturation step, where the microbial colonies attached to substrate grow and develop into the 3-D structure forming a channel that

comprises of the cluster of packed cells. The last fifth step is the detachment stage, where the biofilm disperses cells so that they can move on to initiate the formation of new biofilms [22].

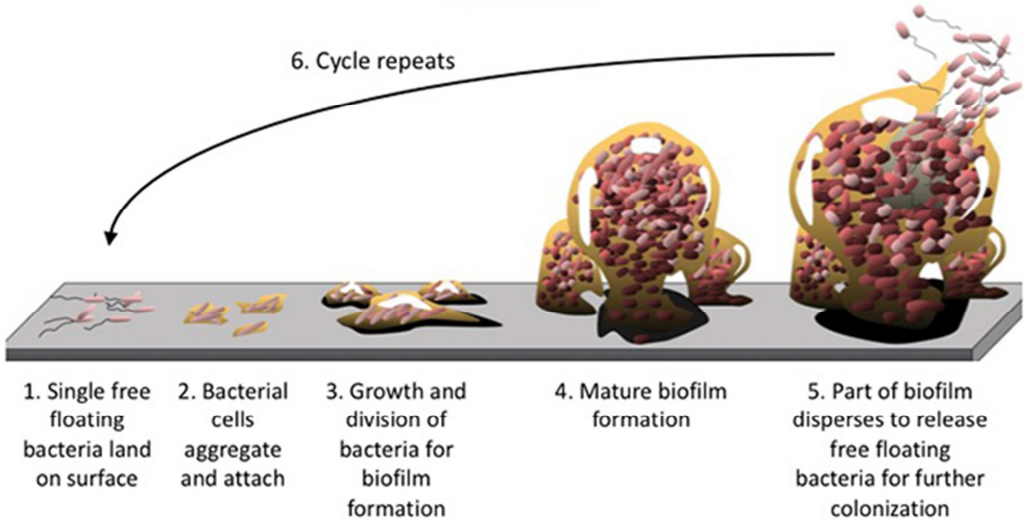

Figure 1. Development of a Biofilm.

2. Biofilm Structure

The application of advanced microscopy, such as confocal laser scanning microscopy, molecular and electrochemical high-resolution methods has provided insights into the structural organization and function of biofilm communities. Therefore, a mature biofilm is seen as a very heterogeneous arrangement, with a basic community structure consisting of microcolonies of microorganisms encased in an extracellular polymeric substance (EPS) matrix separated by water channels. Nevertheless, although some structural attributes can generally be considered universal, every microbial biofilm community is unique [23]. This is due to the fact that a biofilm structure can be influenced by several conditions, such as surface and interface properties, nutrient availability, the composition of the microbial community, the three-dimensional architecture of the matrix (the dense areas, pores and channels), and hydrodynamics, making the exact structure of any biofilm probably a sole feature of the environment in which it develops. A porous architecture, e.g., allows a convectional flow through the depth of the biofilm while within the EPS matrix, only diffusional transport is possible.

Therefore, organisms at the bottom of the biofilm can access nutrients without competing with those at the interface to the bulk water phase. Strong gradients can occur in biofilms, caused, for instance, by actively respiring aerobic heterotrophic organisms, which consume oxygen faster than it can diffuse through the matrix. This generates anaerobic habitats just below highly active aerobic colonies in distances of less than 50 µm. Other gradients, such as pH-value, redox potential and ionic strength are known within biofilms [24].

Hence, the structure of a biofilm can range from a smooth and dense biofilm model to a heterogeneous mosaic model or to one consisting of a more complex organization involving mushroom-like aggregates separated by water channels, normally considered the most typical biofilm architecture [25]. This structure is characteristic of biofilms formed under low nutrient concentration, high hydrodynamic shear stress and the absence of mechanical, abrasive and compressive forces. Concerning the biofilm composition itself, water is considered to be the major component of the biofilm matrix - up to 90% of total volume, being essential as medium for efficient transport of nutrients to microorganisms and also for cell membrane integrity of microbial cells. Microorganisms occupy only between 10% (in most biofilms) and 50% of the total volume of the biofilm, whereas EPS can account for over 90% of the total organic carbon of biofilms [23]. Besides polysaccharides, proteins, or phospholipids, non cellular materials such as mineral crystals, corrosion particles or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix [26].

The matrix is also reservoir of genetic material, namely extracellular DNA, which is now acknowledged as a considerable proportion of the EPS components. The water channels that separate the matrix-enclosed microcolonies are vital to biofilm maintenance, providing a nutrient flow system within it that delivers nutrients deep within the complex community and allows the exchange of metabolic products with the bulk fluid layer [26]. The hydrodynamic flow of liquid over and through the biofilm can also promote the separation of some fragments with viable organisms away from the surface, which can be carried with the flow and

deposited elsewhere for further colonization. Besides protection, EPS matrix immobilizes biofilm cells and keeps them in close proximity, thus allowing cells to exchange information and the formation of synergistic microconsortia by QS molecules - chemical signals, used to regulate cell density-dependent gene expression [27]. Therefore, this complex level of structural organization helps to explain the remarkable metabolic efficiency of microbial biofilms.

2.1. Factors Influencing the Development of Biofilms in Industrial Systems

Industrial biofilms are quite diverse due to a wide range of contributing factors such as microbial species, temperature, nutrient and oxygen availability, flow velocity, surface material type, suspended solids and general water chemistry. Therefore it is difficult to generalize about the types of biofilm that form in these systems, let alone about their control methods [28]. The factors include:

2.1.1. Temperature

The optimum incubation temperature is about 40°C for many bacteria found in cooling water, and this temperature level is the most common in industrial water coolers, especially during the summer months. Microbial activity is very sensitive to temperature, even minor changes in the value of the temperature makes considerable changes in the development of biofilms such as its thickness [10]. Thus, for the evaluation of biocides properly against biofilms in such systems, water temperature should be kept constant at 35°C or 40°C with an immersed electric heater to simulate actual water temperature in the cooling system for maximum microbial growth. Heat transfer surfaces (titanium, admiralty or aluminum brass, and cupronickel 90:10) in an industrial heat exchanger generally experience temperatures in the range 28–45°C for auxiliary cooling systems and 60–70°C for condenser cooling, where bacterial biofilms have been shown to occur [29].

2.1.2. Flow Velocity

In flowing systems, bacterial populations exist as complex, structurally heterogeneous biofilms attached to surfaces. Residence within these complex matrices provides organisms with a higher localized nutrient concentration than that found in normal waters. In the case of heat exchangers, biofilm growth can be controlled if relatively high velocities are imposed, as shear effects are likely to have an impact on biofilm development. Operating at high velocities to achieve increased shear forces also results in erosion of material surface and hence results in increased damage. An optimum shear force and temperature for minimal adhesion is yet to be worked out specifically for heat exchanger operation.

Biofilms have been described as a viscoelastic material with plastic flow properties, based on their response to the modulus of elasticity and yield strength. The viscoelastic property of biofilms makes them mechanically stable and also enables them to resist detachment [30]. The EPS functions as a network of temporary junction points and yield points, which above a certain threshold results in failure of the gel system resulting in a highly viscous fluid. While in most bioreactors there is low velocities (of the order of 0.1 to 50 m h-1) and medium/high substrate concentrations, in real recirculating water systems higher velocities (of the order of 0.1 to 3 m h-1) and low organic substrate concentrations is seen. Higher flow velocities facilitate transport of both planktonic bacteria and the nutrients to the biofilm and promote biofilm formation [7]. Flow velocities of water in pure and cooling water systems govern the development of biofilms.

2.1.3. Nutrient and Oxygen Availability

Recirculating water systems contain oxygen-saturated water due to the aeration provided by continuous circulation of system water and intimate contact of water/air during the cooling process. While oxygen was provided with the aeration, the continuous water flow also provides a continuous supply of nutrients [10]. The major factor controlling biofilm growth is nutrient availability. In industrial and drinking water systems, mass transfer of nutrients to the biofilm will tend to increase with flow velocity. The rough surfaces of biofilms also aid in increased mass transfer of nutrients by as much as threefold compared to a smooth surface. Adsorption of macromolecular substances increases their availability to bacteria. Industrial cooling systems offer a continuous flow of fresh water bringing in nutrients. A 400% increase in biofilm thickness was observed at a given velocity of 1.2 m s –1 for an increase in nutrient level from 4 mg L –1 to 10 mg L –1 (Murthy and Venkatesan, 2008).

2.1.4. Surface Material Type

The type of substratum has a pronounced effect on biofilm accumulation. Smooth surfaces accumulate less biofilm mass than rough surfaces. The mechanism behind this is that individual cells are much smaller than crevices and an irregular rough surface would offer protection for cells from shear effects. However, such surface irregularities have a measurable effect only during the initial stages of biofilm development and biofilms are unavoidable in distribution systems. When biofilm thickness exceeds roughness dimensions, roughness will no longer be of influence for biofilm accumulation; however, it will assist in better anchoring them to surfaces have shown that biofilms of *P. fluorescens* were more pronounced on aluminum plates than on brass and copper [31]. Similarly, more biofilms were

observed on polyethylene pipes than on copper pipes. This is commonly attributed to the toxic effects of copper and brass on microorganisms. However, in industrial situations, heat transfer surfaces of copper, brass and cupronickel alloys have all been shown to accumulate biofilms. Titanium heat exchanger tubes were shown to accumulate more fouling than brass tubes [29].

2.1.5. Suspended Solids

Industrial cooling water drawn from natural sources (seawater or freshwater) contains common particulate material like sand, silt, clay or quartz and to a certain extent metal oxides resulting from the corrosion of equipment upstream. Although in industrial systems the presence of suspended particles is common, studies on their interaction with biofilms are limited. Deposition of these particles onto surfaces from suspension flows is found to occur in consecutive steps. The presence of particles in suspension influences biofilm growth by [31]:

i. Increasing the availability of nutrients to microorganisms, directly influencing their metabolism.
ii. The erosion effects of particles, resulting in removal or suppression of biofilm formation and
iii. The presence of biofilm enhances the capture of particulate matter from flowing systems, increasing accumulation on surfaces.

These mechanisms can be observed and are dependent on the shear force and size of the particles. Particulate material in flowing water influences biofilm thickness and growth. If the particle sizes are large, this results in a sloughing effect on the biofilm whereas smaller particles are known to embed within biofilms.

2.1.6. Microbial Species

Hence bacteria communicate each other with production of diffusible bacterial metabolites, bacteriocins, quorum sensing molecules; they are not randomly distributed in a biofilm but rather settled to best meet the needs of each. Acquirement of new genetic characteristics by intercellular communication and transfer of genetic material within populations of cells and between bacterial populations regulate the diversity and distribution of bacteria in biofilm. Owing to transmissible, genetic elements at accelerated rates in biofilms, bacteria especially pathogens, increase survival capabilities by acquisition of multiple antibiotic resistance, virulence factors and environmental survival capabilities [32, 16, 33].

Examples of industries suffering from the negative effects of biofilms are:

Maritime, dairy, food and beverage, water treatment systems, oil, pulp and paper, cooling towers

2.2. Some Biofilm Forming Organisms Found in Industrial Systems

2.2.1. *Pseudomonas* spp.

Pseudomonads are ubiquitous spoilage organisms. They are found in food processing environments including drains and floors, on fruits, vegetables, meat surfaces and in low acid dairy products. *P. aeruginosa* can be considered a model organism for the study of the development of biofilms and its regulation by quorum sensing. Pseudomonas spp. produce copious amounts of EPS and has been shown to attach and form biofilms on stainless steel surfaces. They coexist within biofilms with Listeria, Salmonella and other pathogens forming multi-species biofilms, more stable and resistant [34].

2.2.2. *Listeria Monocytogenes*

L. monocytogenes is a hardy pathogen with ability to proliferate in cold wet environments that are ideal for biofilm formation. Listeria forms biofilms in pure culture, and can survive and grow in multispecies biofilms [34]. The most prevalent strain of *L. monocytogenes* (strain 1/2c) found in food processing plants has good adhesion ability and requires only a short contact time for attachment. To initiate attachment, *Listeria* utilizes flagella, pili, and membrane proteins [34]. Studies have shown that this pathogen can create a biofilm in slicers and other steel utensils. This fact demonstrates the importance of this biofilm as a factor in cross-contamination. The dairy industry has described the presence of Listeria in milk and dairy products which may be associated with the emergence of clinical outbreaks. It has also been shown that milk protein remains in pipes reduces bacteria, and have a possible inhibitory ability on the biofilm formation in its early stages. However, once established, milk residues in pipes provide a source of nutrients and therefore favor the survival of the biofilm [35].

2.2.3. *Salmonella* spp.

Among the most virulent foodborne pathogens are Salmonella spp. According to the European Food Safety Authority (EFSA), *Salmonella* spp. is the most common cause of foodborne outbreaks in the EU in recent years. Several studies have shown that *Salmonella* can attach and form biofilms on surfaces found in food-processing plants including plastic, cement, and stainless steel [34]. *Salmonella* possess a cell-surface appendage (SEF-17 fimbriae) that facilitates adhesion to inanimate surfaces, and provides cells resistance to mechanical forces. Recent studies on the biofilm formation process have revealed that Salmonella and E. coli, as well as many other species of the Enterobacteriaceae family, produce cellulose as a crucial component of the bacterial extracellular matrix and its formation is essential for

the survival of the bacteria in the environment. Several studies showed significant differences between serovars regarding biofilm formation. The results indicate that the ability to form biofilm is important for the bacteria's persistence in food processing environments. [36]. In 2013 and 2014, an outbreak caused by contaminated pork sausages in Germany affected 145 elderly people [37].

2.2.4. *Escherichia coli*

For the formation of biofilms, E. coli uses flagella, pili, and membrane proteins to initiate attachment. After attaching to the surface it loses its flagella and increases the production of extracellular polymeric substance. Studies have found that some strains of *E. coli* O157: H7 can develop as a result of increased production of exo-polissacharides and curli biofilms. Furthermore, it has been demonstrated that the formation of a biofilm provides greater resistance to E. coli O157: H7 when exposed to solutions of hypochlorite, a frequently used disinfectant in the food industry [38]. In the food industry, this contamination may take place during the pre-harvest period, due to the use of a contaminated water supply when cultivating the vegetables. This contamination may also take place in post-harvest environments, where it may appear after washing and processing the raw material (carcasses, vegetables, etc.), but also due to storage temperatures which allow fast growth of the present bacterial contaminants [39].

2.2.5. *Campylobacter jejuni*

Although *Campylobacter* does not multiply in food, its minimum infective dose is very low, less than in any other pathogen. In addition, experimental research has suggested that *Campylobacter* may have greater potential for dissemination during handling of food for the consumer which increases the risk of cross contamination. One of the mechanisms of survival of *Campylobacter* spp. in the environment is the formation of biofilms. Campylobacter is capable of producing these biofilms in aquatic media or stainless steel and glass surfaces. The microenvironment created within the biofilm prevents inactivation of *C. jejuni* by the presence of oxygen. It has been demonstrated that these bacteria are able to survive within the biofilm for a week at 10°C, with low nutrient levels and under normal atmospheric conditions. The ability of *C. jejuni* to develop biofilms faster under aerobic conditions (20% O_2) than in microaerophilic conditions (5% O_2, 10% CO_2), shows the capacity of this micro-organism to adapt the conditions of the biofilm on their behalf, acting as a reservoir of viable cells. These studies highlight the role of biofilms for the maintenance of *Campylobacter* in environments of processed food, increasing the risk of contamination [40].

2.2.6. *Bacillus* spp.

Bacillus spp. is found throughout dairy processing plants. *Bacillus* survives heat processing and accumulates in pipelines and joints in the processing environment. If hot fluid continuously flows over a surface for over 16 h, Bacillus and other thermoduric bacteria may form a biofilm [34]. A study conducted in a plant producing pasteurized milk in Canada revealed that more than 5.5% of these products contained 10^5 CFU/mL B. cereus and about 4% of these products contained enterotoxins at a level that may result in foodborne illness. The enterotoxin production by B. cereus in this pasteurized milk could occur in only 7–8 days of storage. These higher B. cereus counts were present in products with high butterfat content or in those ones processed with high-temperature, short-time pasteurization treatments [41].

2.2.7. *Legionella* spp

Legionella spp. is an important component in natural and artificial water environments, cooling towers, plumbing systems and evaporators of large air conditioning systems, and remains a health hazard. *Legionella* spp. is known to occur in biofilms in cooling towers, showers, humidifiers [42] and hence knowledge about its response to control measures is important. These Gram-negative aerobic rods have been shown to survive at temperatures of 20–50°C and are inactivated at temperatures above 70°C [43] and in a pH range of 5.5–8.1. The organism is known to occur in stagnant warm water bodies. This aspect is important as power plant exhaust plumes are known sources of *Legionella* deposits. *Legionella* resident within biofilms are a severe problem in cooling tower systems using freshwater.

2.3. Effects of Biofilms in Industrial Systems

Biofilms have adverse effects on all types of instruments, sensors, and equipment used in industrial settings, including power plants, food and beverage production plants, desalination facilities, and paper mills. Biofilm on pipelines, tanks, heat exchangers, RO membranes, and other equipment can cause reduction of heat transfer, increased pressure drop and corrosion of metallic surfaces, and can also be a source for contamination (Figure 2). Biofilms have also shown resistance to treatment, creating an ongoing challenge for industrial engineers and instrument designers. Productivity loss coupled with increased costs of equipment cleaning and replacement has made biofilm prevention a serious problem across industries. Control of fouling in water intakes, piping systems and desalination plants cost over $15 billion per year. In a power plant, biofouling can directly increase the cost of capital equipment, wastewater treatment, and operations.

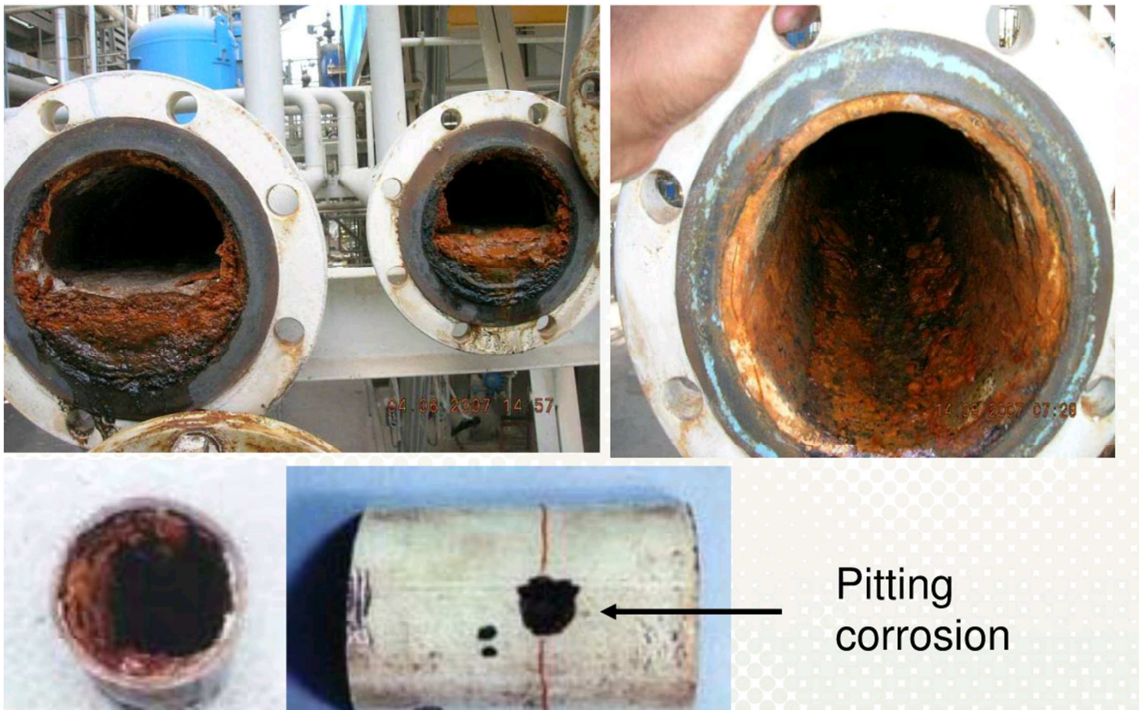

Figure 2. Microbial Influenced Corrosion in an Oil Refinery.

Biofouling of industrial water systems is the phenomenon whereby surfaces in contact with water are colonized by microorganisms, which are ubiquitous in our environment. Biofouling can be described in terms of its effects on processes and products such as material degradation (bio-corossion), product contamination, mechanical blockages, and impedance of heat transfer. Microorganisms distinguish themselves from other industrial water contaminants by their ability to utilize available nutrient sources, reproduce, and generate intra- and extracellular organic and inorganic substances in water [44]. Fouling caused by marine organisms is also an issue of concern for industry and boating. After attaching to a surface, biofouling organisms can form a conditioning layer that provides an active platform for diatoms and algae, which results in increased operational and maintenance costs and the accelerated degradation of abiotic materials. Likewise, membrane fouling hampers pressure-driven membrane processes, such as reverse osmosis, microfiltration, ultrafiltration, and nanofiltration, used for water treatment and desalinization. Membrane biofouling is caused by *Aeromonas, Arthrobacter, Bacillus*, *Corynebacterium*, *Flavobacterium*, and *Pseudomonas* spp. and to a lesser extent by other microorganisms, like, fungi [45].

The formation of biofilms in potable water systems may clog pipes, decreasing velocity and carrying capacity, resulting in increased energy utilization. Biofilm formation in heat exchangers and cooling towers may also reduce heat transfer and efficiency. The ability of bacteria persist in biofilms formed on metal surfaces in a processing facility could cause corrosion of the surface due to acid-production by the bacteria [34].

In the dairy industry and other food industries, ultrafiltration and reverse osmosis systems are used during the fractionation of milk and other liquids as well as during the clarification of fruit juices. These membrane filters have very small pores that are continuously in contact with food; the microbial attachment would block the pores and cause silting of the filter. This causes a reduction in the flow with consequent losses of capacity and product. Effective cleaning procedures are necessary for the prevention of dangerous and costly damage bacterial biofilms can cause. The ability of many bacteria to adhere to surfaces and to form biofilms has major implications in a variety of industries including the food industry, where biofilms create a persistent source of contamination. Therefore, biofilm formation has substantial implications in fields ranging from industrial processes like oil drilling, paper production and food processing. At water and sewage treatment facilities, biofilms (biofouling) are also problematic: they cause metal corrosion, increased risk of contamination of products, decreased quality of water, and reduced efficacy of heat exchange [44, 46, 47].

The major effects of biofilms in industries include;

a. Increased Pressure Drop

b. Reduction of Heat Transfer Processes

c. Product Spoilage

d. Mechanical Blockage

e. Increased Corrosion of Metallic Surfaces

f. Biofouling i.e. contamination of fluids flowing through the tubes and channels.

g. Contamination of Food Products with Pathogenic Organisms in Food Industrial Systems

2.4. Biofilms in the Dairy Industry

Microorganisms forming biofilms in the dairy industry will often cause problems because dairy products are very sensitive towards contamination. Biofilms on surfaces in contact with raw milk stored at low temperature are dominated by psychrotrophic microorganisms typically *Pseudomonas* spp. such as *Pseudomonas fluorescens*, which is one of the most studied biofilm forming organisms. They can grow at low temperature, but higher temperature increases their growth rate and accelerates the formation of biofilms in farm cooling tanks and in silo milk tanks at dairy factories. Heat treatment will normally eliminate these bacteria, but their very heat stable, extracellular lipase and protease enzymes will in active form end up in the final products where they will reduce the shelf life by causing strong off-flavors such as bitterness, rancidity or aged taste. Thermoduric and thermophilic bacteria especially species of *Bacillus, Clostridium, Micrococcus, Streptococcus*, and *Lactobacillus* able to survive heat treatment will often colonize stainless steel surfaces downstream from the pasteurization section and 2 will rapidly grow to large numbers found up to 10^6 bacteria per cm 2 in regenerative sections of pasteurizers after 12 hours of operation. This will cause contamination for example with *Streptococcus thermophilus*, which is reported to reach a contamination level of about 10^7 cells per mL in milk after biofilms have developed for 8 hours in a pasteurizer [48].

In other environments biofilms may take several days or weeks to develop. Endospores produced by genera like *Bacillus* and *Clostridium* are able to survive high heat treatment. These spores can germinate in the manufactured products when conditions become favorable or they can colonize the process equipment and become a significant source of steady contamination. *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. In addition, 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. Several pathogens like *Listeria monocytogenes, Bacillus cereus, Escherichia coli* O157: H7 and species of *Salmonella* and *Staphylococcus* can also produce biofilms or be part of biofilm communities. These organisms are of concern, but also *Enterobacter sakazakii*, which grows well in biofilms, has been highlighted as an important safety risk in the milk powder industry, particularly in relation to production of infant formulas.

2.5. Biofilms in Breweries

Biofilms found in breweries are important reservoirs of spoiling organisms although beer is generally a hostile environment for most microorganisms due to the low pH value and the high content of ethanol and bitter hop components. Bacteria adapted to brewery environments such as species of *Lactobacillus, Pediococcus, Pseudomonas*, and *Bacillus* and yeasts of the genera *Saccharomyces, Candida,* and *Debaryomyces* are found in biofilms in tanks, filters, pasteurizers and fillers where they can cause spoilage of beer for example sour taste and buttery off-flavor. The most common beer spoilage organisms appear to be *Lactobacillus brevis* and *Pediococcus damnosus* found in fermenting wort in the mashing process where they slow down yeast performance [49]. It has been reported that about 25% of the biofilms found in a number of German breweries contained microorganisms able to multiply in beer. This clearly demonstrates that brewery biofilms will influence the quality of the products. On the other hand, pathogenic organisms are seldom found in standard beer products, where they are generally unable to grow [50].

2.6. Biofilms in Heat Exchangers and Cooling Towers

In industrial systems, biofilms frequently grow on cooling water tubes and heat exchanger channels (Figure 3). They cause increased pressure drop and reduced heat transfer efficiency, which ultimately lead to an increase in the costs of production and maintenance, as well as to public health concerns and environmental impacts [6]. In cooling water circuits, the presence of biofilms can restrict flow in pipelines, decrease heat transfer in heat exchangers, increase pressure drop, enhance corrosion and alter surface roughness, which in turn can increase fluid frictional resistance resulting in decreased flow and act as a source of contamination. Two main problems encountered in heat exchanger systems due to fouling by biofilms are reduction in heat transfer (loss of thermal efficiency) and pressure drop across the heat exchangers due to flow reduction by deposits. The restrictions to flow imposed by the presence of biofilm deposits in heat exchanger surfaces increases fluid frictional resistance and, for a given throughput, the velocity will have to increase, which means additional pumping costs. In addition, the presence of biofilms may accelerate corrosion of materials in contact. Other operating costs may accrue

from the presence of biofilm deposits, such as increased maintenance requirement and unplanned shutdowns for cleaning [31].

Circulating cooling systems are key enablers of any industrial process. In such systems, microbial clusters or layers are predominant due to reasons such as the presence of nutrients and water, suitable temperatures, high surface-volume ratio. Biofilm layers are potential niches for pathogenic organisms, especially *Legionella pneumophila* [9] and can lead to problems such as decrease in heat transfer rate and significant energy losses due to increased heat transfer resistance, increase in frictional resistance and blockage in pipes, local corrosion of metallic substrata (microbiologically induced corrosion [MIC]). As a result of adverse effects of biofouling, serious economic losses and hygiene problems including those associated with *Legionellae* may occur. Serious expenses required to eliminate biofouling in systems, increase the capacity of the equipment and premature replacement of corroded equipment before the schedule. Costs for biofouling prevention are estimated to reach billion dollars for individual countries or industries per year [7].

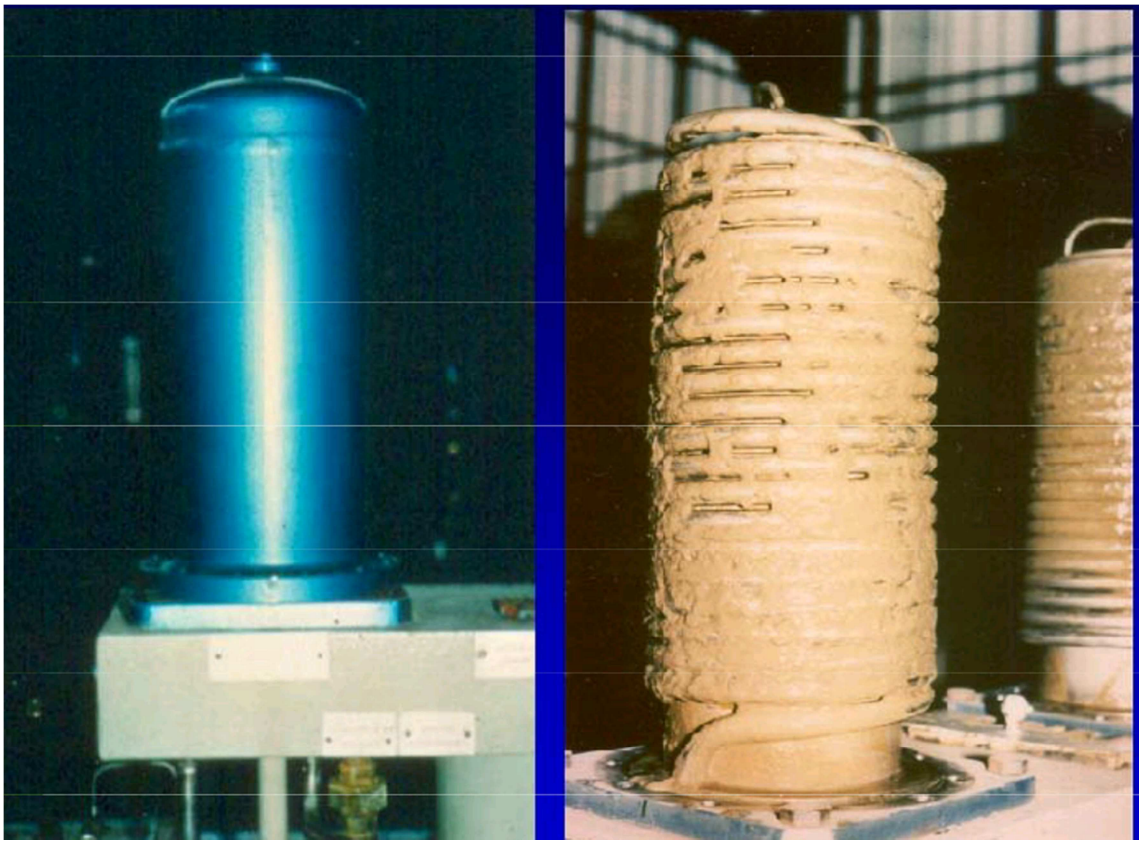

Figure 3. Biocorrosion of a Heat Exchanger.

2.7. Biofilms in Food Industries

The presence of biofilms is common in food industry. Biofilms can exist on all types of surfaces in food plants ranging from plastic, glass, metal, wood, to food products [34]. The attachment of bacteria with subsequent development of biofilms on food industry surfaces has important consequences. The occurrence of such structured microbial communities in food processing plants represents a reservoir of microorganisms and serves as a potential source of contamination of raw materials and processed products as they pass through various stages of food production operations. Moreover, the presence of biofilms may lead to food spoilage, economical losses, and reduced shelf life of products or transmission of pathogens [51-53].

The first report published on food-borne bacterial biofilm described the adhesive properties of *Salmonella sp*., and since then, many bacteria have been identified to form biofilm in the food industry premises, such as *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni*, *Staphylococcus spp*. and *Escherichia coli* O157:H7. *L. monocytogenes* is commonly found in food processing environment, and it has been isolated from both meat and dairy processing plants. This microorganism can adhere rapidly and firmly to inert surfaces and may survive in the sessile form for a long period of time. *Staphylococcus aureus* is one of the most frequent food-borne pathogens in the food industry. Some researchers have observed the ability to

adhere and form biofilm by *Staphylococcus genus*. *E. coli* O157:H7 is known to produce exopolysaccharides (EPS) and to form biofilm on food-contact surfaces and equipment used in beef processing plant. Lactic acid bacteria (LAB) may cause biofilms and in this sense, it represents a concern for the food industry undesirable changes in foods. One example is *Lactobacillus curvatus*, a nonstarter lactic acid bacterium that is capable of forming biofilm and produce an D-(−)-lactic acid, an isomer responsible for the formation of calcium lactate crystals that could lead to sensorial defects in cheeses. Also, in situ biofilm formation by *Bacillus* has been previously reported, e.g., in milk powder and whey processing plants, indicating a risk factor for food-borne diseases [54].

3. Advances in the Control of Industrial Biofilms with Nanotechnology

3.1. Physical and Chemical Control of Industrial Biofilms

In industry, the operations of cleaning and disinfection are essential parts of the production process and the efficiency with which these operations are performed greatly affects the final product quality. Most cleaning regimes include removal of loose soil with cold or warm water followed by the application of chemical agents, rinsing, and disinfection. High temperatures can reduce the need for physical force. Chemical agents, usually surface active agents or alkali compounds, used as detergents, suspend and dissolve contaminant residues by decreasing surface tension, emulsifying fats, and denaturing proteins [55]. These chemical agents are currently used in combination. Many situations require the occasional use of acid cleaners to clean surfaces soiled with precipitated minerals or having high mineral content. Mechanical action (water turbulence and scrubbing) are recognied as being highly effective in eliminating biofilms [34, 55].

An effective cleaning procedure must break up or dissolve the extracellular polymeric matrix associated with the biofilm so that disinfectants can gain access to the viable cells. The cleaning process can remove 90% or more of microorganisms associated with the surface, but cannot be relied upon to kill them. Bacteria can redeposit at other locations and, given time, water and nutrients can form a biofilm. Therefore, disinfection must be implemented [6]. Disinfection is the use of antimicrobials chemicals to destroy microorganisms. This is required, since wet surfaces provide favorable conditions for the growth of microorganisms [55]. The aim of disinfection is to reduce the surface population of viable cells after cleaning and prevent microbial growth on surfaces before restart of production. Disinfectants do not penetrate the biofilm matrix left on a surface after an ineffective cleaning procedure, and thus do not destroy all the living cells in biofilms. Disinfectants are more effective in the absence of organic material (fat, carbohydrates, and protein based materials). Interfering organic substances, pH, temperature, water hardness, chemical inhibitors, concentration and contact time generally control the efficacy of disinfectants.

Although various methods of biofilm control exist, these methods present limitations high cost and often fail to remove biofilms from surfaces, contributing to the dissemination of resistant microorganisms. The control of undesirable biofilms often includes the use of chemical products with antimicrobial properties such as biocides and surfactants. However, these products can be harmful to the environment and consequently they should be used in small quantities as possible [6]. However, new problems are being associated to them. Besides requiring a considerable economic effort and expensive infrastructures [11, 12], the chemical disinfectants are responsible for the production of harmful disinfection by-products (DBP). Chemicals such as free chorine, chloramines and ozone can react with diverse natural water constituents thus forming DBPs, many of which are toxic and/or carcinogenic [12, 11]. For these reasons, and in order to successfully control industrial biofilms, it is imperative the development of new biofilm control strategies.

3.2. Nanotechnology in Industrial Biofilm Control

Advances in the nanotechnology field promoted significant interest in its environmental and biological applications. Nanotechnology can be defined as the engineering and utilization of material, structures, devices and systems at the atomic, molecular and macromolecular scale. Nanomaterials and nanostructures have nanoscale dimensions roughly between 1 and 100 nm and frequently exhibit novel and significantly physical, chemical and biological changed proprieties and functions resulting from their small structures [56]. They are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity [12]. Some of these nanomaterials are engineered to perform specific tasks, being able even to respond to outside signals by changing their structure and properties. For this, they are label as “smart” materials. The field that applies this technology and particles to understand and transform biosystems can be defined as nanobiotechnology [56].

Several engineered and natural nanomaterials have shown

strong antimicrobial properties, including titanium dioxide, silver nanoparticles and chitosan. Chitosan derives from chitin (a natural polysaccharide abundant in arthropod sells) and has been recently engineered into nanoparticles. One of the antimicrobial mechanisms proposed for chitosan involves the interaction of positively charged chitosan molecules with negatively charged cell membranes, leading to an increased of membrane permeability and ultimately cell membrane rupture with consequent linkage of intracellular constituents [12, 57]. Metal nanoparticles such as palladium, platinum, silver and gold nanoparticles are very attractive due to their unique physical and chemical properties. In fact, gold nanoparticles have been widely used to construct biosensors because of their ability to immobilize biomolecules [57]. Nanoparticles present other advantages like high reactivity, unique interactions with biological systems, small size and large surface to volume ratio optimized for mass loading and carrying of antimicrobials [13]. Antimicrobials can be loaded into nanoparticles by physical encapsulation, adsorption or chemical conjugation and this can present several advantages such as significantly improve the activity of the antimicrobial, in contrast to the free product, and release of antimicrobial at a sustained and controlled manner [14, 15].

From several types of nanoparticles for antimicrobial delivery application, polymeric nanoparticles present the advantages of being structurally stable and of having on their surfaces functional groups that can easily be chemically modified with both antimicrobials and targeting ligands [15]. Nanoscience and its application are very recent fields and fundamental properties of nanoparticles are being discovered every day. Further studies and investigation are still needed but the ability of nanoparticles to penetrate the biofilm, enter the cells and affect their biochemical functions makes them potential tools in biofilm control.

The various nanotechnological methods used in industrial biofilm control include;

a. Use of nanoparticles such as titanium dioxide, silver nanoparticles, gold nanopartcles and chitosans
b. Use of nanoparticles with immobilized biocides such as calcium carbonate nanoparticles nanocoated with biocide e.g. BDMDAC
c. Nanofibers and Nanobiocides
d. Nanozymes
e. Nanofiltration
f. Coating Industrial Systems With Nanoparticles e.g. silver nanoparticle

3.3. Nanotechnological Methods in Industrial Biofilm Control

Nanotechnology is the engineering of a functional system that mainly deals with the manipulations on the scale of individual atoms and molecules and with tolerance of less than 100 nm. Recently, nanotechnologies have become a promising tool for biofilm prevention and control. A number of methods have been identified and used to achieve industrial biofilm control and are discussed below.

3.3.1. Antimicrobial and Antibiofilm Mechanisms of Nanoparticles

The mechanisms underlying the antimicrobial effects of NPs are not completely understood and vary from the productions of oxidative and/or free radical formation stressors to DNA damage. Table 1 summarizes published findings on the antibacterial and antibiofilm properties of nanostructured materials, ranging from metals, polymers, and their composites. Mechanisms responsible for the antibacterial activity of NPs might involve particle size, shape, surface charge, or composition, and are believed to involve, cell membrane alterations, loss of respiratory activity, lipid peroxidation, ROS generation, DNA unwinding, nitrosation of protein thiols, or disruptions of metabolic pathways [58]. The table below shows the actions of different nanoparticles against biofilms.

Table 1. Actions of different nanoparticles against biofilms (Adapted from [58].

Material	Nanomaterial Description	Antimicrobial Mechanism of NPs
Inorganic NPs	Silver NPs	Released silver ion interacts with sulfhydryl groups of bacteria and interferes with cell membrane integrity, enzyme activities, respiratory chains, and cell proliferations.
Organic NPs	Surface engineered gold NPs Quaternary ammonium chitosan NPs PEG stabilized lipid NPs Ag-Ti composites Silver or antibiotic conjugated NPs	Highly positive surface charge disrupts the network of EPS. Long cationic polymer chains penetrate the cell membrane and can induce ion exchange to disrupt biofilm.
Metallic/metal-polymer nanocomposites	Silver conjugated silicone NPs Diamond like carbon-metal nanocomposites Silicone containing antibiotic loaded liposome Polymeric silver NPs Silver nanoparticle coated surfaces Polycationic NPs	Highly positive surface charge disrupts the network of EPS Silver ions bound with deoxyribonucleic acid and interfere with electron transport, injuring bacterial enzymes and causing biofilm disruption

3.3.2. Inorganic Nanoparticles

(i) Silver Nanoparticles

Antimicrobial activity of silver, copper and other metal ions is well known and, of all the elements, silver has been described as the one with the highest levels of toxicity for microorganisms and the lowest toxicity for animal cells. In fact, this metal has a broad antimicrobial activity spectrum against both Gram-positive, and Gram-negative bacteria, as well as yeasts. In the case of bacteria it is known to inhibit replication by binding to the microbial DNA and it also switches off important enzymes, leading to microbial death. The nanoscale materials have recently appeared as new antimicrobial agents due to their high surface area to volume ratio and unique chemical and physical properties [59, 60]. Silver nanoparticles (NPs), which are clusters of silver atoms, exhibit very strong bactericidal activity against both Gram-positive and Gram-negative bacteria, including multiresistant strains [59]. There are few studies regarding the mechanism behind the ability of silver NPs or nanocoatings to control yeast biofilm. According to the literature, the silver NPs have affinity for proteinaceous compounds, where they combine with SH groups inducing protein denaturation and corresponding enzyme inactivation [61].

On a model for *Saccharomyces cerevisiae* it was demonstrated that a nanosilver based treatment induces the formation of clusters at the cell wall periphery composed by silver and sulphur, with significant levels of phosphorus, showing a specific reactivity of silver species to phosphorus-containing compounds [62, 63]. It was suggested that the carbonyl groups from amino acid residues of proteins are able to bind to metal and, additionally, Ag+ ions interact with amide groups with a preferential binding to amide carbonyl oxygen [69]. This may cause disarrangement on the secondary structures of proteins, indicating the presence of inactive conformations [63].

Given the increasing microbial resistance and consequent development of resistant strains to traditional antimicrobial agents, silver NPs or nanocoatings constitute nowadays an important antimicrobial agent with numerous applications in industrial biofilm control (Figure 4). Indeed, silver NPs have not been shown to cause bacterial resistance, which is presumably due to the fact that, unlike antibiotics, silver NPs do not exert their antibacterial effects only in a particular site but at several degrees such as bacterial wall, proteosynthesis and DNA [(59, 64, 65]. These nanosilver coatings exert their antimicrobial properties in vivo by slowly releasing Ag+ ions. The considerable surface-to-volume ratio of the NPs enables a constant local supply of Ag^{+} ions at the coating-tissue interface and also allows an improved contact with the microorganisms [66, 60]. As a result, prevention of microorganisms' adhesion and biofilm formation is more prolonged than in other antimicrobial approaches involving silver ions or metallic silver impregnation. Thus, the advantage of impregnation of industrial equipments and machines with silver NPs is that it protects both outer and inner surfaces of devices and there is continuous release of silver ions providing antimicrobial activity.

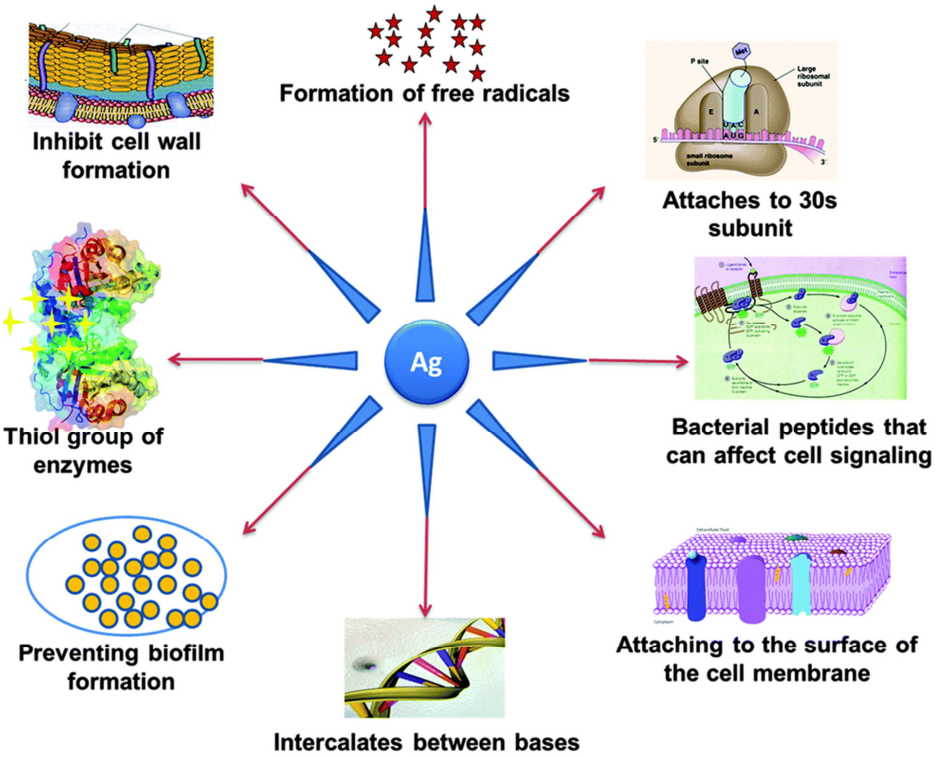

Figure 4. Mechanism of Action of Silver Nanoparticles on Biofilms.

(ii) Metal Oxide Nanoparticles

The antibacterial activities of metal oxide NPs have also been studied; examples include zinc oxide (ZnO), copper oxide (CuO), titanium dioxide (TiO_2), iron oxide (Fe_2O_3), cerium oxide (CeO), magnesium oxide (MgO), and aluminum oxide (Al_2O_3). ZnO NPs have been found to have better antibacterial activities and low toxicities and to be more effective at inhibiting biofilm formation and the growth of *E. faecalis, S. aureus, S. epidermidis, B. subtilis,* and *E. coli* than the NPs of other metal oxides [67, 68].

Nanosized TiO_2 is also considered as nontoxic antibacterial material due to its inert nature as compared with other metal oxides. Usually, it considered a photocatalyst and is used for various environmentally related applications, such as self-cleaning and antifogging effects. Numerous reports have been issued on photocatalytic biofilm inhibition by TiO_2 NPs. The mechanism behind the antimicrobial effect of TiO_2 NPs involves the production of ROS in microbial cells, oxidation of internal enzymes, and lipid peroxidation, which reduces respiratory activity and leads to cell death. It has also been reported that mesoporous TiO_2 NPs facilitate sustained release of attached bioactive materials and thus provide long-term antibiofilm activity [69].

(iii) Gold Nanoparticles

Gold nanoparticles (AuNPs) provide stable, non-toxic and biocompatible alternative, which can be easily synthesized in various morphologies, such as nanospheres, nanorods, nanoshells and nanocrystals. Since AuNPs exhibit biocompatibility, surface plasmon resonance and photothermal effect, they have found wide applications in nanosensors. Although few reports describe the antibacterial activity of AuNPs, Salunke and coworkers reported poor efficacy of chemical and phytogenic AuNPs to inhibit and disrupt *A. baumannii* biofilm. However, these particles are known to carry therapeutic payloads, such as antibiotics, bound to them by covalent bonding, electrostatic adsorption, encapsulation or non-covalent interactions. These moieties are triggered through internal and external stimuli. For example, vancomycin-bound AuNPs showed successful hyperthermic killing of Gram-positive and Gram-negative pathogens including PDR *A. baumannii* via near infra-red irradiation. AuNPs alter membrane potential, decrease intracellular ATP levels, and inhibit activity of ATP synthase and tRNA-binding subunit of ribosome (Cui *et al.,* 2012). Surface modification of AuNPs has also been suggested to control their inhibitory effects.

Gold NPs alone have little or no antibacterial activity. Nevertheless, gold NPs bound to antibiotics, active compounds, or biomolecules show considerable bactericidal and biofilm inhibitory activities against a variety of pathogens, including multidrug resistant strains. Since gold NPs are nontoxic to cells, they have been conjugated with targeting molecules to achieve specific antibiofilm activities. Metal nanoparticles such as palladium, platinum, silver and gold nanoparticles are very attractive due to their unique physical and chemical properties. In fact, gold nanoparticles have been widely used to construct biosensors because of their ability to immobilize biomolecules [57].

(iv) Multi-Metallic Nanoparticles

Use of bi- and tri-metallic nanoparticles is a great approach whereby, instead of a single metal, properties of two or more metals can be exploited. Such nanoparticles exhibit enhanced control efficacy and are required in low concentrations to achieve a similar bactericidal effect as that with mono-metallic ones. Phytogenic silver-gold bimetallic nanoparticles from root extract of *P. zeylanica* showed significant inhibition and disruption of preformed *Acinetobacter* biofilm. In another report, gold-silver core-shell nanoparticles from medicinal plant *Dioscorea bulbifera* inhibited biofilm formation among both Gram-positive and Gram-negative bacteria, including *A. baumannii*. The bactericidal effect from these nanoparticles is due to cell-wall damage causing efflux of cellular materials, which may be attributed to the presence of silver [71]. Once the pores are made in the cell wall, silver and gold interact with cellular components and DNA to cause more destruction to bacteria. Although no report describes the efficacy of tri-metallic nanoparticles in control of *Acinetobacter* biofilm, the study of Mahmoodi and Serpooshan confirmed that chemically prepared tri-metallic SPIONs, consisting of gold and silver shells onto iron core, have profound anti-biofilm potency against *S. aureus* and *S. epidermidis* [72].

3.4. Organic Nanoparticles

Polymeric NPs and polymer based devices are engineered to provide antibacterial properties by releasing antibiotics, antimicrobial agents, or bacteriostatic peptides or by modifying their surfaces with alkyl pyrimidines or quaternary ammonium compounds to cause contact-killing. The polycationic groups responsible for antimicrobial activity cause cell damage perhaps via an ion exchange interaction between bacteria and charged polymer surfaces resulting in the disruption of cellular membranes. The polysaccharides of EPS interact with SO_4 − groups of functionalized polystyrene NPs by hydrophobic complexation, which disrupts bacterial biofilm formation [73].

A nanoporous polymer matrix composed of sodium dodecyl sulfate was found to have significant antibiofilm activity against *E. coli*. Likewise, vitamin E-conjugated cationic

polymers cross linked biodegradable hydrogels exhibit bactericidal and antifungal effects. Levofloxacin (an antibiotic,) conjugated poly (lactic-co-glycolic acid) NPs coated with phosphatidyl choline nanohybrids exhibited enhanced antibiofilm activity against *E. coli.* In addition, physicochemical surface modifications of titanium using polymers, such as polymethacrylic acid, polyurethane acetate, polyethylene oxide, or polyethylene glycol (PEG), prevented protein absorption and inhibited bacterial adherence. Nitric oxide (NO) releasing silica NPs have been utilized for their bactericidal effects on planktonic *P. aeruginosa* cells and used to treat biofilm-related contaminations and reduced bacterial loads of MRSA, *A. baumannii*, and *C. albicans* [58].

3.4.1. Liposomes

Liposomes are self-assembled lipid bilayers containing phospholipids, sterols, glycolipids, membrane proteins and hydrophilic polymers. They resemble biological cell membranes, and can therefore act as effective drug-delivery systems. Antimicrobials can be encapsulated within the lipid bilayer (if hydrophobic), entrapped in the inner core (hydrophilic) or sequestered between the inner and outer bilayer interface (hydrophilic) of the liposome [70]. Liposomal antibiotic delivery studies have been pursued mainly in biofilm-forming *P. aeruginosa*. However, in an interesting study, lipidic nanocapsules loaded with a mixture of carvacol and eugenol (phenols), cinnamaldehyde (aldehyde) and/or beta-caryophyllene (alkene) showed excellent in vitro antibacterial activity against *Acinetobacter baumannii* [74].

3.4.2. Polymeric Nanoparticles

Polymers are multifunctional biomaterials that can be engineered for wide properties suitable for applications in the control of biofilms. While some polymers possess antimicrobial activity due to specific functional groups, such as halogens, guanidine or quaternary nitrogen atom, few of the other polymers can also be loaded with antimicrobial agents. The functional groups on polymers can also be loaded with antimicrobial agents. The functional groups on polymer nanoparticles can be modified or novel synthetic analogs can be designed to increase their specific activity and selectivity. Properties of biocompatible polymers can also be harnessed for in vivo applications. However, very few reports are available on inhibition and disruption of *Acinetobacter* biofilms through polymers. Maleic anhydride-based amphiphillic polymers, containing amide side chains, disrupt surface established *A. baumannii* biofilms. Poly (lactic-co-glycolic acid) polymeric nanoparticles have been used for effective delivery of antibiotics to treat biofilm-forming microorganisms. Nylon-3-polymers and antimicrobial polymeric hydrogels can also be employed for the control of bacterial and fungal biofilms [74].

3.5. Dendrimers

Dendrimers are three-dimensional structures with the ability to encapsulate hydrophilic and hydrophobic entities into the void spaces of their highly branched structures [75]. Synthesized low molecular weight peptide dendrimers showed antimicrobial activity against *E. coli and S. aureus* without additional antibiotics and other studies demonstrated the disruption of *P. aeruginosa* attachment and prevention of its biofilm formation were due to the attachment of fucose-specific lectins (LecB) to fucose-peptide dendrimer ligands [76].

3.6. Removal of Biofilms with Nanozymes

Sessile communities of bacteria encased in extracellular polymeric substances (EPS) are known as biofilms and causes serious problems in various areas, amongst other, the medical industry, industrial water settings, paper industry and food processing industry. Although various methods of biofilm control exist, these methods are not without limitations and often fail to remove biofilms from surfaces. Biofilms often show reduced susceptibility to antimicrobials or chemicals and chemical by-products may be toxic to the environment, whereas mechanical methods may be labor intensive and expensive due to down-time required to clean the system. This has led to a great interest in the enzymatic degradation of biofilms. Enzymes are highly selective and disrupt the structural stability of the biofilm EPS matrix.

Due to the structural role of proteins and polysaccharides in the EPS matrix, a combination of various proteases and polysaccharases may be successful in biofilm removal. The biodegradability and low toxicity of enzymes also make them attractive biofilm control agents. Regardless of all the advantages associated with enzymes, they also suffer from various drawbacks given that they are relatively expensive, show insufficient stability or activity under certain conditions, and cannot be reused. Various approaches are being used to increase the stability of enzymes, including enzyme modification, enzyme immobilization, protein engineering and medium engineering. Although these conventional methods have been used frequently to improve the stability of enzymes, various new techniques, such as self-immobilization of enzymes, the immobilization of enzymes on nano-scale structures and the production of single-enzyme nanoparticles, have been developed. Self-immobilization of enzymes entails the cross-linking of enzyme molecules with each other and yields final preparations consisting of essentially pure proteins and high concentrations of enzyme per unit volume. The activity,

stability and efficiency of immobilized enzymes can be improved by reducing the size of the enzyme-carrier. Nano-scale carrier materials allow for high enzyme loading per unit mass, catalytic recycling and a reduced loss of enzyme activity [78]. Furthermore, enzymes can be stabilized by producing single-enzyme nanoparticles consisting of single-enzyme molecules surrounded by a porous organic-inorganic network of less than a few nanometers thick. All these new technologies of enzyme stabilization make enzymes even more attractive alternatives to other biofilm removal and control agents [79].

4. Conclusion

Biofilms still pose as a critical issue for the industrial community, as most of the traditional control methods are not effective, due to the recalcitrant cells within these communities and the emergence of new highly resistant strains. New nanotechnological strategies are being developed in order to overcome the problems associated with bacterial or/and fungi biofilm formation. At the moment only a few of these methods are normally used in industries. Even so, the nanotechnology approaches seem to be at the moment the most promising field of research to control/eradicate industrial biofilms, most especially for the multiresitant microorganism strains. Despite the advances made in the development of novel antibiofilm agents, devised biofilm control strategies are limited by their high costs and complexities, which means urgent development is required to identify cost-efficient alternatives. Recent developments in nanotechnology-based approaches which have aimed at preventing and controlling bacterial biofilm contaminations are worthy of serious consideration. Different nanoparticle types and composites with demonstrated potential bactericidal and fungicidal properties have been shown to be efficient alternatives to antibiotics in terms of mechanism of action and effects in biofilm disintegration. Nanomaterials are used as constituents of coatings, industrial machines and equipments, and biocide delivery vehicles and research remains active in these areas. Nanomaterial impregnations of antibiofilm devices are believed to provide extended antimicrobial effects and to be minimally toxic as compared with small molecule antimicrobials, which exhibit short term activities and are environmentally toxic. However, key issues like NP resistance and surface interactions between NPs, biofilms, and hosts need to be resolved to ensure successful clinical applications. By engineering structures on the nano or micro scale, we can more intimately control the interaction between biofilms and the surfaces they inhabit, thereby reducing the maintenance cost of industrial systems.

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