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**RESEARCH ARTICLE**

# BIOFILM: BOON AND BANE

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**ABSTRACT**

Bacteria are pioneer of life on earth. Habitation of bacteria can be traced back to trillions of years and since then these unique living forms are evolving on our planet. Biofilm is the complex structure formed by microbes by adhering to substrate (support structure) and flourishing communally. This type of growth system allows them to interact (quorum sensing) and survive efficiently under adverse conditions (influence of antibiotics, other physico-chemical factors). These characteristics of surviving and flourishing exhibited by microbes can be used in human welfare and at the same time can be hazardous if not handled carefully. It can be rightly stated that the biofilm is a double sided sword. In the present study a detailed account has been given about the structure of biofilm, its composition, stages of formation of biofilm, its characteristics like biofilm as a boon to the mankind viz. treatment of nuclear waste, effluent treatment, production of nano-particles, improvement of soil properties and remediation of environment by treating toxic factors affecting the Mother Nature. The bane of biofilm cannot be ignored. Biofilm possesses a great threat to human health by causing severe infections and also to industrial sectors especially food production units.



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## INTRODUCTION

Biofilm is a densely packed diverse complex multicellular community of microorganisms which generally develops in the presence of water on living or inert surfaces irreversibly associated with the surface. It is embedded in a self-produced extracellular polymeric matrix (EPS) which generally accounts for 50% to 90% of the total organic carbon of biofilms (Flemming *et al.*, 2000).It is comprised of polysaccharides, proteins and extracellular DNA (eDNA) and coated by a protective layer. The anionic property is conferred due to the presence of uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked pyruvates. This property is important because it allows association of divalent cations such as calcium and magnesium, which can cross-link with the polymer strands. This provides greater binding force in a developed biofilm. In case of Gram-negative bacteria EPS is made up of neutral or polyanionic polysaccharides. In case of some Gram-positive bacteria, such as *Staphylococci*, the chemical composition of EPS may be different and cationic. Variations in chemical and physical properties of EPS exist. It was found that the slime of coagulase-negative bacteria consists of teichoic acid mixed with small quantities of proteins (Gloag *et al.,* 2013).This matrix, termed as glycocalyx, is synthesized by the bacteria.

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One of its functions is to permanently anchor bacterial cells adsorbed to a substratum (Kinner *et al.,* 1983). It may also show presence of non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles, blood components, depending on the environment in which the biofilm has developed (Donlan, 2002). Biofilms are coordinated and cooperative groups, analogous to multicellular organisms due to their structural and physiological complexity (Nadell *et al.,* 2009). Biofilms are dynamic communities of interacting sessile cells which are irreversibly attached to a solid substratum, as well as to each other (Costerton *et al.,* 1994). The organisms associated with biofilm 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, medical devices, industrial or potable water piping system, or natural aquatic systems and are greatly ubiquitous (Kokare *et al.,* 2009). (Fig.1)

### Factors influencing formation of biofilm

Biofilm formation is influenced by environmental factors like: temperature, salinity, water dynamism, concentration of key nutrients that can change the growth, density and thickness of biofilms. Other factors like adhesins, surfactants and bacterial motility have considerable influence on formation of biofilm (Hassan *et al.,* 2011). (Fig 2)

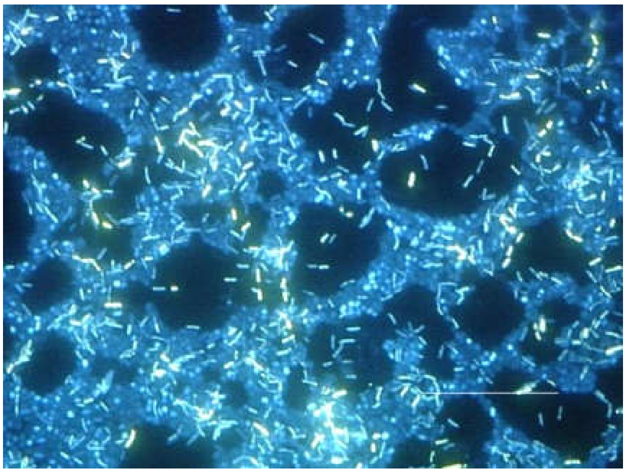

**Fig. 1. Polymicrobic Biofilm on a Stainless Steel Surface**

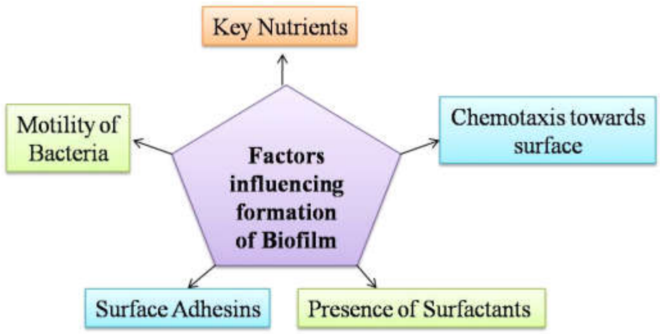


**Fig. 2. Factors Influencing Formation of Biofilm**

## Development of Biofilm

Formation of biofilm occurs step by step such as formation of conditioning layer, bacterial adhesion, bacterial growth and biofilm expansion. According to Gotz biofilm formation is a survival strategy in adverse environments as a result of change of planktonic cells to sessile ones. (Gotz, 2002; Oliveira *et al.,* 2014).Cellulose is an exopolysaccharide produced by microbial cultures which is involved in cell adhesion and formation of biofilm. Recent studies have revealed that some species of the family Enterobacteriaceae (e.g., *Citrobacter*spp, *Enterobacter* spp. and *Klebsiella* spp.) produce cellulose as a major component of the bacterial extracellular matrix which was not observed in earlier studies (Lasa *et al.*, 2006; Seto *et al.,*2006; Adetunji *et al.,* 2014). Special conditions are required for few organisms such as C*.renale*, *C. pseudotuberculosis*, *M. haemolytica*, *P. multocida*, and *A. pyogenes.* They require the addition of fetal bovine serum and incubation under 10% $CO_2$ to grow as biofilms and also longer culture time (Olson *et al.,* 2002)**.**During formation of a biofilm planktonic cells undergo dynamic changes for transition from free living organisms to biofilm cells which involves the specific production of secondary metabolites and a significant increase in the resistance towards biological, chemical and physical conditions (Morikawa, 1995; Das *et al.,* 2012;). In most of the natural and artificial habitat the majority of microbial populations form biofilms on solid surfaces. Microorganisms in biofilms get differentiated for the transformation from the planktonic form to the biofilm cell. This involves a series of developmental stages in a definite sequence that includes: formation of complex, multicellular structures known as micro colonies. These micro colonies get surrounded by a network of water channels. The steps are adhesion, growth, motility and extracellular polysaccharide production. Once the initial biofilm is established, cell to cell communication via quorum sensing with the help of extracellular signalling molecules controls the changes that lead to development of the biofilm (Atkinson and Fowler, 1974; Costerton *et al.,* 1995; Tolker *et al.,* 2000; Klapper *et al.,* 2002; Kjelleberg and Molin 2002; Molin *et al.,* 2003).Bacteria forming biofilms on any surface is bathed in a nutrient-containing fluid. The three major components involved in biofilm formation:

- Bacterial cells,
- Solid surface
- Fluid medium.

Stages of biofilm formation is depicted in Fig.3. (Vasudevan, 2014; Ikuma *et al.,* 2015)

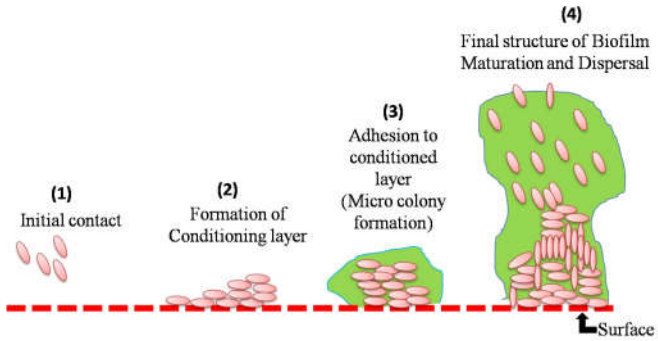


**Fig. 3. Stages of Biofilm Development**

The initial stage in the formation of Biofilm is adsorption on solid surfaces (organic and inorganic) forming a conditioning layer. Once the layer is formed adhesion takes place in three phases:

Phase 1- Transport of microbe to substrate surface
Phase 2 - Initial non-specific microbial–substrate adherence
Phase 3 - Specific microbial substrate adherence

After adhesion, development of biofilm starts and its expansion takes place with the maturation of biofilm (final structure) followed by its dispersal. (Cowan *et al.,* 1987; Costerton *et al.,* 1999; Avinash *et al.,* 2014 ;)

## Formation of Biofilm in Gram negative and Gram positive bacteria

Biofilm is formed in different stages as discussed above. Biofilm is considered as the sole reason for the dispersal of microbes and maintaining their integrity in the harsh and extreme conditions. In Gram positive bacteria a set of surface protein (ssp-1, ssp-2) adhesion molecules Aap (accumulation associated protein) and Alt-E leads to adhesion to the surface. Intra cellular aggregation of biofilm takes place with the help of proteolytic enzymes of host leading to the maturation of biofilm and dispersal at the last stage. On the other hand Gram negative bacteria follow stages with slight modification from the Gram positive organisms. Adherence to the surface/substrate takes place in reversible/irreversible manner. Irreversible attachment takes place mainly by type-I fimbria and also by conjugative pilli, Curli also known as culi fimbria promotes cell adhesion further.

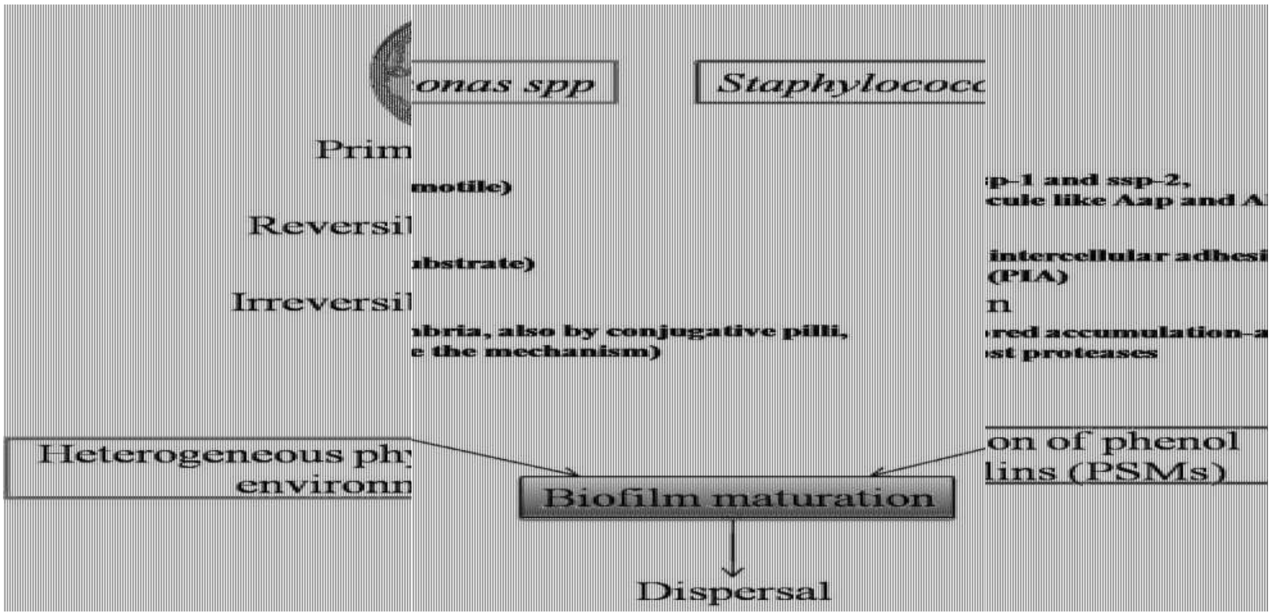


Fig. 4. Pathway for Formation of Biofilm in Gram negative and Gram positive Bacteria

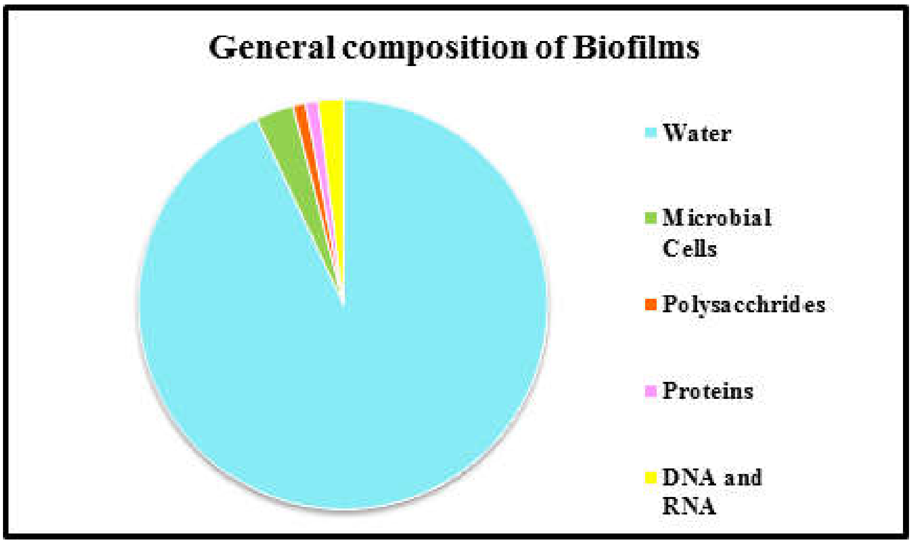


Fig. 5. Composition of Biofilm

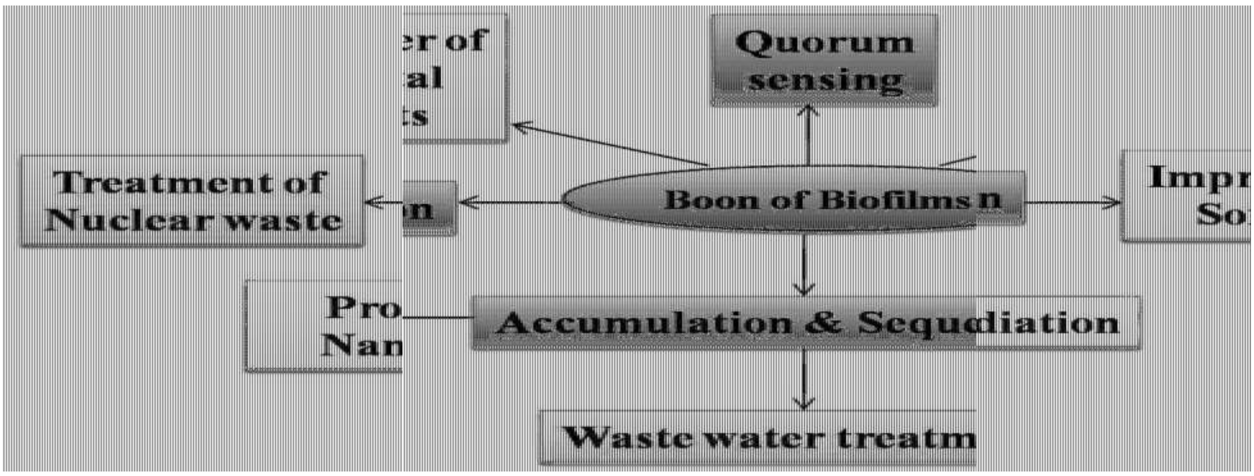


Fig. 6. Boon of Biofilm

Maturation and dispersion of biofilm takes place in heterogeneous physicochemical environment (Vasudevan, 2014). (Fig.4)

**Composition of Biofilm**

A fully developed biofilm is a heterogeneous arrangement of microbial cells on a solid surface. Surface-adherent bacterial cells help in formation of the basic structural unit, that is microcolonies or cell clusters (Wingender *et al.,* 1999; Nivens *et al.,* 2001; Whitchurch *et al.,* 2002; Vasudevan, 2014; Jhajharia *et al.,* 2015).(Fig 5)

**Characteristics of biofilm**

The microorganisms living in a community must have the following four basic criteria (Caldwell *et al*., 1997; Avinash *et al.,* 2014*)*

**Autopoiesis**: Organisms involved in biofilm formation should get self-organized
**Homeostasis:** Should overcome environmental influences
**Synergy**: The synergic relationship of biofilm should be more effective than planktonic cells
**Communality:** The organisms involved in formation of biofilm should respond to environmental changes as a unit rather than as isolated individuals (Avinash *et al.,* 2014).

Based on these criteria the biofilm can be a boon or bane to Mother Nature and all its stakeholders.

**Boon and Bane of Biofilm:** The beneficial and harmful aspects of biofilm have been discussed at length to understand where and how we can exploit this phenomenon to make the environment ecofriendly.

**Boon of Biofilm:** The boon of biofilms is depicted schematically in Fig 6 (Ikuma *et al.,* 2015) and the examples to support the view is listed in Table 1.

**Advantages of Biofilm production**

**1) Protection from environment**

Bacteria get shelter and homeostasis when they are located within a bio lm (Vasudevan, 2014). The main component in providing protection is the surrounding extra polymeric substance matrix. (EPS) This matrix is composed of a mixture of EPS, protein, nucleic acids and other substances. Most bacteria are able to produce polysaccharides in either of the two ways:

- Wall polysaccharides (capsules)
- Extracellular excretions into the surrounding environment (EPS)

EPS provides protection from a variety of environmental stresses such as UV radiation, pH shifts, osmotic shock and desiccation. The EPS matrix also has the capacity to physically prevent access of certain antimicrobial agents into the bio lm. It acts as an ion exchange and restricts the diffusion of compounds from the surrounding microenvironment into the bio lm (Chandra *et al.,* 2001;Davey and O'toole, 2000).This characteristic largely depends on the nature of the antimicrobial agent and the EPS matrix. The effect appears to be more prominent with antibiotics which are hydrophilic and positively charged Eg. aminoglycosides. It has also been found to sequester metals, cations and toxins (Sutherland, 1985; Davey and O'toole, 2000). Biofilms afford greatly increased protection from antibiotics, the host's immune system and physical injury (Kokare *et al*., 2009). Bio lm formation helps to evade predation (i.e. human immune system) or to take advantage of alternative metabolic processes. The bio lms have ability to react to transient and long term stresses (Klapper *et al.,* 2002). This allows the bio lm to stay in environmentally favorable environments. In this way bio lms protect the bacteria from disinfectants (Høiby *et al.,* 2010).

**Table 1. Boon of Biofilm**

| S.No. | Boon | Biofilm forming Organisms studied | Reference |
|---|---|---|---|
| 1. | Protection from environment | *Staphylococcus aureus Klebsiella* | Davey and O'toole, 2000 |
| 2. | Nutrient Availability | *Methanogens* | Davey and O'toole, 2000 |
| 3. | Acquisition of new genetic trait | *Pseudomonas aeruginosa* | Anbazhagan *et al., 2012* |
| 4. | Penetration of antimicrobial agents | *Pseudomonas aeruginosa Staphylococcus epidermidis Bacillus subtilis* | Anbazhagan *et al., 2012* |
| 5. | Antibiotic resistance | *Pseudomonas aeruginosa* | Sodano *et al.,* 2010 |
| 6. | Treatment of nuclear industrial waste | *Pseudomonas denitrificans Pseudomonas aeruginosa* | Wragg *et al.,* 2012 |
| 7. | Production of nanoparticles | *Pseudomonas aeruginosa Pseudomonas fluorescens Alteromonas macleodii Lactococcus lactis Stenotrophomonas maltophilia Rhodopseudomonas capsulata* | Li *et al.,* 2011; Zonaro *et al.,* 2015 |
| 8. | Quorum Sensing | *Acinetobacterbaumannii* | Sodano *et al.,* 2010; Anbazhagan *et al.,* 2012 |
| 9. | Ability to enter into latent states during inhospitable conditions | *Vibrio vulnificus* | Sodano *et al.,* 2010. |
| 10. | Waste Water Treatment | *Sphaerotilus* | Kinner *et al.,* 1983 |
| 11. | Bioremediation | *Bacillus subtilis Bacillus cereus Escherichia coli* | Avinash *et al.,* 2014 |
| 12. | Use in industries | *Z. mobilis, Bacillus* | Morikawa, 2006;Todhanakasem *et al.,* 2015 |
| 13. | Modelling and Simulation | *L. monocytogenes* | Khassehkhan and Hermann, 2008. |
| 14. | Improvement of soil properties | *Bacillus, Enterobacter, Pseudomonas* | Redmile *et al.,* 2014. |

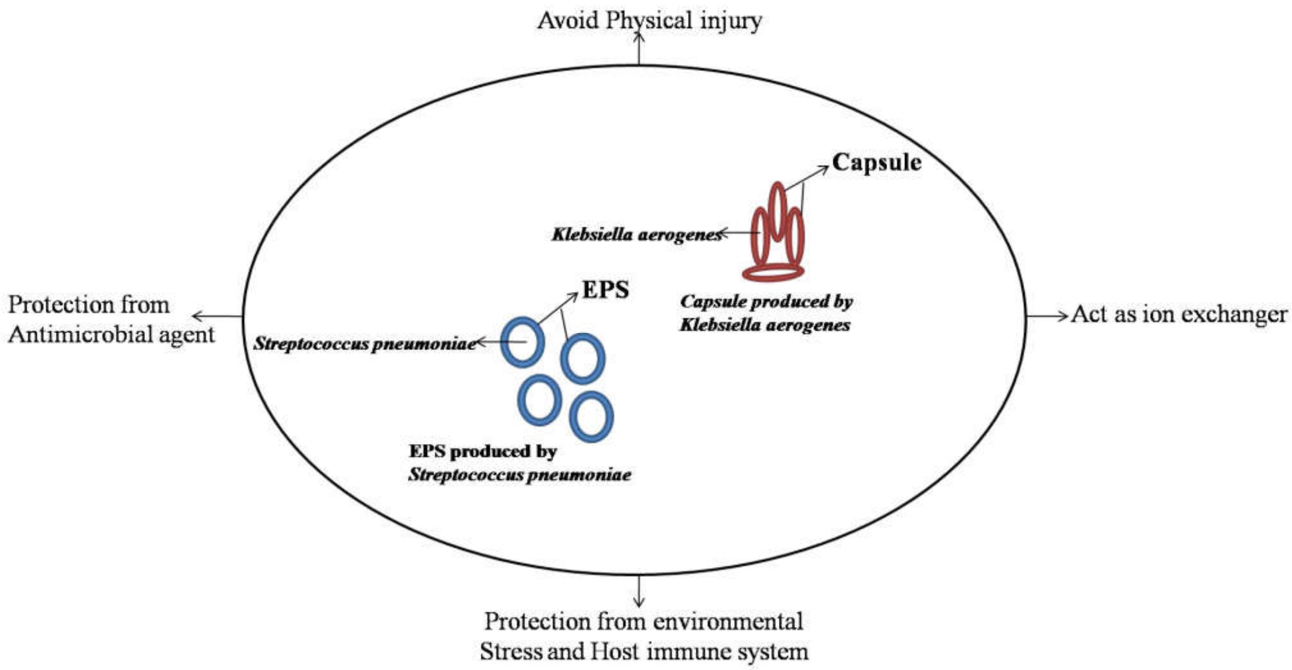


**Fig. 7. Advantages to Bacteria Producing Biofilm**

Several pathogenic bacterial species are capable of forming biofilm on the surfaces (Watnick and Kolter, 2002; Tsuneda *et al.,* 2003). The biofilms protect the cells from host immune response and antimicrobial agents (Gillaspy *et al.,* 1995; Høiby *et al.,* 2010). One of the well-known pathogens that can live in a wide variety of environments is *Staphylococcus aureus*. It also forms biofilms on both biotic and abiotic surfaces (Begur *et al.,* 2007; McCann *et al.,* 2008). Fig 7 summarizes the advantages enjoyed by the microbe producing the biofilm.

**2) Nutrient availability and metabolic co-operativity**

The highly permeable water channels present throughout the biofilm in the areas around the microcolonies provide an effective means of exchanging nutrients and metabolites with the aqueous phase. This increases the availability of nutrients and removal of toxic metabolites (Costerton *et al*., 1995). The metabolic characteristics of bacteria within a biofilm community are different and unique. This provides a possibility for metabolic cooperation. The bacteria are exposed to varied environmental signals within a biofilm. For example, cells situated near the center of micro colonies generally experience low oxygen tensions. Microcolonies of multimember films generally consist of a mixture of species (Hirsch, 1984; MacLeod *et al.,* 1990). These multispecies micro consortia result from an association between metabolically cooperative organisms and their closeness helps in interspecies exchange of substrate and the removal of metabolic products. For example, during anaerobic digestion the degradation of complex organic matter into methane and carbon dioxide requires the interaction of at least three different organisms. Fermentative bacteria produce acids and alcohols by catabolism. Acetogenic bacteria then readily utilize these products as substrates. Finally, the methanogens obtain energy by converting acetate, carbon dioxide, and hydrogen into methane. Hence, there may be development of efficient cooperation and mutual dependence within the biofilm. Biofilms also provide an ideal environment for the establishment of syntrophic relationships.

Syntrophism is a special case of symbiosis in which two metabolically different types of bacteria depend on each other to use certain substrates, mainly for the production of energy. Syntrophic associations are found during methanogenic degradation (Schink, 1997; Davey and O'toole, 2000).

**3) Acquisition of new genetic trait:**

Horizontal gene transfer leads to evolution and genetic diversity of natural microbial community. Acquisition of new genetic trait increases the chances of transcription of necessary genes by the microbial communities. This further helps them to become the active member of the biofilm communities. The main reason is transcription of different genes by biofilm forming communities. Transcription of algC gene which is involved in the production of alginate is increased almost four fold in cells associated with biofilm. It has been observed that synthesis of large amounts of alginate makes the pulmonary isolates *Pseudomonas aeruginosa* mucoid (Costerton *et al.,* 1995; Schink, 1997; Kokare *et al.,* 2009).

**4) Penetration of antimicrobial agents**

Diffusion is the rate-limiting step to inactivate the biofilm forming microbial community by antimicrobial agents. EPS influences the rate of transport of the molecule to the interior of the biofilm or the reaction of antimicrobial agents with the matrix material and hence acts as diffusion barrier for these molecules.

The advantages of biofilm mode of growth to the microbial community are:

- All the energy is used up by the bacteria in making EPS as the growth is restricted. This gives protection to microbial community. *Pseudomonas fluoroscens* produces EPS lyase which degrades the biofilm associated EPS for consumption and release cells from biofilm scaffold to seek more favourable environment.

- As the growth is restricted bacteria will remain in dormant stages that will give protection to the microbial community against antibiotics (Kokare *et al*., 2009).

**5) Antibiotic resistance**

The emergence of bacterial resistance to antibiotics has become a common problem in both hospitals and community settings. As a result, the effectiveness of antibiotic treatment of bacterial infections has progressively decreased (Sievert *et al.,* 2013). The problem is particularly seen in the treatment of biofilm-associated infections as bacteria grown in biofilm mode are more tolerant to conventional antibiotics and biocides compared to free swimming cells (Stewart and Costerton, 2001). Therefore, it is becoming necessary to develop and test new antimicrobial compounds capable of bactericidal activity even in biofilm growth mode towards multidrug resistant bacterial species (Zonaro *et al.,* 2015). In human medicine *Pseudomonas aeruginosa* has been recognized to form antibiotic resistant biofilms both on implanted devices and within tissues (Mah and O'toole, 2001; Olson *et al.,* 2002).

**6) Treatment of nuclear industrial waste**

Many industries including nuclear facilities generate effluents containing high content of nitrate. Various stages of the nuclear fuel cycle; fuel fabrication and reprocessing generates nitrate wastes. Treating and disposing of concentrated wastes becomes a major concern. Infants who consume high-nitrate water suffer from methaemoglobinemia also known as blue-baby syndrome. Biological denitrification is an economical and technically feasible method for removal of nitrates. Granular biofilm based sequencing batch reactors (SBRs) allows designing compact and high rate processes which are suitable for the treatment of concentrated effluents (Krishna *et al.,* 2011;Wragg *et al.,* 2012;Prakash *et al.,* 2013) (Fig 8).

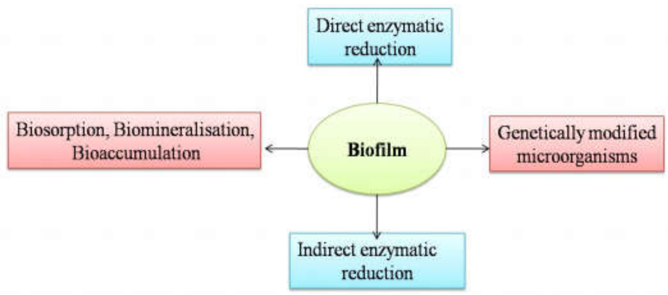


**Fig 8. Different Mechanisms through Which Biofilm Interacts with Radionuclide's**

**7) Production of nanoparticles**

The potential of biofilms to act as a factory for production of nanoparticles has been increasingly recognized in both natural and engineered systems. Natural biofilm plays an important role in the biogeochemical cycling of elements which can lead to formation of nanoparticles. For example, microbes exhibit detoxification mechanism by precipitating metals in the form of nanoparticles. The use of biofilms for nanomaterial synthesis is becoming popular as it is relatively clean, non-toxic, and environmentally safe (Zonaro *et al.,* 2015). *Rhodopseudomonas capsulata* exhibits the mechanism of converting gold (Au) into gold nanoparticle (AuNPs). In *R.capsulata* enzyme NADH-dependent reductase catalyses the process initiating the electron transfer and forming nanoparticles (Li *et al.,* 2011; Pantidos and Horsfall, 2014). (Fig 9)

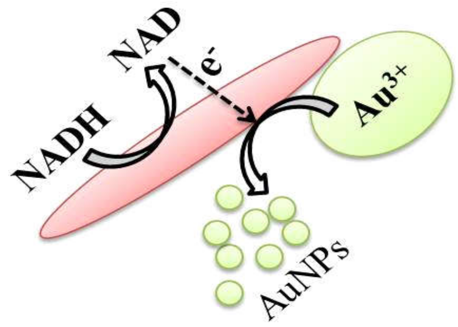


**Fig. 9. Biofilm Converting Gold (Au) to Gold Nanoparticle (AuNP)**

**8) Communication and Coordination**

Cell to cell signalling plays a major role in cell attachment. Bacterial products which are able to diffuse away from one cell and enter into another cell help in intracellular communication between bacteria and create a diffusion limited environment. Quorum sensing molecules are also referred as acyl-homoserine lactone. This signal may introduce new genetic trait or alter protein expression in the neighboring cells (Kokare *et al.,* 2009). (Fig 10)

**Formation of Biofilm in Gram negative and Gram positive bacteria and its association with Quorum sensing**

In Gram negative bacteria signal molecule N-homoserinelactone acts as the auto inducer and in Gram positive bacteria peptide molecule acts as the auto inducer which binds to the QS receptor and this complex binds to QS regulated gene which in turn up regulates the formation of virulence gene which gives rise to formation of biofilm (Vasudevan, 2014).

**9) Waste Water Treatment**

Wastewater biofilms are found to be very complex. They may possess a bacterial component which is thick, overlying, less firmly bound, filamentous (Eighmy *et al.,* 1983; Borkar *et al.,* 2013). The ability of rotating biological contactors to efficiently degrade wastes and withstand environmental fluctuations is generally increased due to the presence of a diverse biofilm community. Deteriorating conditions in the biofilm are indicated by the presence of apparent phage within the microbial cells in the contactor. The bacteriophage reduces their ability to assimilate organic matter from the wastewater and also acts as a natural enemy of biofilm bacteria. The predominant filamentous organism present in a contactor is *Sphaerotilus* species (Kinner *et al.,* 1983).Fig 11 shows the generation of energy from waste water with microbe forming a biofilm. Biofilm processes have proved to be reliable for removal of organic carbon and nutrients without some of the problems of activated sludge processes. Biofilm reactors have proved to be very useful when slow growing organisms like nitrifiers have to be kept in a wastewater treatment process. Biofilm reactor exhibits both nitrification and denitrification process.

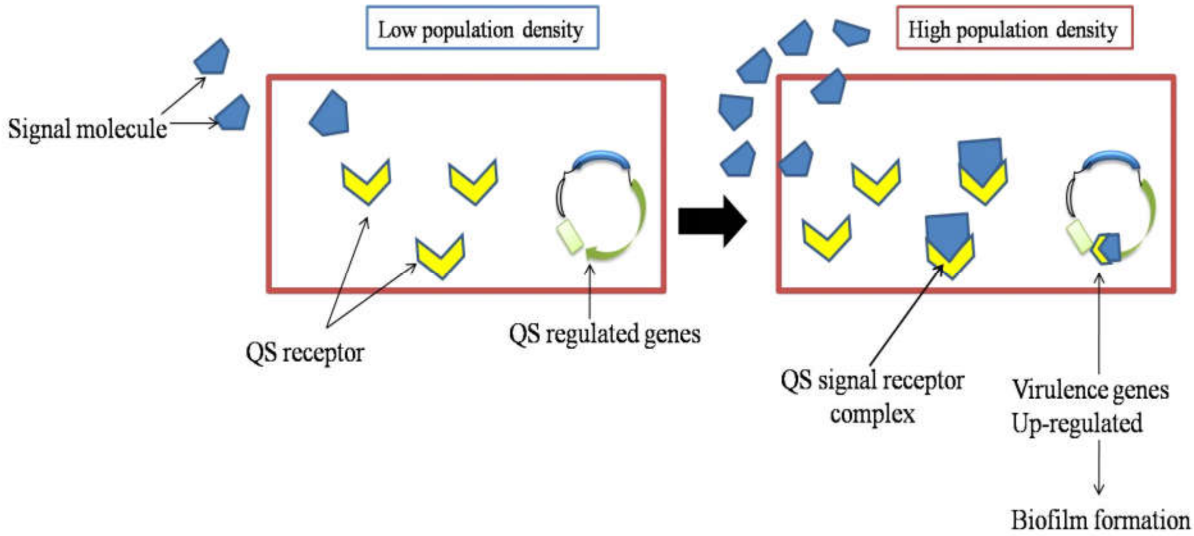


**Fig. 10. Biofilm Formation and Its Role in Quorum Sensing (QS)**

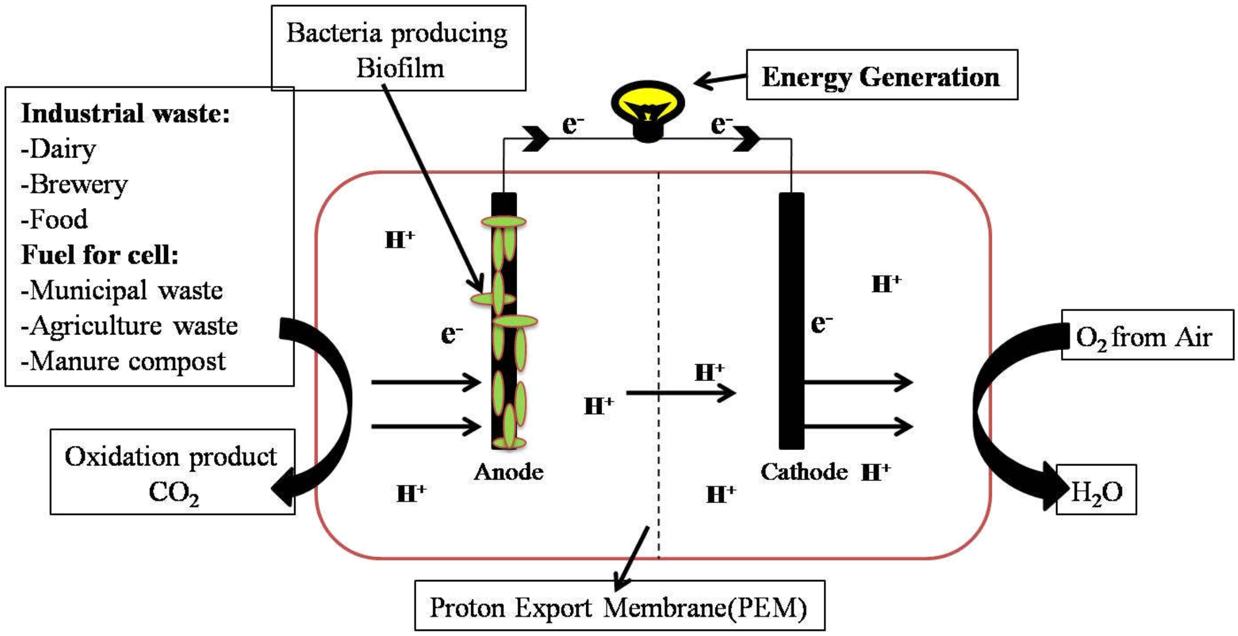


**Fig. 11. Energy Generation from Waste Water with Microbe Forming Biofilm (Khan and Majumder, 2016)**

There are already many different biofilm systems in use such as trickling filters, Rotating Biological Contactors (RBCs), fixed media submerged bio-filters, granular media bio-filters, fluidized bed reactors, etc. having their own advantages and limitations. The MBBR is a complete mix, continuous flow through process which is based on the principle of biofilm that combines the benefits of both the activated sludge process and conventional fixed film systems without their disadvantages (Borkar *et al.,* 2013).

**10) Bioremediation**

Bioremediation is referred as the application of biological processes for the removal of hazardous pollutants present in the environment (Gianfreda and Rao, 2014). It is the process of using microorganisms to clean up a contaminated site (Zylstraa and Kukor, 2005). Biofilms can be applied for the bioremediation of waste waters. There are successful examples of the positive use of biofilms that are called beneficial biofilms which offer protection of environment from the hazardous effects of toxic pollutants.

Biofilm reactors also play a major role in the remediation of xenobiotic compounds (Lazarova and Manem, 2000, Wolfaardt *et al.,* 2000). Immobilization, concentration and partitioning to an environmental compartment helps in heavy metal remediation, thereby minimizing the anticipated hazards (Barkay and Schaefer, 2000; Llyod, 2003). Biofilm developed using consortium of *Bacillus subtilis* and *Bacillus cereus* on coarse sand is able to remove chromium (Das *et al.,* 2012). (Fig 12)

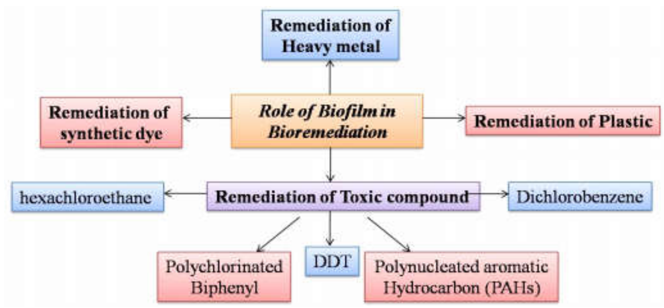


**Fig. 12. Role of Biofilm in Bioremediation of Environment**

**11) Use in industries**

*Z. mobilis* is a useful ethanologenic bacterium that can form biofilms of single species (Breidt and Flemming, 1998). The phenomenon of *Z. mobilis* biofilm formation has increased cell tolerance to a toxic substrate and allows continuous biotransformation for the productions (Maksimova, 2014). Hence it has a great potential towards various industrial applications (Todhanakasem *et al.,* 2015). *Bacillus subtilis* is an industrially important bacterium which exhibits developmental stages. It forms rough biofilms at the air-liquid interface due to the aerotaxis of the cells. Biofilm formation by *B. subtilis* and related species reduce mild steel corrosion, and also help in exploration of novel compounds (Morikawa, 2006).

**12) Modeling and Simulation**

The structure of a biofilm has been simulated in two dimensions (2D) without regard to its function. Each discrete time step of the simulation contains the following phases:

- Diffusion of nutrients
- Growth and death of microbes
- Consumption of nutrients by microbes.

These concepts provide the background for diffusion and release of microbial products, attachment to the biofilm of a microbe that is wandering in free space and detachment of microbes from the biofilm, as well as applications in quite different areas (Shiflet and Shiflet, 2012). An example of mathematical modelling is control of *Listeria monocytogenes* by *Lactococcus lactis* which are frequently developed as probiotics and added to dairy products as health promoting functional foods (Breidt and Flemming, 1998; Khassehkhan and Hermann, 2008; Shiflet and Shiflet, 2012; Horn and Lackner, 2014).(Fig 13)

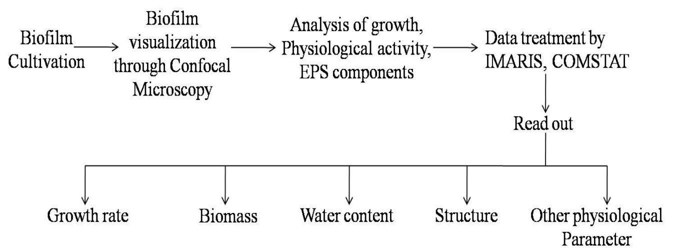


**Fig. 13. Flow Sheet for the Process of Modelling and Simulation of Biofilm**

IMARIS- Software for visualizing 3D/4D images, COMSTAT (COMputer STATistics) Program to analyze stacks of Biofilm recorded by Confocal Microscopy.

**13) Improvement of soil properties**

EPS produced *in situ* improves soil properties, for example, it increases heterogeneity and improves stability of soil aggregates (Redmile *et al.,* 2014). Agronomically, studies have proved that the inoculation of wheat seedlings with EPS-producing *Bacillus* and *Enterobacter* species has been seen to afford saline tolerance and survival rates of sunflowers in drought are found to improve by inoculation with EPS-producing strains of *Pseudomonas* (Kavazanjian and Karatas, 2008;Redmile *et al.,* 2014).(Fig 14)

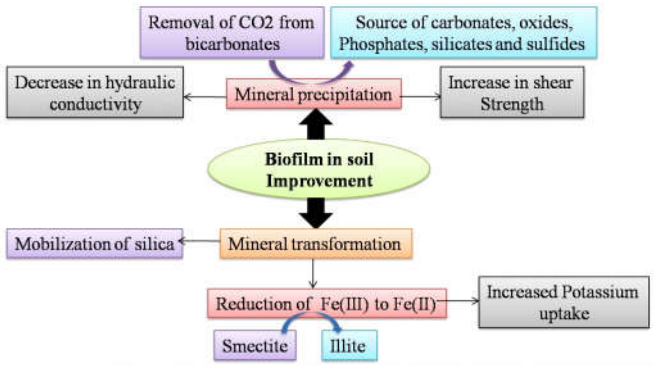


**Fig. 14. Role of Biofilm in Soil Improvement**

**14) Ability to enter into latent states during inhospitable conditions**

The microbes like *Vibrio vulnificus* in biofilm communities can enter into a viable but unculturable state in the midst of inhospitable conditions such as nutrient starvation (Sodano *et al.,* 2010; Oliver 2014).

**Bane of Biofilm**

The disadvantages of Biofilm are diagrammatically explained in Fig.15 and Table 2 lists out the different arena where biofilms cause harmful effects.

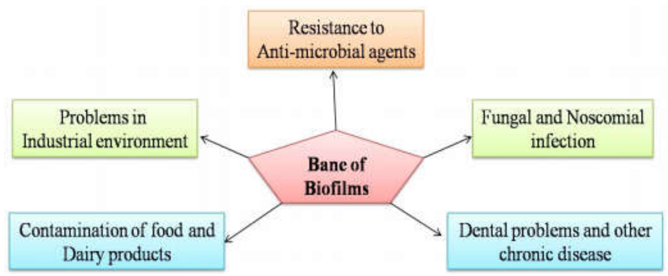


**Fig. 15. Bane of Biofilm**

**Disadvantages of Biofilm Production**

**1) Contamination of food and Dairy Industry:**

The ability of many bacteria to form biofilms has major implications in a variety of industries including the food industry. The biofilms create a persistent source of contamination in such industries. The factors responsible for formation of a biofilm are the nature of the attachment surface, characteristics of the bacterial cell and environmental factors (Noiri *et al.,* 2002). Growth of biofilms in the food processing environment increases the chances of microbial contamination of the processed food. *Listeria monocytogenes*is the most common strain found in the food processing environment. It has a good ability of adhesion and requires very short time for attachment (Kokare *et al.,* 2009). Bacteria in biofilms are a major source for contamination of food, which leads to outbreak of foodborne diseases. *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* and *Salmonella enteritidis* are some of the important foodborne pathogens worldwide and many foodborne outbreaks have been linked to them as they are capable of adhering to and forming biofilms on a variety of surfaces commonly used in food environment such as stainless steel, metals, plastics, rubber, glass, polypropylene mesh etc (Adetunji *et al.,* 2014).

**Table 2. Bane of Biofilm**

| Sr. No. | Bane | Organisms involved | Reference No |
|---|---|---|---|
| 1. | Contamination of food | *Listeria monocytogenes* *Escherichia coli* *Staphylococcus aureus* *Salmonella enteritidis* | Van and Michiels, 2010 |
| 2. | Dairy Industry | *Listeria monocytogenes* *Yersinia enterocolitica* | Garrett *et al.*,2008 |
| 3. | Cause of chronic diseases | *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Group A β-hemolytic Streptococci, enteric bacteria, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* | Hoiby *et al.,* 2010. |
| 4. | Cause of nosocomial infections | *Staphylococcus epidermidis* | Brown *et al.,* 2014 |
| 5. | Cause of dental problems | *E. faecalis* | Davey and O'toole, 2000; Jhajharia *et al.,* 2015; |
| 6. | Cause of plant diseases | *Xylella fastidosa* | Molhoek *et al.,* 2011. |
| 7. | Problems in industrial environment | *Listeria monocytogenes* | Michiels, 2010. |
| 8. | Problems in oil industry | *Sulphate reducing bacteria* | Garrett *et al.,* 2008. |
| 9. | Resistance to antimicrobial agents | *Staphylococcus,Pseudomonas aeruginosa* | Hoiby *et al.,* 2010 |
| 10. | Fungal Infections | Candida albicans, Candida glabrata | Brown *et al.,* 2014; Colomba *et al.,* 2014 |

The main sources of contamination of dairy industry are generally due to improper cleaning and disinfection of equipment. It is very important to sanitize the processing equipment. Bacterial EPS and milk residues like proteins and calcium phosphate predominate dairy biofilms. The formation of biofilms on equipment of dairy industry can lead to serious hygiene problems and economic losses due to spoilage of food and impairment of equipment. Metal corrosion in pipelines and tanks is catalysed due to chemical and biological reactions in biofilms. If biofilms become sufficiently thick at plate heat exchangers and pipelines, it reduces the heat transfer efficacy. Pathogens associated with biofilms include *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni*, *Salmonella* spp, *Staphylococcus* spp and *E coli*.

It has been found out that undesirable microorganisms such as *Lactobacillus curvatus* and *Lactobacillus fermentum* can persist on milk residues in cheese processing plants even after repeated cleaning. *Bacillus cereus* accounts for more than 12% of the biofilms constitutive flora in a commercial dairy plant (Simoes *et al.,* 2010). Food spoilage, hygienic risks and diseases are caused due to formation of bio lm on production surfaces (Verran, 2002 ;Trachoo, 2003) A relevant example is *Listeria monocytogenes* that is found in dairy products. It is a hardy, harmful, food borne, bio lm forming pathogen (Stecchini *et al.,* 1995; Ryser and Marth, 2007) and causes the often fatal infection listeriosis. Prevention and eradication of bio lms is necessary for food safety, hygienic and public health concern. (Khassehkhan and Hermann, 2008). (Fig 16)

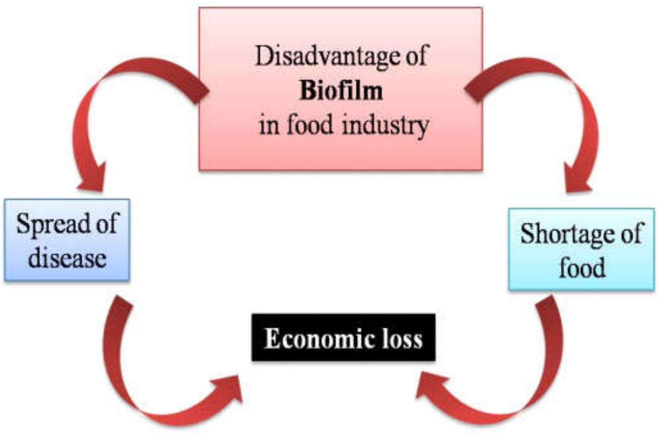


**Fig. 16. Adverse Effects of Biofilm in Food and Dairy Industries**

## 2) Biofilm involved in Health Hazards

### a) Chronic diseases

Bacterial biofilms cause chronic infections due to their increased tolerance to antibiotics and disinfectant chemicals as well as their capacity to resist phagocytosis and other components of the body's defence system. For example, the persistence of *Staphylococcal* infections is due to biofilm formation. Biofilm-growing mucoid strains of *Pseudomonas aeruginosa* causes chronic lung infection in cystic fibrosis patients. Growth of biofilm is linked with an increased level of mutation as well as with quorum-sensing-regulated mechanisms (Høiby *et al.,* 2010). Biofilms of *Staphylococcus* on native heart valves may result in valve tissue damage or production of emboli. Otitis media (OM) is caused by a number of different organisms, including *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis*, group A beta-hemolytic streptococci, enteric bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and other organisms (Feigin *et al.,* 1992; Donlan and Costerton, 2002).Immuno compromised individuals may be especially susceptible to the nontubercular *Mycobacterium avium* organism. The best example of a pulmonary disease that is caused by biofilms is meloidosis, which causes pulmonary infections and the causative pathogen is *Burkholderia pseudomallei* (Costerton *et al.,* 2003; Garrett *et al.,* 2008; Michiels and Houdt, 2010)

### b) Nosocomial infections

*Staphylococcus epidermidis* is a major cause of nosocomial infections. It leads to infections related to indwelling medical devices such as intravascular catheters, cerebrospinal fluid shunts, peritoneal dialysis catheters, intraocular lenses, cardiac pacemakers and prosthetic joints. In mature biofilms these communities are encased in an extracellular matrix composed of bacterial polysaccharides It is an important member of the human skin and mucous membrane micro flora and can be easily transmitted to the surfaces of these devices when they are implanted or manipulated. It adheres to and grows on medical devices, which may lead to formation of mature biofilm consisting of cells embedded in a sticky extracellular slime composed of bacterial polysaccharides, proteins and DNA. This matrix causes resistance to antibiotics and host defence system. It is difficult or almost impossible to eradicate these biofilms (Molhoek *et al.,* 2011). Hospital infection is a serious public health problem and factors like presence of insects influence the occurrence of these infections. The main bacteria responsible for nosocomial infections and transmitted by insects is *Staphylococcus aureus*. It is a versatile pathogen capable of causing a wide variety of human diseases. Nosocomial infections cause high morbidity and mortality and it is a major challenge to control such infections (Oliveira *et al.,* 2014).

**c) Dental problems**

Oral biofilm formation involves three steps: formation of pellicle, colonization by bacteria and maturation of biofilm. The microorganisms of the biofilm are surrounded by the organic substance which contains carbohydrates, proteins, and lipids (Richards *et al.,* 1999; Marsh, 2004; Jhajharia *et al.,* 2015).The development of biofilm *of E. faecalis* on the dentin of root canal involves three stages as follows:

**Stage 1:** Formation of micro colonies
**Stage 2:** Localized increase in the calcium and phosphate ions
**Stage 3:** Mineralization or calcification of the biofilm (Distel *et al.,* 2002; Noiri *et al.,* 2002; Hubble *et al.,* 2003)

d) **Resistance to antimicrobial agents**

Microorganisms growing in a biofilm are more resistant to antimicrobial agents than planktonic cells. High antimicrobial concentrations are required to inactivate organisms growing in a biofilm, as antibiotic resistance increases almost 1,000 fold (Hassan *et al.,* 2011). Recalcitrant infections are caused by biofilm producing bacteria and are very difficult to eradicate. They exhibit resistance to antibiotics by different methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes (Høiby *et al.,* 2010; Hassan *et al.,* 2011). The nature of biofilm structure and the physiological attributes of biofilm organisms confer resistance to antimicrobial agents such as antibiotics, disinfectants or germicides. Mechanisms responsible for resistance may be one or more of the following:

- Delay in penetration of the antimicrobial agent through the biofilm,
- Alteration in the growth rate of biofilm organisms, and
- Other physiological changes due to the biofilm mode of growth (Donlan, 2002).

**e) Fungal Infections**

*Candida glabrata* is monomorphic yeast that commonly colonizes the gastrointestinal and genitourinary tracts. Earlier it was considered a relatively non-pathogenic saprophyte of the normal flora of healthy individuals and was not readily associated with serious infections in human. Following the widespread and ever increasing use of immunosuppressive therapies and broad spectrum antibiotic treatment, the frequency of mucosal and systemic infections caused by *Candida glabrata* has increased significantly in hospitalized patients. After *Candida albicans*, *Candida glabrata* currently represents the second most cause of fungal infections of the bloodstream, oropharynx, urinary tract etc. *Candida glabrata* is able to attach and colonize host tissues as well as abiotic surfaces on expression of adhesins where it develops as multi-layered biofilm structures. Development of biofilm contributes to increased resistance to antifungal agents and results in persistent infections (Colombo *et al.,* 2014). *Candida albicans* has the ability to exist in sessile community form which provides tolerance to stress conditions (Ramage *et al.,* 2006). Surface induced gene expression initiates the formation of community growth (Kumamoto and Vinces, 2005). Formation of biofilms takes place due to adhesion of cells to tissue surfaces or solid surface which activates cellular events (Chandra *et al* 2001).Biofilms possess drug resistance which poses a serious threat to immune compromised patients (Jabra *et al.,* 2004; Aparna and Yadav, 2008; Shinde *et al.,* 2013).

Fig 17 gives the cumulative connotation of health hazards caused by the formation of biofilms.

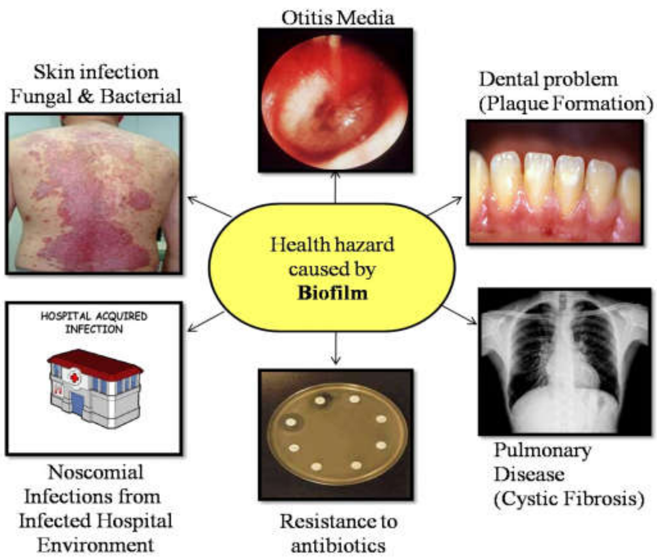


**Fig. 17. Biofilm Involved in Health Hazards**

**3) Plant diseases**

*Xylella fastidosa* (Xf) is a Gram negative phyto-pathogen capable of forming biofilms which are more sensitive to iron levels in the environment. It disrupts water and nutrient transport by blocking the xylem. It is the causative agent of Pierce's disease. It affects a variety of commercially important plants such as citrus, coffee and grapes (Toney and Koh, 2006).

**4) Generalized problems in industrial environment**

Growth of biofilm is observed in many industrial and domestic domains. In most of the cases the growth of biofilms has been detrimental. Many industries suffer the ill-effects of growth of biofilm which leads to heavy costs of cleaning and maintenance. Examples of such industries include the paper (Klahre and Flemming, 2000), opticians (Liesegang, 1997), dentistry (Marotta *et al.,* 2002) and hospitals (Halabi *et al.,* 2001) etc Domestic environment is the place where people are exposed most frequently to biofilms (Scott *et al.,* 1982; Barker and Bloomfield, 2002).Detrimental effects of biofilms include spoilage of products, reduction in efficiency of production, corrosion, unpleasant odours (malodours), unsightliness, infection, blockages of pipes, failure of equipments etc (Garrett *et al.,* 2008) (Fig 18). The oil industry encounters many problems resulting from formation of biofilm by sulphate-reducing bacteria (SRB). It might lead to pipe and rig corrosion, blockage of filtration equipment and oil spoilage.

Contamination by SRB can result from temperature-resistant organisms originating from hydrothermal vents (McCoy and Brown, 1998). The effects of oil spills can result in alterations in the microbial flora which affects growth and reproduction of fish and invertebrates (Voordouw *et al.,* 1996; Garrett *et al.,* 2008).

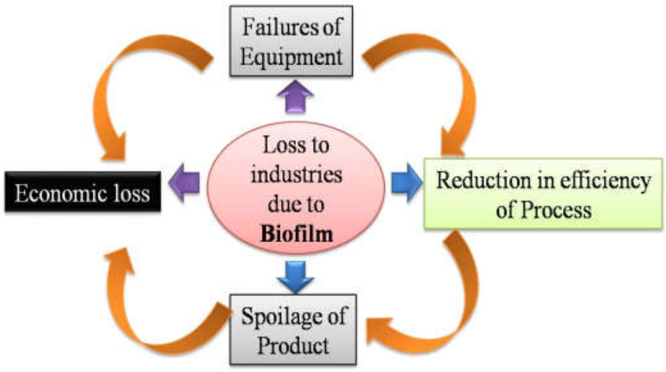


**Fig. 18. Hazardous Effect of Biofilm in Industry**

**Future Prospects**

Study of microbial biofilms in diverse fields is the latest arena of research, especially with molecular approach for elucidation of the genes specifically expressed by biofilm-associated organisms. Various control strategies are evaluated for either preventing or remediating biofilm colonization on medical devices, and development of new methods for assessing the efficacy of these treatments, (including medical devices treated with antimicrobial agents and antimicrobial locks). Role of biofilms in antimicrobial resistance, biofilms as a reservoir for pathogenic organisms and the role of biofilms in chronic diseases is the present focus. The universality of the biofilm phenotype has been accepted by the field of microbiology. Researchers in the fields of health sector, water treatment plants, food industries and environmental microbiology have begun to study microbiologic processes in terms of biofilm formation and its effects. Pharmaceutical and health-care industries have embraced this approach and new strategies formulation is in process. Complete understanding of what makes the biofilm phenotype so different from the planktonic phenotype will help to get an insight for avoiding (in infections) or using biofilms in various applications (Donlan, 2002). Recent research indicates that strain characteristics and growth period are important factors for formation of biofilm and contamination. Study of these strains with respect to genetic compositions and optimum growth conditions may help in understanding the formation and prevention of biofilm. Contamination and biofilm formation on food is a major problem and is responsible for spoilage of food which can be prevented by proper sanitary procedures to remove biofilms or prevent their formation. Policies, guidelines, standards and regulations should be developed which will help to find solutions to food safety problems in the world at large (Adetunji *et al.,* 2014).

## REFERENCES

Adetunji, V.O., Adedeji, A.O. and Kwaga, J., 2014. Assessment of the contamination potentials of some foodborne bacteria in biofilms for food products. *Asian Pacific journal of tropical medicine*, *7*, pp.S232-S237.

Alleman, J.E., Veil, J.A. and Canaday, J.T., 1982. Scanning electron microscope evaluation of rotating biological contactor biofilm. *Water research*,*16*(5), pp.543-550.

Anbazhagan, D., Mansor, M., Yan, G.O.S., Yusof, M.Y.M., Hassan, H. and Sekaran, S.D., 2012. Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of Acinetobacter spp. *PloS one*, *7*(7), p.e36696.

Aparna, M.S. and Yadav, S., 2008. Biofilms: microbes and disease. *Brazilian Journal of Infectious Diseases*, *12*(6), pp.526-530.

Atkinson, B. and Fowler, H.W., 1974. The significance of microbial film in fermenters. In *Advances in Biochemical Engineering, Volume 3* (pp. 221-277). Springer Berlin Heidelberg.

Avinash, T., Sandhya, T., Bhalerao, S., Uma, M., Salunkhe, G. and Sonali, R., 2014. Study of Microflora in Pulp Tissue of Carious Teeth Using Gram Stain. *Int. J. Curr. Microbiol. App. Sci*, *3*(9), pp.539-551.

Barkay, T. and Schaefer, J., 2001. Metal and radionuclide bioremediation: issues, considerations and potentials. *Current opinion in microbiology*, *4*(3), pp.318-323.

Barker, J. and Bloomfield, S.F., 2000. Survival of Salmonella in bathrooms and toilets in domestic homes following salmonellosis. *Journal of Applied Microbiology*, *89*(1), pp.137-144.

Begun, J., Gaiani, J.M., Rohde, H., Mack, D., Calderwood, S.B., Ausubel, F.M. and Sifri, C.D., 2007. Staphylococcal biofilm exopolysaccharide protects against *Caenorhabditis elegans* immune defenses. *PLoS Pathog*,*3*(4), p.e57.

Borkar, R.P., Gulhane, M.L. and Kotangale, A.J., 2013. Moving bed Biofilm reactor- a new perspective in wastewater treatment. *Environ. Sci. Toxicol. Food technol.,* 6(6), pp.15-21.

Breidt, F. and Fleming, H.P., 1998. Modeling of the Competitive Growth of *Listeria monocytogenes* and *Lactococcus lactis* in Vegetable Broth. *Applied and Environmental Microbiology*, *64*(9), pp.3159-3165.

Brown, A.J., Brown, G.D., Netea, M.G. and Gow, N.A., 2014. Metabolism impacts upon Candida immunogenicity and pathogenicity at multiple levels. *Trends in microbiology*, *22*(11), pp.614-622.

Caldwell, D.E., Atuku, E., Wilkie, D.C., Wivcharuk, K.P., Karthikeyan, S., Korber, D.R., Schmid, D.F. and Wolfaardt, G.M., 1997. Germ theory vs. community theory in understanding and controlling the proliferation of biofilms. *Advances in dental research*, *11*(1), pp.4-13.

Chandra, J., Kuhn, D.M., Mukherjee, P.K., Hoyer, L.L., McCormick, T. and Ghannoum, M.A., 2001. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *Journal of bacteriology*, *183*(18), pp.5385-5394.

Colomba, C., Trizzino, M., Imburgia, C., Madonia, S., Siracusa, L. and Giammanco, G.M., 2014. *Candida glabrata* meningitis and endocarditis: a late severe complication of candidemia. *International Journal of Infectious Diseases*, *29*, pp.174-175.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R. and Lappin-Scott, H.M., 1995. Microbial biofilms– Annu. *Rev. Microbio*, *49*, pp.711-745.

Costerton, J.W., Lewandowski, Z., DeBeer, D., Caldwell, D., Korber, D. and James, G., 1994. Biofilms, the customized microniche. *Journal of bacteriology*, *176*(8), p.2137.

Costerton, J.W., Stewart, P.S. and Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. *Science*, *284*(5418), pp.1318-1322.

Costerton, W., Veeh, R., Shirtliff, M., Pasmore, M., Post, C. and Ehrlich, G., 2003. The application of biofilm science to the study and control of chronic bacterial infections. *The Journal of clinical investigation*, *112*(10), pp.1466-1477.

Cowan, M.M., Taylor, K.G. and Doyle, R.J., 1987. Energetics of the initial phase of adhesion of *Streptococcus sanguis* to hydroxylapatite. *Journal of bacteriology*, *169*(7), pp.2995-3000.

Das, N., Geetanjali Basak, L.V. and SalamJ Abdul, A.M., 2012. Application of biofilms on remediation of pollutants–an overview. *J Microbiol Biotech Res*,*2*(5), pp.783-790.

Davey, M.E. and O'toole, G.A., 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiology and molecular biology reviews*, *64*(4), pp.847-867.

Distel, J.W., Hatton, J.F. and Gillespie, M.J., 2002. Biofilm formation in medicated root canals. *Journal of endodontics*, *28*(10), pp.689-693.

Donlan, R.M. and Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical microbiology reviews*, *15*(2), pp.167-193.

Donlan, R.M., 2002. Biofilms: microbial life on surfaces. *Emerg Infect Dis*,*8*(9).

Eighmy, T.T., Maratea, D. and Bishop, P.L., 1983. Electron microscopic examination of wastewater biofilm formation and structural components. *Applied and environmental microbiology*, *45*(6), pp.1921-1931.

extracellular and wall polysaccharides. *Annual Reviews in Microbiology*, *39*(1), pp.243-270.

Feigin, R. D., Kline, M. W., Hyatt, S. R. and Ford III, K. L., 1992. Otitis media. *Textbook of pediatric infectious diseases,, 1*, pp. 174-189.

Flemming, H.C., Wingender, J., Griegbe, T. and Mayer, C., 2000. Physico-chemical properties of biofilms. *Biofilms: recent advances in their study and control. Amsterdam: Harwood Academic Publishers*, pp.19-34.

Garrett, T.R., Bhakoo, M. and Zhang, Z., 2008. Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*, *18*(9), pp.1049-1056.

Gianfreda, L. and Rao, M.A., 2004. Potential of extra cellular enzymes in remediation of polluted soils: a review. *Enzyme and Microbial Technology*,*35*(4), pp.339-354.

Gillaspy, A.F., Hickmon, S.G., Skinner, R.A., Thomas, J.R., Nelson, C.L. and Smeltzer, M.S., 1995. Role of the accessory gene regulator (agr) in pathogenesis of Staphylococcal osteomyelitis. *Infection and immunity*, *63*(9), pp.3373-3380.

Gloag, E.S., Turnbull, L., Huang, A., Vallotton, P., Wang, H., Nolan, L.M., Mililli, L., Hunt, C., Lu, J., Osvath, S.R. and Monahan, L.G., 2013. Self-organization of bacterial biofilms is facilitated by extracellular DNA. *Proceedings of the National Academy of Sciences*, *110*(28), pp.11541-11546.

Gotz F., 2002. Staphylococcus and biofilms, MolMicrobiol. , 43:1367-78.

Halabi, M., Wiesholzer-Pittl, M., Schöberl, J. and Mittermayer, H., 2001. Non-touch fittings in hospitals: a possible source of *Pseudomonas aeruginosa* and *Legionella spp*. *Journal of hospital infection*, *49*(2), pp.117-121.

Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A. and Iqbal, M., 2011. Evaluation of different detection methods of biofilm formation in the clinical isolates. *Brazilian Journal of Infectious Diseases*, *15*(4), pp.305-311.

Hirsch, P., 1984. Microcolony formation and consortia. In *Microbial Adhesion and Aggregation* (pp. 373-393). Springer Berlin Heidelberg.

Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S. and Ciofu, O., 2010. Antibiotic resistance of bacterial biofilms. *International journal of antimicrobial agents*, *35*(4), pp.322-332.

Horn, H. and Lackner, S., 2014. Modeling of biofilm systems: a review. In *Productive Biofilms* (pp. 53-76). Springer International Publishing.

Hubble, T.S., Hatton, J.F., Nallapareddy, S.R., Murray, B.E. and Gillespie, M.J., 2003. Influence of *Enterococcus faecalis* proteases and the collagen-binding protein, Ace, on adhesion to dentin. *Oral microbiology and immunology*, *18*(2), pp.121-126.

Ikuma, K., Decho, A.W. and Lau, B.L., 2015. When nanoparticles meet biofilms—interactions guiding the environmental fate and accumulation of nanoparticles. *Frontiers in microbiology*, *6*.

Jabra-Rizk, M.A., Falkler, W.A. and Meiller, T.F., 2004. Fungal biofilms and drug resistance. *Emerging infectious diseases*, *10*(1), pp.14-19.

Jhajharia, K., Parolia, A., Shetty, K.V. and Mehta, L.K., 2015. Biofilm in endodontics: a review. *Journal of International Society of Preventive and Community Dentistry*, *5*(1), p.1.

Khan, S. and Majumder, D., 2016. Natural eco-friendly processes by omnipresent microorganism to reduce carbon footprint. *Journal of environmental Science, Computer Science and Engineering & Technology*, 5(2), pp. 267-288.

Khassehkhan, H. and Eberl, H.J., 2008. Modeling and simulation of a bacterial biofilm that is controlled by pH and protonated lactic acids. *Computational and Mathematical Methods in Medicine*, *9*(1), pp.47-67.

Kinner, N.E., Balkwill, D.L. and Bishop, P.L., 1983. Light and electron microscopic studies of microorganisms growing in rotating biological contactor biofilms. *Applied and environmental microbiology*, *45*(5), pp.1659-1669.

Kjelleberg, S. and Molin, S., 2002. Is there a role for quorum sensing signals in bacterial biofilms?. *Current opinion in microbiology*, *5*(3), pp.254-258.

Klahre, J. and Flemming, H.C., 2000. Monitoring of biofouling in papermill process waters. *Water Research*, *34*(14), pp.3657-3665.

Klapper, I., Rupp, C.J., Cargo, R., Purvedorj, B. and Stoodley, P., 2002. Viscoelastic fluid description of bacterial biofilm material properties. *Biotechnology and Bioengineering*, *80*(3), pp.289-296.

Kokare, C.R., Chakraborty, S., Khopade, A.N. and Mahadik, K.R., 2009. Biofilm: Importance and applications. *Indian Journal of Biotechnology*, *8*(2), pp.159-168.

Krishna Mohan, T.V., Nancharaiah, Y.V., Venugopalan, V.P., Narasimhan, S.V. and Satyasai, P.M., 2010. Development of mixed microbial granular biofilms for denitrification of concentrated wastes. In *International conference on Asian nuclear prospects 2010*.

Kumamoto, C.A. and Vinces, M.D., 2005. Alternative *Candida albicans* lifestyles: growth on surfaces. *Annu. Rev. Microbiol.*, *59*, pp.113-133.

Lasa, I. and Penadés, J.R., 2006. Bap: a family of surface proteins involved in biofilm formation. *Research in microbiology*, *157*(2), pp.99-107.

Lazarova, V. and Manem, J., 2000. Innovative biofilm treatment technologies for water and wastewater treatment. *ChemInform*, *31*(32).

Li, X., Xu, H., Chen, Z.S. and Chen, G., 2011. Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*, *2011*, p.8.

Liesegang, T.J., 1997. Contact lens-related microbial keratitis: Part II: Pathophysiology. *Cornea*, *16*(3), pp.265-273.

Lloyd, J.R., 2003. Microbial reduction of metals and radionuclides. *FEMS microbiology reviews*, *27*(2-3), pp.411-425.

MacLeod, F.A., Guiot, S.R. and Costerton, J.W., 1990. Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. *Applied and Environmental Microbiology*, *56*(6), pp.1598-1607.

Mah, T.F.C. and O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. *Trends in microbiology*, *9*(1), pp.34-39.

Maksimova, Y.G., 2014. Microbial biofilms in biotechnological processes. *Applied Biochemistry and Microbiology*, *50*(8), pp.750-760.

Marotta, M., Martino, A., De Rosa, A., Farina, E., Cartenı, M. and De Rosa, M., 2002. Degradation of dental plaque glucans and prevention of glucan formation using commercial enzymes. *Process Biochemistry*, *38*(1), pp.101-108.

Marsh, P.D., 2004. Dental plaque as a microbial biofilm. *Caries research*, *38*(3), pp.204-211.

McCann, M.T., Gilmore, B.F. and Gorman, S.P., 2008. *Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management. *Journal of Pharmacy and Pharmacology*, *60*(12), p.1551.

McCoy, D.L. and Brown, K.M., 1998. Hydrocarbon pollutants alter short-term recruitment in the barnacle *Balanus eburneus*. *Marine environmental research*, *45*(3), pp.209-224.

Molhoek, E.M., Van Dijk, A., Veldhuizen, E.J., Haagsman, H.P. and Bikker, F.J., 2011. A cathelicidin-2-derived peptide effectively impairs *Staphylococcus epidermidis* biofilms. *International journal of antimicrobial agents*, *37*(5), pp.476-479.

Molin, S. and Tolker-Nielsen, T., 2003. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Current opinion in biotechnology*, *14*(3), pp.255-261.

Moller, S., Korber, D.R., Wolfaardt, G.M., Molin, S. and Caldwell, D.E., 1997. Impact of nutrient composition on a degradative biofilm community. *Applied and environmental microbiology*, *63*(6), pp.2432-2438.

Morikawa, M., 2006. Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. *Journal of bioscience and bioengineering*, *101*(1), pp.1-8.

Nadell, C.D., Xavier, J.B. and Foster, K.R., 2009. The sociobiology of biofilms. *FEMS microbiology reviews*, *33*(1), pp.206-224.

Nivens, D.E., Ohman, D.E., Williams, J. and Franklin, M.J., 2001. Role of alginate and its O acetylation in formation of *Pseudomonas aeruginosa* microcolonies and biofilms. *Journal of bacteriology*, *183*(3), pp.1047-1057.

Noiri, Y., Ehara, A., Kawahara, T., Takemura, N. and Ebisu, S., 2002. Participation of bacterial biofilms in refractory and chronic periapical periodontitis. *Journal of endodontics*, *28*(10), pp.679-683.

Oliveira, P.S., Souza, S.G., Campos, G.B., da Silva, D.C., Sousa, D.S., Araújo, S.P., Ferreira, L.P., Santos, V.M., Amorim, A.T., Santos, A.M. and Timenetsky, J., 2014. Isolation, pathogenicity and disinfection of *Staphylococcus aureus* carried by insects in two public hospitals of Vitória da Conquista, Bahia, Brazil. *Brazilian Journal of Infectious Diseases*, *18*(2), pp.129-136.

Oliver, J.D., 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS microbiology reviews*, *34*(4), pp.415-425.

Olson, M.E., Ceri, H., Morck, D.W., Buret, A.G. and Read, R.R., 2002. Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Canadian Journal of Veterinary Research*, *66*(2), p.86.

Pantidos, N. and Horsfall, L.E., 2014. Biological synthesis of metallic nanoparticle by bacteria, fungi and plants. *Journal of Nanomedicine & Nanotechnology, 2014.*

Prakash, D., Gabani, P., Chandel, A.K., Ronen, Z. and Singh, O.V., 2013. Bioremediation: a genuine technology to remediate radionuclides from the environment. *Microbial biotechnology*, *6*(4), pp.349-360.

Ramage, G., Martínez, J.P. and López-Ribot, J.L., 2006. Candida biofilms on implanted biomaterials: a clinically significant problem. *FEMS yeast research*, *6*(7), pp.979-986.

Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T. and Hirsch, P.R., 2014. Measuring the soil-microbial interface: Extraction of extracellular polymeric substances (EPS) from soil biofilms. *Soil Biology and Biochemistry*, *72*, pp.163-171.

Richards, M.J., Edwards, J.R., Culver, D.H. and Gaynes, R.P., 1999. Nosocomial infections in pediatric intensive care units in the United States. *Pediatrics*, *103*(4), pp.e39-e39.

Robertson, S.A. and McLean, R.J., 2015. Beneficial biofilms. *AIMS Bioengin*, *2*(4), pp.437-448.

Rosche, B., Li, X.Z., Hauer, B., Schmid. and Buehler, K., 2009. Microbial biofilms: a concept for industrial catalysis. *Trends in biotechnology, 27(11),* pp.636-643.

Ryser, E.T. and Marth, E.H. eds., 2007. *Listeria listeriosis and food safety.* CRC Press.

Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and molecular biology reviews*, *61*(2), pp.262-280.

Scott, E., Bloomfield, S.F. and Barlow, C.G., 1982. An investigation of microbial contamination in the home. *Journal of Hygiene*, *89*(02), pp.279-293.

Seto, A., Saito, Y., Matsushige, M., Kobayashi, H., Sasaki, Y., Tonouchi, N., Tsuchida, T., Yoshinaga, F., Ueda, K. and Beppu, T., 2006. Effective cellulose production by a coculture of *Gluconacetobacter xylinus* and *Lactobacillus mali*. *Applied microbiology and biotechnology*, *73*(4), pp.915-921.

Shiflet, A.B. and Shiflet, G.W., 2010. Simulating the formation of biofilms in an undergraduate modeling course. *Procedia Computer Science*, *1*(1), pp.895-901.

Shinde, R.B., Raut, J.S., Chauhan, N.M. and Karuppayil, S.M., 2013. Chloroquine sensitizes biofilms of *Candida albicans* to antifungal azoles. *Brazilian Journal of Infectious Diseases*, *17*(4), pp.395-400.

Simoes, M., Simões, L.C. and Vieira, M.J., 2010. A review of current and emergent biofilm control strategies. *LWT-Food Science and Technology*, *43*(4), pp.573-583.

Sodano, W.L., Ron Grisanti, D.C. and DABCO, M., 2010. Functional Medicine University's Functional Diagnostic Medicine Training Program.

Stecchini, M.L., Aquili, V. and Sarais, I., 1995. Behavior of Listeria monocytogenes in Mozzarella cheese in presence of *Lactococcus lactis*. *International Journal of Food Microbiology*, *25*(3), pp.301-310.

Stoodley, P., Sauer, K., Davies, D.G. and Costerton, J.W., 2002. Biofilms as complex differentiated communities. *Annual Reviews in Microbiology*, *56*(1), pp.187-209.

Sutherland, I.W., 1985. Biosynthesis and composition of gram-negative bacterial

Todhanakasem, T., Narkmit, T., Areerat, K. and Thanonkeo, P., 2015. Fermentation of rice bran hydrolysate to ethanol using *Zymomonas mobilis* biofilm immobilization on DEAE-cellulose. *Electronic Journal of Biotechnology*, *18*(3), pp.196-201.

Tolker-Nielsen, T. and Molin, S., 2000. Spatial organization of microbial biofilm communities. *Microbial ecology*, *40*(2), pp.75-84.

Toney, J.H. and Koh, M.L., 2006. Inhibition of *Xylella fastidosa* biofilm formation via metal chelators. *Journal of the Association for Laboratory Automation, 11(1),* pp.30-32

Trachoo, N., 2003. Bio lms and the food industry. *Biofilms* 25(6), p808.

Tsuneda, S., Aikawa, H., Hayashi, H., Yuasa, A. and Hirata, A., 2003. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. *FEMS microbiology letters*, *223*(2), pp.287-292.

Van Houdt, R. and Michiels, C.W., 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of applied microbiology*, *109*(4), pp.1117-1131.

Vasudevan, R., 2014. Biofilms: microbial cities of scientific significance. *J Microbiol Exp*, *1*(3), p.00014.

Verran, J., 2002. Biofouling in food processing: biofilm or biotransfer potential? *Food and Bioproducts Processing*, *80*(4), pp.292-298.

Voordouw, G., Armstrong, S.M., Reimer, M.F., Fouts, B., Telang, A.J., Shen, Y. and Gevertz, D., 1996. Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. *Applied and Environmental Microbiology*, *62*(5), pp.1623-1629.

Watnick, P. and Kolter, R., 2000. Biofilm, city of microbes. *Journal of bacteriology*, *182*(10), pp.2675-2679.

Whitchurch, C.B., Tolker-Nielsen, T., Ragas, P.C. and Mattick, J.S., 2002. Extracellular DNA required for bacterial biofilm formation. *Science*, *295*(5559), pp.1487-1487.

Wingender, J., Neu, T.R. and Flemming, H.C., 1999. What are bacterial extracellular polymeric substances? In *Microbial extracellular polymeric substances* (pp. 1-19). Springer Berlin Heidelberg.

Wolfaardt, G.M., Korber, D.R., Karthikeyan, S. and Caldwell, D.E., 2000, January. Biodegradation by biofilm communities. In *SYMPOSIA-SOCIETY FOR GENERAL MICROBIOLOGY* (pp. 279-294). Cambridge; Cambridge University Press; 1999.

Wragg, J., Harrison, H., West, J.M. and Yoshikawa, H., 2012. Comparison of microbiological influences on the transport properties of intact mudstone and sandstone and its relevance to the geological disposal of radioactive waste. *Mineralogical Magazine*, *76*(8), pp.3251-3259.

Zonaro, E., Lampis, S., Turner, R.J., Qazi, S.J.S. and Vallini, G., 2015. Biogenic selenium and tellurium nanoparticles synthesized by environmental microbial isolates efficaciously inhibit bacterial planktonic cultures and biofilms. *Frontiers in microbiology*, *6*.

Zylstra, G.J. and Kukor, J.J., 2005. What is environmental biotechnology? *Current Opinion in Biotechnology*, *16*(3), pp.243-245.

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