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Biofilms: Formation, Properties, Impact on Industries, Strategies for Control

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Abstract

Microbes being one of the oldest inhabitants on the earth, have successfully survived the harsh environments by a number of survival strategies. One such strategy is the formation of a multicellular structure formed by the adherence of the microorganisms to a surface, known as biofilm. This structure is formed by first reversible and then irreversible linkages with the solid support. These are the porous structures with micro colonies, enclosed by exopolysaccharides and are connected by interstitial voids called water channels to promote the influx of nutrients, oxygen and other vital molecules and the efflux of metabolic wastes. Biofouling – formation of biofilms in an industrial setting has been a major problem in the wide range of industries with deleterious effects. The challenges brought about by biofilms in various sectors of the food industry, paper manufacturing and marine industries as well as other technological problems are discussed. Over the years several strategies are used targeting various stages and site of biofilm formation have been formulated to rid industries of biofilms once and for all. Though there exists no 'perfect solution' for complete biofilm eradication to date, some of the more recent and potential strategies will be discussed

Keywords: Biofilms, Biofouling, Micro colonies, Extracellular polysaccharide substance (EPS), Biocorrosion.

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1. Introduction

Microbes are fascinating organisms that were the first living organisms to inhabit the earth and till today continue to dominate in their presence, being found in almost any environment. One of the reasons for their success in surviving even the harshest of environments is the number of survival strategies that they have evolved to adopt one of which includes the development biofilms. Biofilms as defined by Reynolds and Fink [1] are '*Multicellular structures formed by aggregation and adherence of microorganisms to a surface*'. Microbes adhere and colonize on any surface that is conditioned with nutrients, ions or other organic molecules that aid in their viability and growth, forming a biofilm. To protect themselves from hostile situations like starvation, desiccation and the effect of antimicrobial, toxins or biocides, they secrete and hide under a polysaccharide layer called the Extracellular polysaccharide substance (EPS).

For decades man has shared a love-hate relationship with biofilms. These complex microbial structures are ubiquitous in nature and are studied in various disciplines of science including water engineering, biomedicine and evolutionary biology[2]. Biofilms are a representation of a population of cells that are functionally interdependent on each other to collectively bring about microbial activities that neither of them can achieve individually [3] . Biofilms associated with the human body manifest themselves on the skin and mucosal surfaces protecting the host from pathogenic attacks. In the gut, these sessile congregations ensure the well being of the host by overseeing metabolic activities and preventing infections from pathogenic strains. In nature biofilms participate in terrestrial and benthic nutrient cycling while also keeping in check the level of xenobiotics and other environmental pollutants through biodegradation and bioremediation [3]. Modern science has also harnessed these microbial structures in various fermentation processes and in waste water treatment plants. On the downside however, biofilm formation in industries can clog pipes, corrode equipment and reduce heat transfer causing large economical and energy losses. Biofilm formation of pathogenic, spoilage and corrosive bacteria contaminate food products and increase health risks and equipment spoilage due to corrosion. In the body,

pathogenic strains can form recalcitrant and highly resistant biofilms leading to very strong infections not easily be treated. Fungi from the genus *Candida,* particularly *Candida albicans* can establish both superficial and systemic infections called *Candidiasis. Candida albicans* can form biofilms on many indwelling medical devices like pacemakers, artificial joints, vascular bypass grafts, catheters and dental implants. Despite the presence of antifungal treatments, invasive Candidiasis has still resulted in a 40% mortality rate among patients thus posing a devastating problem in the medical world [2]. To compound the problem, eradication of biofilms is not completely possible due to the increased resistance and uniqueness of the microbes in a biofilm.

The story of biofilms and its various aspects is extensive and impossible to be portrayed completely in this review. Hence, though the beneficial aspects of biofilms are many, this review will focus on some aspects of the formation, characteristics and structure of biofilms and zoom in on their adverse impact on industries. Novel and potential strategies to control and prevent biofilm formation will also be discussed in brief detail.

2. Formation of a Biofilm

Over the years various theories have been proposed to explain biofilm formation. However, the principle remains the same and can be explained most simply by the 2 step model. This model summarizes biofilm formation with 2 major steps, reversible and irreversible adhesion. A summary of biofilm formation with the various terms used to address the different stages are depicted in figure 1.

Biofilm formation is initiated when bacterial cells are transported to a surface via diffusion, turbulence or convective currents. They bind weakly or 'reversibly' to the surface with weak forces like van der Waals forces, electrostatic forces and hydrophobic interactions which can easily be disrupted through shear forces like rinsing and turbulence [4, 5]. To overcome electrostatic repulsion, cells then start to bind 'irreversibly' through stronger more specific interactions like covalent and hydrogen bonds and hydrophobic interactions.

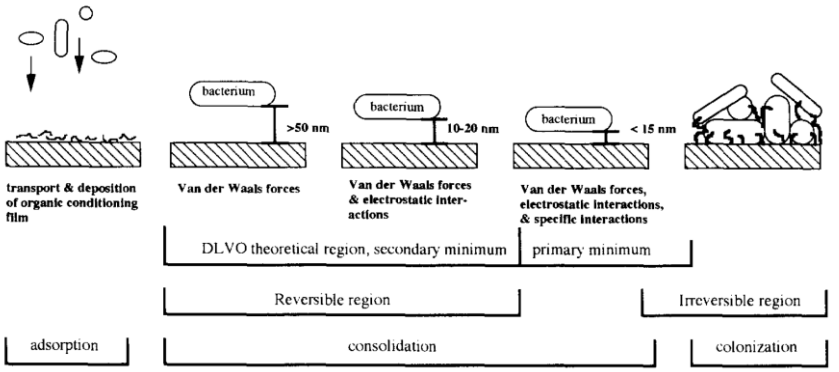

Fig 1: A diagrammatic representation of the various stages of biofilm formation and the various names that were conferred to identify these stages over the years [6]

Polymeric adhesins and other cell surface features like flagella and fimbriae stabilize the cells on the surface. They also start to secrete exopolysaccharide substances which embed the cells and aid in attachment. The adhered cells then divide producing sister cells that are bound within the exopolysaccharide matrix, initiating the formation of adherent micro colonies. Further growth of the biofilm is brought about by cell division and recruitment of planktonic bacteria from the surrounding bulk fluid. Finally a mature biofilm forms, consisting of single cells, micro colonies of sister cells and entrapped macromolecules from the surrounding all embedded within a primarily anionic exopolysaccharide matrix [7]. As cell density and size of the biofilm increases, dispersal of parts of the biofilm closer to the solid-liquid interface occurs through quorum sensing or due to lack of nutrients and shearing due to flow dynamics or shedding of daughter cells from metabolically active cells [8].

3. Characteristics of a biofilm

3.1 Structure

The biofilm matrix comprises largely of the EPS, which accounts for 50 to 90% of the total organic carbon content. This polysaccharide matrix is also highly hydrated comprising of 80-95% of water. Inorganic particles like mineral crystals, corrosion particles, clay and slit particles or blood components can be found in the matrix depending on the environment in which the biofilm is developed. The population of microbes embedded in the matrix can be either viable or non-viable and single or multi-specied. While many pathogenic bacteria and fungi can form both pure and polymicrobial cultures in the human body, biofilms formed in nature are primarily polymicrobial, harboring bacteria that fill distinct niches, share genetic material at a high rate and play a role in the survival of the biofilm [9, 10]. Donlan [8] demonstrated this point by comparing the biofilm from an industrial water system and on a medical device. Structurally, biofilms are porous structures with micro colonies, covered with copious amounts of exopolysaccharides. Between and under these micro colonies are interstitial voids called water channels that promote the influx of nutrients, oxygen and other vital molecules and the efflux of metabolic wastes [11]. A schematic representation of the basic structure of a biofilm is as in figure 2.

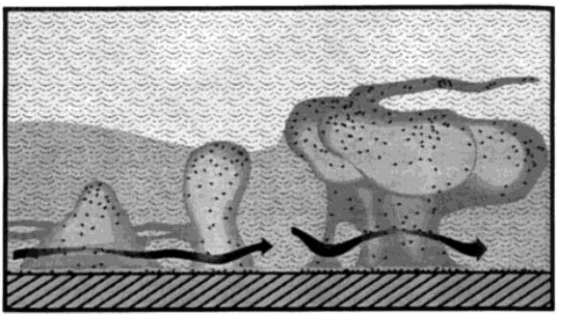

Fig 2: A pictorial depiction of bacterial biofilm formation, representing its complex 3-dimensional structure. The microcolonies are surrounded with large amounts of exopolysaccharide and have water capillaries between and under them [12]

Microbes in a polymicrobial biofilm tend to occupy distinct niches within the biofilm based on their oxygen requirements and

metabolic activity. Generally, metabolically active cells are found closer to the solid liquid interface where oxygen is more readily available. Areas deeper within the biofilm normally are oxygen deprived and are therefore normally colonized by anaerobic bacteria[12]. The overall structure of biofilms is unique to every environment and is heterogeneous both spatially and temporally, changing constantly due to its internal and external environment. The microbes of the biofilm, both pure and polymicrobial also influence the structure of the biofilm. Biofilm thickness is sometimes influenced by the type and number of component organisms.

Dual species biofilms of *P. aeruginosa* and *K. pneumoniae* are thicker than their respective pure culture biofilms owing to the mutual stability they provide each other[13]. Bacterial motility showed altered biofilm structures in *P. aeruginosa* and *P. putida* [14].

3.2 Extracellular polysaccharide substance

In addition to microbial species, the EPS also partly determines the structure and characteristics of the biofilm. The EPS is primarily made up of polysaccharides, but its physical and chemical structures vary from species to species. Gram negative bacteria generally produce neutral or polyanionic EPS layers, owing to the presence of uronic acids or ketal-linked pyruvates [15]. However the EPS of gram positive bacteria like coagulase negative *Staphylococci* is a mixture of 80% of teichoic acid and 20% proteins which is primarily cationic[16]. The charge of the EPS also influences the ability of the cells to adhere to a surface[17]. Other than contributing structurally, the EPS also serves as one of the many factors that bring about increased resistance in biofilms.

3.3 Resistance in biofilms

Understanding the contributing factors to increased resistance in biofilms is crucial for the development of more effective eradicatory and preventive strategies. Increased resistance in biofilms is attributed to numerous contributing factors like the presence of efflux pumps, the EPS, modifying enzymes, target mutations, communication through cell signaling and so on. Mah

and O'Toole [18], proposed three hypotheses to explain resistance in biofilms, as seen in figure 3.

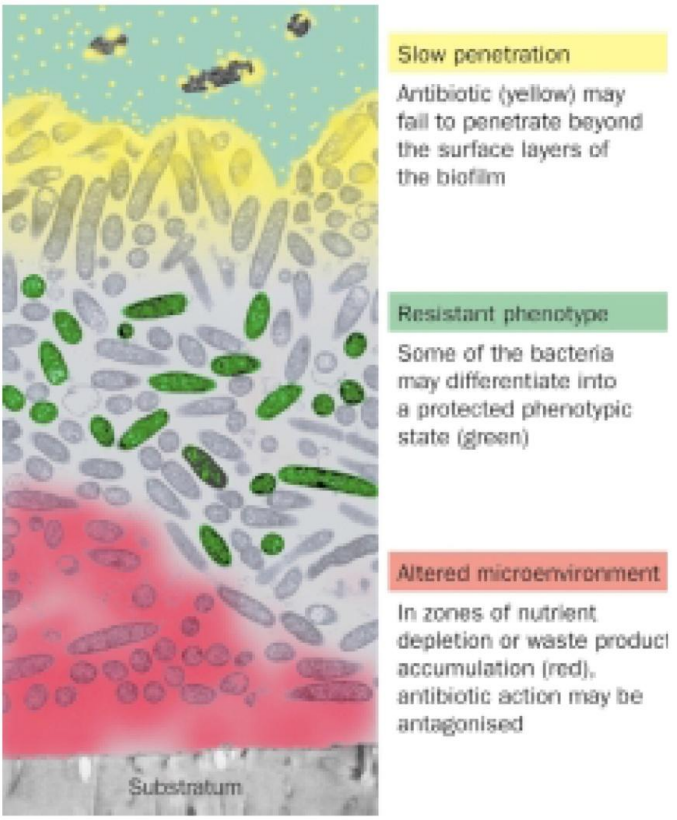

Fig 3 : The three main hypotheses of resistance in biofilm as proposed by Mah and O'Toole [19].

The first hypothesis suggests the slow or incomplete penetration of antimicrobials through the biofilm matrix. EPS serves as an initial protective barrier slowing down the penetration and contacting of various antimicrobial with the resident bacterial cells. *P. aeruginosa* biofilms developed on one side of a dialysis membrane reduced the diffusion of piperacillin across the membrane suggesting that the biofilm was responsible for preventing the diffusion of the antibiotic. However, *Staphylococcus epidermidis* showed resistance to Rifampicin and Vancomycin which can penetrate the biofilm

matrix [18]. Kumon *et.al.* [20] using *Klebsiella pneumonia* demonstrated that slow penetration of the antimicrobials was due to inactivating enzymes like β-lactamases. However, contradictory results reported β-lactamase negative strains of *Klebsiella pneumonia* could withstand for more than 4 hours treatment with 5000 µg/ml of ampicillin [21]. Binding of positively charged agents like aminoglycosides to the negatively charged anions of the matrix suggests that the charge of the EPS also contributes to hindering penetration [22].

The second proposed hypothesis considers the chemically altered microenvironment found within the biofilm. As a biofilm grows, physiological gradients develop in the metabolic activity and oxygen availability. Generally, cells in the less metabolically active, stationary phase have lower exposure to oxygen and are found deeper within the biofilm. Imipenem and ciprofloxacin, which are antibiotics effective against non-growing cells, had greater activity against *E. coli* biofilms compared to β-lactam antibiotics that are effective against actively growing bacteria. Accumulation of waste products within the matrix, bring about a difference in pH between the bulk fluid and biofilm interior which could also antagonize the antimicrobial agent [23].Furthermore, exposure to osmotic stress could initiate a stress response, altering the relative proportions of porins and reducing cell wall permeability to antimicrobials [24].

The third hypothesis proposes the existence of a persistant phenotype within a biofilm that confers its increased resistance. According to Lewis, biofilms that are too thin to prevent penetration of the antimicrobials through their matrix had persistant phenotypes which formed atleast 1% of the total biofilm, leading to an increase in biofilm resistance. He also proposed that antimicrobials kill the cells indirectly by causing cell damage which leads to the cell death. With regards to this statement, he suggested that one possible reason for resistance could be that these persistor variants could have a defective programmed cell death program thus making antimicrobial ineffective against them [25, 26].

In addition to the above mechanisms, other factors like the activation of a general stress response increase resistance to dessication, oxidation and DNA damage and up-regulation of the production of proteins invovled in metabolism, exopolyasccharide

production, oxidative damage, phospolipid biosynthesis, membrane transport and secretion, amino acid metabolism, anaerobic processes and tricarobxylic acid cycle [27]. The key to increased resistance however lies in the multicellular nature of biofilms and the interdependancy of these cells on each other. The dormant persistant variant depends on their metabolically active neighbours for genome propagation, while the metabolically active cells depend on their dormant neighbours to reseed the community incase of a catastrophic killing. The development of resistance due to the multicelluar nature also explains the rapid revertion of cells dispersed from a biofilm to their susceptible planktonic phenotype [19].

4. Factors affecting Biofilm formation

The establishment of a bacterial biofilm is triggered by the initial adhesion of the bacterial cell to a surface. The nature and strength of this initial adhesion is however influenced by a number of contributing factors some which include substrate properties, presence of a conditioning film and properties of the cell surface [28].

4.1 Substrate properties

Microbial adhesion to a surface is one of the most crucial steps of biofilm formation. Bacterial attachment depends on the physiochemical interactions occurring between the bacterial membrane, substrate surface and polymeric substance [28]. Understanding the relationship between substrate properties and bacterial adhesion can aid in preventing biofilm formation through substrate modification. In most cases, microbial population occurs on rougher surfaces, due to the higher surface areas and diminished effect of shear forces exhibited on these surfaces [8]. Ong *et. al* and Fletcher and Loeb reported that surface properties like hydrophobicity, surface charge influenced bacterial adhesion [29]. Hydrophobic surfaces like rubber and plastics showed greater attachment when compared to hydrophilic substrates like stainless steel [30]. Gottenbos *et.al* established the effect of surface charge on initial adhesion in their study, reporting that positively charged surface allowed rapid adhesion of cells but inhibited further

bacterial growth on the surface while negatively charged surfaces delayed initial adhesion slightly but subsequently allowed growth of bacterial cells on its surface [31].

4.2 Formation of conditioning films

Surface properties of the substrate like their hydrophobicity, surface free energy and electrostatic charges are further modified by the formation of a conditioning film, which in result affect microbial attachment [32]. Loeb and Neihof were the first to document the formation of conditioning films on solids exposed to sea water. This organic film formed almost immediately on a variety of surfaces, lowering the surface energy and imparting a moderately negative charge to the surface, making it conducive for biofilm formation [33]. In the food industry, conditioning films are formed on surfaces that contact the food product and consist of organic molecules like proteins from dairy and meat products[32]. However microbial attachment does not occur on all conditioned surfaces and the type of proteins that constitute the conditioning film play and influential role on microbial attachment [28, 34-36].

4.3 Cell surface properties

Factors on the cell surface like hydrophobicity, surface energy, surface charge and outer membrane proteins interact with the substrate to determine adhesion. Hydrophobic interactions increase with the non polar nature of the surfaces, playing a role in cell adhesion to the substrate surface. While most bacteria are negatively charged, they possess structures like flagella, fimbriae and other residues which alter its hydrophobicity [9]. Stenstrom measured the hydrophobicity of the cell surface of numerous strains including *E. coli*. He concluded that high hydrophobicity coincided with enhanced adhesion and negative charge played no role in adhesion while positive cell surface charges enhanced adhesion [37]. Rivas *et.al* however showed that presence of bacterial hydrophobicity, surface charge and cell surface structures did not influence the attachment in various strains of shigatoxigenic *E. coli* on stainless steel. [38]

Cell surface structures like flagella and fimbriae contribute to cell hydrophobicity. High levels of amino acid residues on fimbriae

enhance the hydrophobicity of the cell surface. Fimbriae contribute to cell adhesion by overcoming the electrostatic repulsion barrier between the cell and substrate[8]. Expression of a wiry, thin fiber called curli fimbriae have been observed under certain growth conditions in *E. coli*. Expression of curli fimbriae enhanced ability of environmental non pathogenic *E. coli* to attach to abiotic surfaces [39]. However, this idea was opposed by Ryu *et.al* and they showed no difference in adhesion to stainless steel between curli and non curli producing *E. coli* O157:H7 [40].

Flagella provide motility to cells allowing them to move towards a surface. Upon initial attachment to a surface however, flagellin synthesis is down regulated indicating that it is not required for further biofilm formation [11]. The controversy on the role of flagella on biofilm formation however, is still alive with some authors like De weger *et.al* [41] who support its role biofilm formation while others like Rivas *et.al* who were not in favor of this idea [38].

It is apparent that there is still a lot of controversy on the various factors and their influence on biofilm characteristics and formation. This could be due to the differences in growth media used, various methods used to measure factors like hydrophobicity and surface charge and the differences in the characteristics of the various bacterial strains and species employed in these studies [38]. With further studies and more specialized techniques, it will hopefully be possible to conclude with evidence the actual role of these factors on the overall establishment of biofilms.

5. Adverse effects of biofilms in Industries

Biofilms have for decades posed a challenge in a wide range of industries. In an industrial setting, the word 'biofouling' is used to refer to any undesirable formation of biofilm or products of its decomposition, which have deleterious effects on industries[6]. Common sites of biofilm formation in industries include the floors, drains, waste water pipes and pipe bends, rubber gaskets, conveyor belts, Teflon and buna-N- rubber seals and stainless steel surfaces [32]. The challenges brought about by biofilms in various

sectors of the food industry, paper manufacturing and marine industries as well as other technological problems are discussed.

Microbial contamination in the food industry has led to the loss of millions of dollars annually. In 2000, the potential cost incurred due to food-borne infections in New Zealand was $88.8 million [42]. In 2006 and 2007, cases of *Escherichia coli* O157:H7 were associated with fresh produce like spinach and packed salad, and also ground meat. One such case was the recalling of 21.7 million pounds of ground meat patties in New Jersey. Until recently, biofilms were not the focus of microbial disinfection in the food industry. However, it has now been established that these microbial communities are one of the leading causes of contamination and lowered shelf-life of food products.

Over the past decade, food production has become a complicated process involving automated systems to produce large volumes of food with higher shelf lives. The rise in health awareness has increased pressure to produce products with lesser usage of chemical preservatives and preservation mechanisms. This has led to the need for more stringent hygienic condition and sanitary practices and therefore, the use of more chemical detergents in industries. While commonly used sanitizer like chlorine and iodophor are effective against planktonic cells, biofilms are more resistant to these agents [43]. The frequency of emerging strains with a low-level resistance to Quaternary ammonium compounds (QACs) is considerably high for *Listeria monocytogenes, Staphylococcus aureus, Pseudomonas* spp. and lower in lactic acid bacteria and coliforms obtained from food and food industries. Sharma and Anand [44] demonstrated the prevalence of biofilms of pathogenic and spoilage microflora in the pre- and post-pasteurization line of a dairy plant even after cleaning-in-place and sanitizing procedures were completed. They concluded that it is vital to have biofilms evaluations as part of the HACCP and ISO: 9000 specifications.

Many pathogenic and spoilage organisms can adhere strongly to materials commonly used in the food industry like stainless steel, glass and HDPE. Coupled with their increased resistance to disinfectants, eradication of these microbial communities has lead to huge economical implications. Biofilms of chlorine and iodophor

resistant *Salmonella* can form on High density polyethylene (HDPE), stainless steel and cement making conventional cleaning procedures ineffective over them [43]. A large number of product recalls in the US and EU are due to the presence of *L. monocytogenes*. The tolerance level for *L. monocytogenes* in many countries is zero thus making contamination of products by this organism a huge economic concern [45]. While pasteurization and cooking in the meat industry generally prevent the occurrence of *Listeria* in the finished product, this organism still prevails in the processing environment within biofilms for years, posing the risk of contamination during slicing, packaging etc [46]. Strongly adherent strains of *Listeria monocytogenes* isolated from a meat processing plant were capable of adhering equally well to glass, stainless steel, plastic and rubber and at temperatures ranging from 10 to 40°C and were detected more in ready to eat meats more frequently than their weakly adherent counterparts [47].

In the dairy industry pathogenic bacteria vegetative microbes are usually killed during pasteurization. However, some heat resistant vegetative cells and spores can survive this temperature and prevail in the milk. Milk proteins start to denature and aggregate as soon as milk is heated and these aggregated proteins deposit on surfaces downstream the heat exchanger providing a conducive environment for the attachment and proliferation of the surviving spores and cells. Studies have shown the ability of pathogenic strains like *Klebsiellae, Mycobacteria, Bacillus, Legionellae, E. coli, Campylobacter* and coliforms to adhere onto and colonize on established biofilms of autochthonous species [48, 49]. Hydrodynamic effects of the fluid disperse the pathogenic microbes from these autochthonous biofilm causing downstream contamination of the products. Non starter lactic acid bacteria (NSLAB), like *Lactobacillus* spp., have become growing concern in dairy industries. NSLAB biofilm in cheese manufacturing industries can survive routine cleaning, posing a threat to the quality and consistency and flavor of the cheese. Surveys detected a number of NSLABs on the floors, drains, cleaning vats, hoops and vacuum packaging machines in the dairy environment [50].

The popularity of ready to drink beverages, bottled water and functional and fortified drinks are on a constant rise. An increase in

global bottled water consumption by 34% between 2000 and 2005 and total sales of $40 billion in 2005 for functional drinks was reported. While factors like low pH, low temperature and low water activity can largely suppress bacterial colonization, yeasts can tolerate these conditions and are associated with spoilage of cola type beverages, soft drinks, ciders, wines and fruit juices. Spoilage yeasts *Zygosaccharomyces rouxii* and *Zygosaccharomyces bailii* and Lactic acid bacteria are highly problematic in fruit juices, alcoholic beverages and carbonated soft drink industries [51]. The adherence of yeasts like *S.cerevisiae* to bacteria like *Lactobacillus plantarum, Escherichia, Klebsiella, Salmonella and Enterobacter* in a mannose sensitive manner has been reported in the past [52, 53]. Though it has not been reported till date, adherence of spoilage yeasts like *Zygosaccharomyces* to indigenous but harmless biofilms in the beverage industry could be a possible cause in cases of persistent yeast contaminations in the products. The environment in the brewing industry also makes it favorable to biofilm formation, particularly on the equipment used in the filling process. Biofilm formation by acetic acid bacteria and enterobacteria are not necessarily harmful to the final product, they do however secrete a slime that allows the survival of other contaminating microbes in the surrounding areas. Undisturbed product residues can allow the growth of wild yeasts which produce metabolites enhancing the growth of LABs which in turn allow the growth of *Pectinatus* spp., leading to higher risks of contamination. The impact of biofilms in brewing industries is such that it costs the German Brewing Industry an estimated €250 million annually [54].

Biofilms in the paper industry normally manifest themselves as thick slimy layers, forming on the interfaces of the machine and liquid. Microbial growth is highly favorable in a paper manufacturing industry due to the high nutrient level and warm temperature. Various types of microbes gain entry into the processing line due to the large number of raw materials used. The microbial population may also differ based on the different dyes, starches, pigments and coatings used in manufacturing various paper types. Examination of the slime found in the paper industry showed a large range of aerobic and anaerobic bacteria with *Bacillus* spp. and *Sphaerotilus natans* predominating [55]. Biofilms in

the paper industry cause wet end breaks that disrupt the runnability of the processing lines, produce foul odors in the mill and on finished paper products and cause sheet defects like blotches, holes and spots due to the sloughing off part of the biofilm in the processing line [56]. An estimated 10 to 20% of downtime is also contributed to treating slime formed on machines, thus causing a great and adverse economical impact on this industry [55].

Even the marine transportation system face challenges from biofouling. Shipping hulls form a favorable environment for biofilm formation being colonized mostly by algae, diatoms and bacteria [32]. Biofilm formation of a 100μm thickness on shipping hulls could increase frictional resistance from 5 to15%. According to D. C. White, the US Navy spends more than 500 million US dollars for additional fuel due to frictional resistance from biofilm formation [57]. The economical impact of biofilms on the marine and naval transport, indirectly affect other industrial sectors which depend on marine ways for import and export of goods [32].

Other than affecting product safety and quality, biofilms also impose large technological problems on industries. Cooling towers are an essential part of many industries like petrochemical, oil, wastewater treatment, beverage industries and power plants. They are also prime sites for biofilm formation due to the availability of nutrients, optimum temperatures, high residence time etc. Biofouling in cooling towers have major adverse economical impacts on the industry. High microbial density leads to build up of odors and slime along the circulating line. Depositions of the biofilms and inorganic molecules result in increased fluid frictional resistance and low thermal conductivity therefore resulting in loss of energy and heat transfer. Microbiologically induced corrosion (MIC), in the oil and gas industry resulted in estimated losses of $100 million in USA alone, excluding costs for remediation treatments. Sulphate reducing bacteria (SRB) occupy anaerobic niches in a biofilm and can corrode cast iron, carbon steel, stainless steel and other alloys used in industries resulting in a decreased production due to downtime required to clean, treat and replace fouled equipment[58]. Furthermore, cooling towers form conducive environment for the proliferation of *Legionella pneumophila* and

have in the past been connected to outbreaks of Legionnaires disease in nearby areas [55, 59]. Biofilm formation also has detrimental effects on the efficiency of heat exchangers. Slime formation on these surfaces act as an insulator allowing heat transfer only through diffusion rather than convective currents resulting in high frictional resistance, increase in energy consumption, MIC and finally spoilage of the equipment, causing large economical setbacks [57].

Membranes used for the desalination of water and in the ultra filtration of milk products are subject to high amounts of fouling by mineral salts and inorganic molecules and proteins and other organic molecules respectively. Fouling of these membranes result in a decrease in flux and thus decrease in efficiency and increase in energy consumption of the membrane filtration process. Fouling of membranes can serve as highly nutritious substrates for bacterial attachment and biofilm formation. Biofouling of membrane pose a recalcitrant problem resulting in clogging of the membrane pores and contamination. Unlike other fouling, biofouling is difficult to eradicate and involves high expenditure for its removal [57].

From the above cases it can be seen that biofilms pose a serious problem either directly or indirectly on industries as they are capable of forming in almost any environment. Contamination can sometimes be unforeseen due to the ability of pathogenic and contaminating strains to hide within autochthonous biofilms. Hence industries must constantly stay alert spending large amounts on preventive and eradicatory measures. In the cases where biofilms have established, large losses are incurred due to higher energy consumption, spoilage and corrosion of equipment, downtime to treat and remove biofilms and product call-backs due to contamination by pathogenic or spoilage organisms.

6. Potential strategies for Biofilm control and Prevention

Due to their recalcitrance and high resistance, biofilm eradication and prevention has become an issue of extreme importance. There are currently three main approaches to overcome biofouling: mechanical detachment of the biofouling organism or adsorbed biomolecules, use of biocides, antibiotics and cleaning chemicals to

inactivate or kill biofilms, and the modification of substrate surface into one with non-adhesive properties. Over the years several strategies are used targeting various stages and site of biofilm formation have been formulated to rid industries of biofilms once and for all. Though there exists no 'perfect solution' for complete biofilm eradication to date, some of the more recent and potential strategies will be discussed.

6.1 Modification of surface and substrate properties

Biofilm formation on a surface is triggered by the adsorption of macromolecules like protein onto substrate surfaces. Theoretically biofilm formation can be controlled by preventing the adhesion of proteins on the surface. The surface energy and wettability of a solid surface play a major role in determining bacterial and protein attachment and bacterial detachment. Bacterial detachment can be induced by rapid and dramatic changes in the hydrophobicity of a substrate surface [60]. Modification of surfaces properties to prevent adhesion of proteins and bacteria by altering various properties like surface energy, surface charge, topography and hydrophobicity is currently under research.

The properties of Polyethylene glycol (PEG), like their highly flexible chains and ability to cause entropic repulsion of protein molecules, have made it potential antifouling agent. PEG is highly water soluble and is capable of creating a water-molecule cluster shield around the PEG macromolecular chains resulting in a resistance to bacterial adhesion. PEG adsorbed onto stainless steel was successful in reducing the adherence of *Pseudomonas* spp. by between 2 and 4 orders of magnitude [61]. Plasma-enhanced coating has shown great potential in reducing protein adhesion to surfaces. Stainless steel samples coated with 1,4,7,10-tetraoxacyclodecane (12-crown-4) -ether and tri (ethylene glycol) dimethyl ether (triglyme) – when studied under radio frequency for plasma enhanced coating, showed the deposition of PEG-like (-CH_2-CH_2-O-) structures on the surface. Plasma coating conferred a lower rough surface value and higher hydrophilic nature to the stainless steel resulting in a significant reduction in bacterial attachment and biofilm formation in mixed cultures of *Salmonella typhimurium, Staphylococcus epidermidis* and *Pseudomonas flourescens*.

It also altered the chemical characteristics of the biofilm thus showing a promising alternative for biofilm prevention in food-processing and medical industries [62]. Other studies on influence of surface modification on bacterial and protein adhesion to surfaces are reported as well [23, 63, 64]. While some of these multistep modificatory processes require the use of chemical reagents harmful to the environment, processes like Cold-plasma technology are more efficient and serve as a more promising alternative for surface modifications [62]. Further studies and optimization of this technique must also be conducted to achieve stable layers of the polymer sufficient graft density and surface uniformity [61].

6.1. Neutral Electrolyzed Water (NEW)

Biofilms, particularly in the food industry are controlled through constant cleaning and disinfection. The spectrum of disinfectants used in the food industry includes quaternary ammonium compounds, amphoteric products, biguanides, iodophores, peroxo acids and chlorine- containing compounds. More often compounds with chlorine like NaClO are used due to its shorter contact time and higher effectiveness against bacteria. However, besides the developing resistance of a number of strains to NaClO, they are also potentially hazardous to workers and highly unstable due to the decrease in active chlorine with storage [65]. More recently, the development of neutral electrolyzed water (NEW) has led to the possible substitution of NaClO with these solutions as sanitizers. NEW is a neutral solution more stable solution, which at a concentration of 63 mg^{-1} active chlorine reduced *E.coli, P. aeruginosa, S. aureus* and *L. monocytogenes* at an initial population of about 8 log CFU 50 cm^{-2} on both glass and stainless steel surfaces to 1 log CFU 50 cm^{-2} after treatment for 1 minute. These results showed the efficacy and bactericidal effect of NEW to be similar to that of NaClO treatment and slightly higher in the case of *S. aureus* on stainless steel [66].. Kim, *et.al.*, [67] also reported a significant reduction of *Listeria* biofilms on stainless steel to non-detectable levels after treatment with electrolyzed water (56 ppm residual chlorine) for 300s. Thus NEW could serve as a more safe and effective alternative to NaClO and other harsh chemicals.

6.2. Biological approaches

The harsh side effects of detergents and chemicals on the environment and the increased resistance brought about by the overuse of these chemicals have sparked the search for new environmentally friendly alternatives to biofilm prevention and eradication. The idea of inhibiting biocorrosion through the establishment of beneficial bacterial biofilms (BBB) is a novel one still under testing [68]. Zuo [69] in his review summarized the various mechanisms through which BBB could inhibit biofilm formation by corrosive bacteria. According to him, metabolic activities of the cells in the BBB like aerobic respiration can remove agents like oxygen which promote corrosion, the growth of BBBs with antimicrobial activity can act as a strong natural inhibitor against their corrosive counterparts and BBB formation can serve as a protective layer on the surface preventing colonization and corrosion by corrosive bacteria. The establishment of gramicidin-S-producing *Bacillus brevis* on mild steel prevented growth biofilm formation of iron oxidizing *Leptothrix discophora* SP-6 and corrosion causing *Desulfosporosinus orientis* resulting in a 20-fold decrease in corrosion rate when compared to non gramicidin-S- producing *P. polymyxa.*

The antimicrobial properties of secondary metabolites like flavanoids, alkaloids, terpenoids, phenolics, quinones and polyacetylenes in plants have drawn the attention of a number of researchers to plant extracts as a potential for biofilm inhibition [70]. In specific, plant extracts that can inhibit biofilm formation without affecting cell growth, thus preventing selection pressures, resistance and development of persistent strains among the biofilm formers are of particular interest. These extracts inhibit biofilm formation not through killing of the cell but by disrupting various processes required for biofilm formation like quorum sensing, motility and so on. Ursolic acid from the tree *Diospyros dendo* from Africa proved to be non-toxic inhibitor of biofilm formation in the pathogens *E.coli* (6-20 fold inhibition), *P. aeruginosa* (>87%) and *V. harveyi* (57%). Ursolic acid at low concentrations (10µg) altered the gene expression of the cells in the treated biofilm affecting motility and sulphur metabolism which were required for biofilm

formation [71]. Various plants have also shown anti-quorum sensing activity, indicating its potential as a biofilm formation inhibitor [70, 72]. Studies by Hellio, *et.al* confirmed the potential use of extracts from various species of marine algae as antifouling agents in shipping hulls. Extracts from *Enteromorpha intestinalis, Polysiphonia lanosa* and *Sargassum muticum* showed inhibition of fouling at both a micro and macro level suggesting its potential use as an active ingredient in anti-fouling paints [73].

7. Conclusion

The aspects of biofilms and their impact on industries discussed here are equivalent to only a tiny drop in the ocean. On reading this article however, it does become evident that biofilms are highly complex structures that, even after over half a century of research, continue to confound researchers. Minute though they may seem, the adverse economical, health and technological impact microbes in a biofilm pose on industries is huge and cannot be neglected. Though there has been extensive research to come up with measures to prevent and eradicate biofilm formation, the 'perfect' solution that battles all aspects of biofilm resistance has yet to be found. Of the many existing preventive measures, it would be a good idea to focus on the development of biological strategies like plant extracts that are eco-friendly, and target aspects of biofilm formation that do not lead to selective pressures and development of resistance. Overall, the strength of a biofilm shows truth in the saying 'together we stand, divided we fall'.

References

[1] T. B. Reynolds and G. R. Fink, "Bakers' yeast, a model for fungal biofilm formation," *Science,* vol. 291, pp. 878-81, Feb 2 2001.

[2] J. Chandra, D. M. Kuhn, P. K. Mukherjee, L. L. Hoyer, T. McCormick, and M. A. Ghannoum, "Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance," *J Bacteriol,* vol. 183, pp. 5385-94, Sep 2001.

[3] A. J. McBain, R. G. Bartolo, C. E. Catrenich, D. Charbonneau, R. G. Ledder, A. H. Rickard, S. A. Symmons, and P. Gilbert, "Microbial characterization of biofilms in domestic drains and the establishment of stable biofilm microcosms," *Appl Environ Microbiol,* vol. 69, pp. 177-85, Jan 2003.

[4] M. C. van Loosdrecht, J. Lyklema, W. Norde, G. Schraa, and A. J. Zehnder, "Electrophoretic mobility and hydrophobicity as a measured

to predict the initial steps of bacterial adhesion," *Appl Environ Microbiol,* vol. 53, pp. 1898-901, Aug 1987.

[5] K. C. Marshall, R. Stout, and R. Mitchell, "Selective sorption of bacteria from seawater," *Can J Microbiol,* vol. 17, pp. 1413-6, Nov 1971.

[6] E. A. Zottola and K. C. Sasahara, "Microbial biofilms in the food processing industry--should they be a concern?," *Int J Food Microbiol,* vol. 23, pp. 125-48, Oct 1994.

[7] W. Costerton, K. Cheng, G. Geesey, T. Ladd, C. Nickel, M. Dasgupta, and Marrie, "Bacterial Biofilms in Nature and Disease," *Annual Reviews in Microbiology,* vol. 41, pp. 435-464, 1987.

[8] R. M. Donlan, "Biofilms: Microbial Life on Surfaces," *Emerging infectious diseases,* vol. 8, pp. 881-890, 2002.

[9] R. M. Donlan, "Biofilm formation: a clinically relevant microbiological process," *Clin Infect Dis,* vol. 33, pp. 1387-92, Oct 15 2001.

[10] P. Watnick and R. Kolter, "Biofilm, city of microbes," *J Bacteriol,* vol. 182, pp. 2675-9, May 2000.

[11] L. A. Pratt and R. Kolter, "Genetic analyses of bacterial biofilm formation," *Curr Opin Microbiol,* vol. 2, pp. 598-603, Dec 1999.

[12] L. Poulsen and . "Microbial Biofilm in Food Processing," *Lebensm.-Wiss. U.-Technology,* vol. 32, 1999.

[13] G. James, L. Beaudette, and J. Costerson, "Interspecies bacterial interactions in biofilms," *Journal of Industrial Microbiology,* vol. 15, pp. 262-267, 1995.

[14] T. Tolker-Nielson, U. Brinch, P. Ragas, J. Andersen, C. Jacobsen, and S. Molin, "Development and Dynamics of Pseudomonas sp. Biofilms," *Journal of Bacteriology,* vol. 182, pp. 6482-6489, 2000.

[15] I. Sutherland, "Biofilm exopolysaccharides: a strong and sticky framework," *Microbiology,* vol. 147, pp. 3-9, Jan 2001.

[16] M. Hussain, M. H. Wilcox, and P. J. White, "The slime of coagulase-negative staphylococci: biochemistry and relation to adherence," *FEMS Microbiol Rev,* vol. 10, pp. 191-207, Apr 1993.

[17] P. Becker, W. Hufnagle, G. Peters, and M. Herrmann, "Detection of differential gene expression in biofilm-forming versus planktonic populations of Staphylococcus aureus using micro-representational-difference analysis," *Appl Environ Microbiol,* vol. 67, pp. 2958-65, Jul 2001.

[18] T. F. Mah and G. A. O'Toole, "Mechanisms of biofilm resistance to antimicrobial agents," *Trends Microbiol,* vol. 9, pp. 34-9, Jan 2001.

[19] P. S. Stewart and J. W. Costerton, "Antibiotic resistance of bacteria in biofilms," *Lancet,* vol. 358, pp. 135-8, Jul 14 2001.

[20] H. Kumon, K. Tomochika, T. Matunaga, M. Ogawa, H. Ohmori, and T. Usui, "A sandwich cup method for the penetration assay of

antimicrobial agents through *Pseudmonas exopolysaccharides*," *Microbial Immunology,* vol. 38, pp. 615-619., 1997.

[21] T. Maira-Litran, D. G. Allison, and P. Gilbert, "Expression of the multiple antibiotic resistance operon (mar) during growth of Escherichia coli as a biofilm," *J Appl Microbiol,* vol. 88, pp. 243-7, Feb 2000.

[22] J. N. Anderl, M. J. Franklin, and P. S. Stewart, "Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin," *Antimicrob Agents Chemother,* vol. 44, pp. 1818-24, Jul 2000.

[23] T. Zhang and P. Bishop, '', , Vol. 68, Issue 7, Pg. 1107-1115, "Evaluation of substrate and pH effects in a nitrifying biofilm," *Water Environment Research,* vol. 68, pp. 1107-1115, 1996.

[24] C. Prigent- Combarat, O. Vidal, C. Dorel, and P. Lejeune, "Abiotic surface sensing and biofilm-dependant regulation of gene expression in *Escherichia coli*," *Journal of Bacteriology,* vol. 81, pp. 5993-6002, 1999.

[25] K. Lewis, "Programmed death in bacteria," *Microbiol Mol Biol Rev,* vol. 64, pp. 503-14, Sep 2000.

[26] K. Lewis, "Riddle of biofilm resistance," *Antimicrob Agents Chemother,* vol. 45, pp. 999-1007, Apr 2001.

[27] K. Sauer, A. K. Camper, G. D. Ehrlich, J. W. Costerton, and D. G. Davies, "Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm," *J Bacteriol,* vol. 184, pp. 1140-54, Feb 2002.

[28] M. Fletcher, "The effects of proteins on bacterial attachment to polystyrene," *J Gen Microbiol,* vol. 94, pp. 400-4, Jun 1976.

[29] Y. Ong, A. Razatos, G. Georgiou, and M. Sharma, "Adhesion forces between E.coli bacteria and Biomaterial surfaces," *Langmuir,* vol. 15, 1999.

[30] C. Faille, C. Jullien, F. Fontaine, M. N. Bellon-Fontaine, C. Slomianny, and T. Benezech, "Adhesion of Bacillus spores and Escherichia coli cells to inert surfaces: role of surface hydrophobicity," *Can J Microbiol,* vol. 48, pp. 728-38, Aug 2002.

[31] B. Gottenbos, D. W. Grijpma, H. C. van der Mei, J. Feijen, and H. J. Busscher, "Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria," *J Antimicrob Chemother,* vol. 48, pp. 7-13, Jul 2001.

[32] C. G. Kumar and S. K. Anand, "Significance of microbial biofilms in food industry: a review," *Int J Food Microbiol,* vol. 42, pp. 9-27, Jun 30 1998.

[33] G. Loeb and R. Neihof, "Marine conditioning films," *Advances in Chemistry,* vol. 145, pp. 319-335, 1975.

[34] H. al-Makhlafi, A. Nasir, J. McGuire, and M. Daeschel, "Adhesion of Listeria monocytogenes to silica surfaces after sequential and competitive adsorption of bovine serum albumin and beta-lactoglobulin," *Appl Environ Microbiol,* vol. 61, pp. 2013-5, May 1995.

[35] P. S. Meadows, "The attachment of bacteria to solid surfaces," *Arch Mikrobiol,* vol. 75, pp. 374-81, 1971.

[36] J. G. Speers and A. Gilmour, "The influence of milk and milk components on the attachment of bacteria to farm dairy equipment surfaces," *J Appl Bacteriol,* vol. 59, pp. 325-32, Oct 1985.

[37] T. A. Stenstrom, "Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles," *Appl Environ Microbiol,* vol. 55, pp. 142-7, Jan 1989.

[38] L. Rivas, N. Fegan, and G. A. Dykes, "Attachment of Shiga toxigenic Escherichia coli to stainless steel," *Int J Food Microbiol,* vol. 115, pp. 89-94, Apr 1 2007.

[39] C. Prigent-Combaret, G. Prensier, T. T. Le Thi, O. Vidal, P. Lejeune, and C. Dorel, "Developmental pathway for biofilm formation in curli-producing Escherichia coli strains: role of flagella, curli and colanic acid," *Environ Microbiol,* vol. 2, pp. 450-64, Aug 2000.

[40] J. H. Ryu, H. Kim, J. F. Frank, and L. R. Beuchat, "Attachment and biofilm formation on stainless steel by Escherichia coli O157:H7 as affected by curli production," *Lett Appl Microbiol,* vol. 39, pp. 359-62, 2004.

[41] L. De Weger, C. van der Vlught, A. Wijfjes, P. Bakker, and B. Schippers, "Flagella of a plant growth stimulating Pseudomonas flourescens are required for colonization of potato roots," *Journal of Bacteriology,* vol. 169, pp. 2769-2773, 1987.

[42] W. G. Scott, H. M. Scott, R. J. Lake, and M. G. Baker, "Economic cost to New Zealand of foodborne infectious disease," *N Z Med J,* vol. 113, pp. 281-4, Jul 14 2000.

[43] B. Joseph, S. K. Otta, and I. Karunasagar, "Biofilm formation by salmonella spp. on food contact surfaces and their sensitivity to sanitizers," *Int J Food Microbiol,* vol. 64, pp. 367-72, Mar 20 2001.

[44] M. Sharma and S. Kumar, "Biofilm evaluation as an essential component of HACCP for food/dairy processing industry- a case," *Food control,* vol. 13, 2002.

[45] T. Moretro and S. Langsrud, "Listeria monocytogenes: biofilm formation and persistence in food-processing environments," *Biofilms,* vol. 1 pp. 107-121, 2004.

[46] B. Jessen and L. Lammert, ‘’, , Vol. 51, Pg. 265-269., "Biofilm and disinfection in meat processing plants," *International Biodeterioration and biodegradation,* vol. 51, pp. 265-269, 2003.

[47] R. Gamble and P. M. Muriana, "Microplate fluorescence assay for measurement of the ability of strains of Listeria monocytogenes from meat and meat-processing plants to adhere to abiotic surfaces," *Appl Environ Microbiol,* vol. 73, pp. 5235-44, Aug 2007.

[48] G. W. Joshua, C. Guthrie-Irons, A. V. Karlyshev, and B. W. Wren, "Biofilm formation in Campylobacter jejuni," *Microbiology,* vol. 152, pp. 387-96, Feb 2006.

[49] M. LeChavellier, "Coliform regrowth in drinking water: a review," *Journal of American Water Works Association,* vol. 92, pp. 74-86, 1990.

[50] E. Somers, M. Johnson, and L. Wong, "Biofilm formation and contamination of cheese by Nonstarter Lactic Acid Bacteria in the Dairy Environment," vol. 84, pp. 1926-1936, 2001.

[51] K. Lowler, J. Schuman, P. Simpson, and P. Taormina, "Microbiological Spoilage of Beverages' in Food Microbiology and Food Safety," *Compendium of the Microbiological Spoilage of Food and Beverages, Springer New York,* vol. 1st Edition, pp. 245-284, 2009.

[52] I. Adlerberth, S. Ahrne, M. L. Johansson, G. Molin, L. A. Hanson, and A. E. Wold, "A mannose-specific adherence mechanism in Lactobacillus plantarum conferring binding to the human colonic cell line HT-29," *Appl Environ Microbiol,* vol. 62, pp. 2244-51, Jul 1996.

[53] N. Sharon and N. Firon, "Oligomannoside units of cell surface glycoproteins as receptors for bacteria," *Pure Applied Chemistry,* vol. 55, pp. 671-675., 1983.

[54] A. Vaughan, T. O'Sullivan, and D. Sinderen, "Enhancing the Microbial Stability of Malt and Beer – A review," *Journal of the Institute of Brewing and Distilling,* vol. 111, pp. 355-371, 2005.

[55] M. Ludensky, "Control of biofilms in industrial applications,," *International Biodeterioration and Biodegradation,* vol. 51, 2003.

[56] M. Kolari, J. Nuutinen, and M. S. Salkinoja-Salonen, "Mechanisms of biofilm formation in paper machine by Bacillus species: the role of Deinococcus geothermalis," *J Ind Microbiol Biotechnol,* vol. 27, pp. 343-51, Dec 2001.

[57] H. C. Flemming, "Biofouling in water systems--cases, causes and countermeasures," *Appl Microbiol Biotechnol,* vol. 59, pp. 629-40, Sep 2002.

[58] L. L. Barton, T. Ball, R. Villar, and B. Duncan, "Resident continuity clinic: a modest proposal," *Clin Pediatr (Phila),* vol. 46, pp. 446-7, Jun 2007.

[59] N. Ceyhan and G. Ozdemir, "Extracellular polysaccharides produced by cooling water tower biofilm bacteria and their possible degradation," *Biofouling,* vol. 24, pp. 129-35, 2008.

[60] L. K. Ista, V. H. Perez-Luna, and G. P. Lopez, "Surface-grafted, environmentally sensitive polymers for biofilm release," *Appl Environ Microbiol,* vol. 65, pp. 1603-9, Apr 1999.

[61] P. Kingshott, J. Wei, D. Bagge-Ravn, N. Gadegaard, and L. Gram, "Covalent Attachment of Poly(ethylene glycol) to Surfaces Critical for Reducing Bacterial Adhesion," *Langmuir,* vol. 19, pp. 6912-6921, 2003.

[62] A. Denes, E. Somers, A. Wong, and F. Denes, "12-crown-4-ether and Tri(ethylene glycol) Dimethyl-Ether Plasma-Coated stainless steel surfaces and their ability to reduce bacterial biofilm deposition," *Journal of Applied Polymer Science,* vol. 81, pp. 3425-3438, 2001.

[63] G. Cheng, Z. Zhang, S. Chen, J. D. Bryers, and S. Jiang, "Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces," *Biomaterials,* vol. 28, pp. 4192-9, Oct 2007.

[64] A. Terada, A. Yuasa, T. Kushimoto, S. Tsuneda, A. Katakai, and M. Tamada, "Bacterial adhesion to and viability on positively charged polymer surfaces," *Microbiology,* vol. 152, pp. 3575-83, Dec 2006.

[65] G. Qin, Z. Li, X. Chen, and A. Russell, "An experimental study of an NaClO generator for anti-microbial applications in the food industry," *Journal of Food Processing,* vol. 54, pp. 111-118, 2002.

[66] M. A. Deza, M. Araujo, and M. J. Garrido, "Inactivation of Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa and Staphylococcus aureus on stainless steel and glass surfaces by neutral electrolysed water," *Lett Appl Microbiol,* vol. 40, pp. 341-6, 2005.

[67] C. Kim, Y. Hung, R. Brackett, J. Frank, and (), , Vol. 25, Issue 2, and Pg. 91-100., " Inactivation of Listeria monocytogenes biofilms by electrolyzed oxidizing water," *Journal of Food Processing and Preservations,* vol. 25, pp. 91-100, 2000.

[68] H. Videla and L. Herrera, "Understanding microbial inhibition of corrosion: A comprehensive overview," *International Biodeterioration and Biodegradation,* vol. 63, pp. 896-900, 2009.

[69] R. Zuo, "Biofilms: strategies for metal corrosion inhibition employing microorganisms," *Appl Microbiol Biotechnol,* vol. 76, pp. 1245-53, Oct 2007.

[70] D. A. Vattem, K. Mihalik, S. H. Crixell, and R. J. McLean, "Dietary phytochemicals as quorum sensing inhibitors," *Fitoterapia,* vol. 78, pp. 302-10, Jun 2007.

[71] D. Ren, R. Zuo, A. F. Gonzalez Barrios, L. A. Bedzyk, G. R. Eldridge, M. E. Pasmore, and T. K. Wood, "Differential gene expression for investigation of Escherichia coli biofilm inhibition by plant extract ursolic acid," *Appl Environ Microbiol,* vol. 71, pp. 4022-34, Jul 2005.

[72] G. Tinaz, S. Ulusoy, A. Ugur, and O. Ceylan, "Inhibition of quorum sensing–regulated behaviors by Scorzonera sandrasica," *Current Microbiology,* vol. 55, pp. 114-118, 2007.

[73] C. Hellio, D. De La Broise, L. Dufosse, Y. Le Gal, and N. Bourgougnon, "Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints," *Mar Environ Res,* vol. 52, pp. 231-47, Sep 2001.