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Trail of Biofilm Research: As Microbial Tool for Ecological and Industrial Application

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ABSTRACT: Biofilms are consortium of microbes of different origin embedded in extra polymeric matrix, which is composed of carbohydrates, extracellular DNA and secreted proteins. A biofilm may be of a single species microbe or a combination of different species and/or groups including bacteria, virus and fungus. Bacterial biofilm formation relies on bacterial cells, substrates, surrounding media and its formation is a complex process involving reversible attachment followed by irreversible attachment phase, Extra Polymeric Substance (EPS) production, biofilm maturation phase and a final detachment phase. Biofilms are found and formed in aquatic environments well rather than other terrestrial or xerophytic conditions, where the ecosystem supports microbial growth. Biofilm in nature have both beneficial and detrimental effects of which, negative effects in health care, drinking water distribution systems, food and marine industries etc. are highlighted and studied well, which resulted studies on inhibition and control of biofilms. Despite the harmful effects, biofilms serve beneficial roles in a variety of fields including bioremediation, waste water treatment, corrosion inhibition, heavy metal remediation and so on. This review elaborates the positive and negative aspects of biofilms of bacterial origin in various fields and highlights the need to encourage the formation of beneficial bacterial biofilms.

Keywords: Biofilms, Bioremediation, Extra Polymeric Matrix, Bacterial consortium.

INTRODUCTION

The principle of unity is strength is the universal law practiced by living organisms to tackle the emerging environmental constraints. Our nature is changing day by day, so to resist these changes or to acclimatize with these changing environmental conditions is a major problem faced by the flora and fauna of ecosystem. Microbial communities develop in both organic (leaves and wood) and inorganic (sand, gravel, cobbles, rocks) stream benthic substrata. Microorganisms either in natural or artificial habitat exhibit two modes of life- a planktonic free livinsg form and an attached form termed as biofilms, based on the physiological and physiochemical conditions prevailing in their surroundings. Biofilm formation appears to be an adaptable attribute of microbes that provides them with better options compared to their planktonic cells. In this mode of life they show greater access to nutritional resources and greater environmental stability (Dang and Lovell 2016). Moreover, this age-old survival mechanism provides them with stronger ability to grow in oligotrophic environments, improved survival to biocides (Fleming *et al*., 2016) and enhanced organism productivsity and interactions (Roder *et al*., 2018). Dental plaques, surfaces of slippery stones and pebbles in a stream, slimy coatings in showers or on boat hulls, surface of infected wounds or the mass clogging water distribution pipes are examples of biofilms that may be encountered in one's everyday life.

Bill Costerton has been regarded as the founding father of the field of biofilms which is the study of microorganisms attached to surfaces. The term biofilm can be defined as a consortium of microbes (bacteria, algae, fungi, and protozoa) embedded in extra polymeric substances (EPS) matrix which in turn is composed of carbohydrates, proteins and DNA. Depending upon the prevalence of different microbial groups in the substratum, the community structure and function of the biofilm also varies (Romani and Sabater 2002). According to Flemming and Wuertz (2019) about 40-80% of bacterial cells on earth can form biofilms and their formation is detrimental in several situations. For example biofilms persist on medical device surfaces as well as on patient's tissue leading to persistent infections as suggested by Percival *et al*. (2015). Moreover, in food industries biofilm formation by pathogenic bacteria causes food spoilage and endangering consumer's health (Galie *et al*., 2018). In the view of negative aspects of biofilms on human health, nowadays researchers have been focusing on the prevention and inhibition of harmful biofilms. Although biofilms are attributed with a variety of positive and negative impacts in the field of microbiology, their applications for beneficial purposes increases globally. For example biofilms serves a key role in bioremediation treatment of hazardous pollutants (Irankhah *et al*., 2019) and for the waste water treatment (Ali *et al*., 2018). The formation of bacterial biofilms is important in agricultural and industrial settings and are also used as biocontrol agents and biofertilizers (Timmusk *et al*., 2017). Despite of these beneficial aspects the understanding of the harmful side of biofilms has been far better for decades (Fig. 1).

In order to provide a comprehensive understanding of bacterial biofilms, this review describes the events involved in the bacterial biofilm formation, emphasizes the negative and positive aspects associated with bacterial biofilms, throws light on the main strategies currently used to regulate the establishment of harmful bacterial biofilms as well as certain strategies employed to encourage the formation of beneficial bacterial biofilms and highlights their future perspectives.

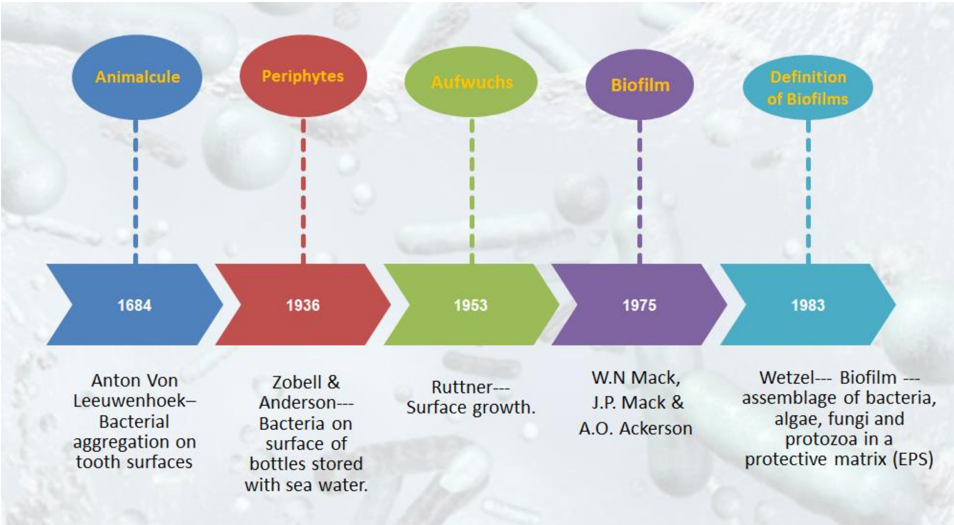

Fig. 1. History of biofilms.

Process of formation of biofilms

Bacteria exhibit the biofilm mode of life in response to severe environmental stresses such as desiccation, UV radiation, nutrient limitation, extreme temperature and pH, high salinity and antimicrobial agents. Generally, the formation of bacterial biofilms which is a multi-step process relies on the interaction between the bacterial cells, their substrates and surrounding media (Van Houdt and Michielis, 2010). The basic steps involved in the formation of a biofilm are reversible attachment followed by irreversible attachment aided by adhesive structures of bacteria, EPS (Extra Polymeric Substances) production, maturation of biofilm and dispersal/detachment (Toyofuku *et al*., 2016) and the regulation mechanism of these various phases varies with bacterial species.

Ecological perspective of biofilms

Environmental biofilms actively participate in organic matter decomposition, nutrient dynamics and biogeochemical cycling which in turn facilitates the smooth functioning of ecosystems (Battin *et al*., 2007, Sabater *et al*., 2002) (Fig. 2).

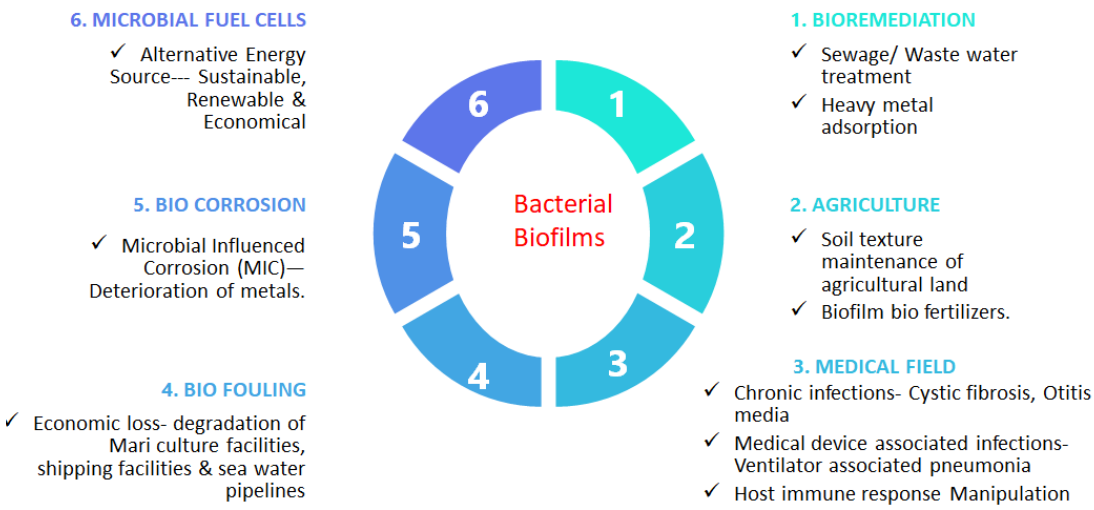

Fig. 2. Role played by biofilms.

Moreover, the attributes of biofilms such as short generation time, rapid response to changing environmental conditions, species richness, and stress recovery had made them effective indicators against a wide range of disturbances (Lowe and Pan, 1996). Numerous ecological studies have focused on biofilm mode of existence of microbes and their role on nutrient recycling in aquatic bodies and as sources of nutrients to the higher trophic levels in the food web. Fungi in biofilms are key factors in decomposition and dissolved organic matter production in aquatic bodies. Meanwhile bacteria play the role of decomposers in benthic sediments and overlying water.

Biofilms in Agriculture

According to Turhan *et al*. (2019) the role played by biofilm in the field of agriculture is due to their fermentation capabilities and also their antimicrobial and biochemical characteristics. With these qualities they improve plant nutrient availability and uptake by their recyclability and also involve in water contents and soil texture maintenance of agricultural land. It has been reported that some bacterial species including Potassium Solubilizing bacteria (KSB) and phosphate solubilizing bacteria (PSB) enhanced soil nutrient levels significantly. The biofilms improve the uptake rate of potassium indirectly by promoting the growth of plants and directly by enhancing potassium solubilisation in the soil which in turn improves crop production (Han and Lee, 2005). The use of chemical fertilizers for profitable crop production and food security adversely affects the soil microbial population and leads to deterioration of soil health by residue left over. Biofertilizers being ecofriendly microbial amendments (Bacteria, Fungi, Algae) ensures sustainability in recharging soil nutrients without any disruption of soil-micro biome interaction will be the most appropriate solution applicable in this scenario. Biofilms which are complex communities of multiple microbial species which are attached to the surfaces or physical interfaces in nature can also be developed in vitro using beneficial microbes which in turn can be used as Biofilm Bio fertilizers (BFBFs). They can repair the bulk network of soil-plant microbe parameters in agro-ecosystems degraded due to excessive use of chemical fertilizers. Sudadi *et al*. (2018) showed that biofilm bio fertilizers are the next generation bioweapons showing better performance in combating both biotic and abiotic challenges as well as increasing the crop yields. According to Donlan (2002), biofilms enhances nutrient cycling and availability, bio control of pests and diseases and improves soil fertility as well as productivity which equips them to become an effective bio fertilizer. As suggested by Ansari *et al*. (2012), the preparation of biofilm fertilizer using suitable microorganisms requires certain mechanisms to provide resistance to biocide agents which in turn are provided by EPS (Extra Polymeric substances) that have barrier properties against UV light and antibiotics. According to Muhsin *et al*. (2015), there are four basic steps involved in the preparation of a biofilm bio fertilizer.

1. Bacterial attachment to a surface and subsequent growth of microorganisms.
2. Multiplication of planktonic bacterial cells within the matrix which leads to the formation of a micro colony.
3. Formation of 3D structure and transcription of specific genes.
4. Detachment of biofilm cells by Quorum Sensing.

The bacterial strain selected for biofilm bio fertilizer production should have the ability to easily adhere on a surface of any substrate, soil particles and plant roots. Moreover the bacterial strains should possess antibiotic resistance properties. *Pseudomonas putida* and *Bacillus amyloliquefaciens* biofilms in chick pea (host plant) enhances the plant growth promoting attributes. The interaction of bacterial strain with diverse fungal structures like spores and hyphae can also be maintained in the colonized plant root as biofilm. These associations can reinstate microbe mediated networks for enhanced cycling of nutrients and their availability to crop growth while improving crop productivity and soil fertility (Seneviratne and Jayasinghearachchi, 2005). For example *Pseudomonas* sp. interaction with *Rhizophagus irregularis* for phosphorus solubilization and *Bacillus thuringiensis* interacts with *Gigaspora margarita* for ethylene production and growth inhibitor for fungal pathogens (Pandi *et al*., 2020). Biofilms not only act as bio fertilizers but as agents to reduce biotic stress in the environment. According to Seneviratne *et al*., 2008, BFBFs facilitates biological nitrogen fixation in non-legumes (eg. rice) while solubilizing phosphorus and other nutrients required for crop growth through beneficial interactions between microbes and the soil.

Biofilms in Bioremediation

Bioremediation process relies on the enzymatic activity of microbes converting toxic environmental waste to less toxic or harmless products such as water and carbon dioxide (Das and Dash, 2014). Generally the process of bioremediation involves a transfer of electrons from donors to acceptors through aerobic or anaerobic counter parts. According to Joutey *et al*. (2015), in contaminated sites several redox reactions utilize trace elements and a change in oxidation number is associated with the toxicity and solubility of metals. For example heavy metals sulphates are converted to sulphide forms thereby facilitating their immobilization and removal from contaminated sites (Beyenal *et al*., 2004). The ability of Extra Polymeric Substances (EPS) to capture harmful cations from the bulk medium made biofilm to become an efficient tool for bioremediation. The binding capacity of EPS can be further improvised using synthetic biology and genetically engineered bacteria that may increase the natural chelating ability of the environment. For example a cadmium chelating and arsenic chelating bacteria that absorb cadmium and arsenic from water was constructed by Bae *et al*. (2000). Recent efforts of bioremediation of persistent organic pollutants showed that biofilm microbial communities have significantly increased the removal rate from contaminated environments. This approach provides carbon and energy sources to the microbes as well as a support system to which they can adsorb, while transforming the contaminants (Petrie *et al*., 2003). In return, the biofilm community converts the organic pollutants to harmless materials. Although using microbial communities to enhance POPs bioremediation is a promising strategy, the lack of specific biodegradative pathways that are specific for the degradation of different POPs might limit the complete mineralization of these compounds. For example it was found in a study that *Geobacter* was useful for bioremediation of metals, but a lack of Fe as the electron acceptor slowed the process. As suggested by Van Dillewijn *et al*. (2009) microorganisms living in biofilms, display greater tolerance to contaminants, higher chance of survival and adaptation as well as stronger ability to decompose different pollutants through catabolic pathways, when compared with their planktonic counterparts. There is an increasing demand in the use of bacterial biofilm mediated remediation for the removal of different kinds of environmental pollutants such as oil spills, explosives, pesticides, pharmaceutical products, contaminated soil and ground water (Edwards and Kjellerup, 2013). The common biofilm forming bacteria that are involved in bioremediation include Pseudomonas, Dehalococcides, Arthrobacter, Bacillus, Alcanivorax, Cycloclasticus, Burkholderia and Rhodococcus (Dasgupta *et al*., 2013 and Yoshikawa *et al*., 2017).

Biofilms in heavy metal adsorption

The contamination of freshwaters with toxic heavy metals has become a matter of concern all the time. The ecological balance of the recipient environment and a diversity of aquatic organisms have been adversely affected by heavy metal contamination. The sources of metals that contaminate the fresh water system results from a variety of different processes such as weathering of soils and rocks, volcanic eruptions and also from certain human activities such as mining, smelting and agricultural activities. Research has revealed that microorganisms associated with biofilms binds metals on their surfaces and produce metal binding extracellular polymers. Accordingly the application of these microorganisms in the removal of heavy metals from waste water has been effective and widely recommended.

Heavy metals uptake by these microbial biomass is a new eco-compatible and economically feasible application that has been developed to remove heavy metals from waste water and studies have shown that interaction of microbial substances with heavy metals reduced heavy metal ion concentrations in solution. This bioremediation option is based on the high metal binding capacity of biological agents, which remove heavy metals from waste water or contaminated sites with high efficiency. Biofilms can decompose or transform hazardous into less toxic metabolites or degrade them to nontoxic end products. They can also survive in contaminated habitats because they are metabolically able to exploit contaminants as potential energy sources. In biological treatment or removal of heavy metals, microorganisms with biological activity such as algae, bacteria, fungi and yeast can be used in their naturally occurring forms.

Based on their experiments on metal accumulation in algal biofilm in lotic streams Meylen *et al*. (2006) and Ogbuagu *et al*. (2011) observed that biofilms are efficient model for removal of metals in solution. Bio sorption has become one of the alternative treatment technologies to remove heavy metals from aqueous solutions. The observation of very high concentration of the trace metals in biofilms than in water column by Doering and Uehlinger (2006) confirms that biofilms are good candidates for bioaccumulation studies. The statistically significant difference between metal concentrations in the biofilms and water columns reinforces the bio concentration and bio magnification potentials of the candidate bio accumulators (Meylan *et al*., 2006) against background water columns.

Many Studies have demonstrated that natural biofilms are important substances that affect the behaviour of trace metals in water (Dong *et al*., 2000; Duong *et al*., 2010; Hua *et al*., 2013). The presence of natural biofilms affects the migration and distribution of contaminants significantly through the mechanisms of adsorption-desorption, accumulation and degradation, etc. (Dong *et al*., 2003; Hua *et al*., 2012b; Meylan *et al*., 2004).

Biofilms in medicine

In medical field the effects of biofilms are seen primarily in 4 ways by facilitating the emergence of antimicrobial drug resistance, generating chronic infections, the modulation of host immune response, and the contamination of medical devices. Biofilm is a critical problem in the medical sector since it is formed on medical implants within human tissue and involved in a multitude of serious chronic infections. Due to the unique ability of tolerance to antibiotics and immune system, biofilms can develop in all medical inserts such as intrauterine tubes, cardiac valves, catheters as suggested by Auler *et al.* (2010). For example *Staphylococcus aureus* and *Staphylococcus epidermis* are commonly associated with biofilms formed on medical devices that cause health care associated infections (Von Eiff *et al*., 2005). As per the studies of Amin (2009) and Bauer *et al*. (2002), there is a rapid growth of biofilms (within 24 h) on endotracheal tubes (ETTs) which is the major cause of Ventilator-associated pneumonia. Multidrug Resistant bacterium (MRSA) and gram negative bacilli such as *K. pneumonia, E. coli, P. aeruginosa* are the bacteria commonly involved in the formation of biofilms on ETTs as suggested by Ramirez *et al*., 2007. According to Donlan (2002), several diseases such as cystic fibrosis, native valve endocarditis, otitis media, periodontitis and chronic prostatitis were caused by biofilm associated microorganisms. Moreover these biofilms harbor pathogens like *Helicobacter pylori, Legionella pneumophila*, and non-tuberculous mycobacteria in potable water systems. Microorganisms within a biofilm are encased within a matrix of extracellular polymeric substances that can act as a barrier and recalcitrant for different hostile conditions such as sanitizers, antibiotics, and other hygienic conditions. The other critical issue with biofilm formation is their antibiotic resistance which makes medication difficult, and they use different physical, physiological, and gene-related factors to develop their resistance mechanisms. In order to mitigate their production and develop controlling methods, it is better to understand growth requirements and mechanisms.

Biofilms in food industry

Food and food processing surface becomes an ideal environment for biofilm formation where there are sufficient nutrients for microbial growth and attachment. Therefore, biofilm formation on these surfaces, especially on food processing surface becomes a challenge in food safety and human health. Generally, they persist and exist in food processing environments where they become a source of cross-contamination and foodborne diseases. According to Han *et al*., 2017 about 60% of food borne disease out breaks are caused by biofilms. One of the most common biofilm forming food borne pathogen is *Lysteria monocytogens* that can cause abortion in pregnant women and complication in immunocompromised individuals (Galie *et al*., 2018). The other examples of biofilm forming food borne pathogens that cause serious illness include Salmonella species, Clostridium Perfringes and *Campylobacter jejuni* (Wirtanen and Salo, 2016), *Pseudomonas* spp.(Rajmohan *et al*., 2002), *Vibrio parahaemolyticus* (Yeung and Boor, 2004) *Bacillus* sp. (Galie *et al*., 2018), *Shewanella putrefacians* (Bagge *et al.*, 2001) and *Geobacillus searothermophilus* (Burgess *et al*., 2017). Biofilms are also responsible for serious technical challenges of food industry such as increasing the fluid frictional resistance at the surfaces, promoting the corrosion rate of surfaces which inturn leads to production efficiency (Chmielewski and Frank, 2003; Meesilp and Mesil, 2019). In short within a food industry biofilms serves the risk of direct contamination of food as well as the risk of contamination of instruments and equipments which can cause serious public health risk to consumers and economic consequences.

Biofilms in human health

In humans, biofilms seems to play a significant role in the persistence and transmission of various diseases. As suggested by Donlan (2002) the reasons for this may be due to the exchange of resistance plasmids between bacterial cells within a biofilm through horizontal gene transfer, reduced susceptibility of bacterial cells to antibiotics which is provided by the EPS and detachment of individual cells from biofilm that may result in the blood stream or urinary tract infections. According to Ramage *et al*. (2006) and Douglas, (2003) about 65% of the hospital infections in humans are of biofilm origin and are difficult to eradicate as majority of our present antimicrobials target the planktonic phase of the bacteria. In a biofilm mode of life, the EPS (Extra Polymeric Substance) prevent the antibacterial agents from reaching the microbe. According to Akyildiz *et al*. (2013) and Masters *et al*. (2019) biofilm forming bacteria contribute a lot of life threatening infections and disease in humans such as cystic fibrosis, otitis media, periodontitis, endocarditis, chronic wounds and osteomyelitis. Moreover, in health care setting biofilms have shown to develop on medical device surfaces such as catheters, prosthetic heart valves, pacemakers, breast implants, contact lenses and cerebrospinal fluid shunts and dead tissues (Alav *et al*., 2018). Both Gram positive and Gram negative bacteria may attach to and develop biofilms on the surfaces of these devices but the most frequently reported biofilm forming bacteria are *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Hall-Stoodley *et al*., 2004; Shokouhfard *et al*., 2015 and Pakharukova *et al*., 2018). In addition to the medical dice surfaces bacterial biofilms (for example *P. aeruginosa*) are also shown to develop on the inner surfaces of metal pipes in hospital water distribution systems (Loveday*et al*., 2014).

Biofilms in Bio-fouling

Biofouling or biological fouling is the accumulation of microorganisms, plants, algae, or small animals where it is not wanted on surfaces such as ship and submarine hulls, devices such as water inlets, pipework, grates, ponds, and rivers that cause degradation to the primary purpose of that item. Such accumulation is referred to as epibiosis when the host surface is another organism and the relationship is not parasitic. Since bio fouling can occur almost anywhere water is present, bio fouling poses risks to a wide variety of objects such as boat hulls and equipment, medical devices and membranes, as well as to entire industries, such as paper manufacturing, food processing, underwater construction, and desalination plants. According to Hopkins and Forrest, 2010 and Schultz *et al*. (2011) biofouling has been a major challenge in the naval industry and for civilian oceangoing ships. Bacteria are among the early microorganisms to settle and colonize substrates in the marine environment and may subsequently facilitate attachment and colonization of larger fouling organisms such as algae, mussels and barnacles which leads to marine biofilm biofouling. Such accumulation of biofoulers by biofilms on ship hulls can increase the hydrodynamic drag of the ships which causes challenges for shipping industry including speed reduction, increased cleaning time and greater fuel consumption (Demirel *et al*., 2017). Anti-fouling is the ability of specifically designed materials (such as toxic biocide paints, or non-toxic paints) to remove or prevent bio fouling, the area in which researchers are focusing on nowadays.

Biofilms in Bio-corrosion

Investigations on the role of biofilms in corrosion of metals and their alloys started in the late 1970's (Geesey *et al*., 2020; Beech *et al*., 2005) but a substantial microbial influenced corrosion (MIC) theory was proposed by Hamilton (2003). MIC has become the subject of numerous studies for the past decades due to their economic and environmental importance. The term 'Microbially Influenced Corrosion or biocorrosion' refers to the accelerated deterioration of metals due to the presence of biofilms on their surfaces. The electro active sessile cells in biofilms can accept electrons from the metals and the interaction of microorganisms in the form of biofilms with the metallic surfaces results in the MIC and bioleaching of the materials. The EPS (Extra Polymeric Substances) of biofilms which is termed as 'dark matter' by Thomas Neu plays a vital role in the biodeterioration and bioleaching of materials and various minerals (Ma *et al*., 2020). Since bacteria are regarded as the primary colonizers of both natural and man- made surfaces, majority of the MIC investigations resulted in the impact of pure and mixed culture bacterial biofilms on corrosion of copper, iron, aluminium and other alloys.

Sulphate Reducing Bacteria (SRB), Sulphur Oxidising Bacteria, Manganese-Oxidising Bacteria are the main types of bacteria that are associated with corrosion of metals (Beech and Coutinho, 2003). Of these SRBs are of interest to many microbiologists as they are efficient in anaerobic degradation of many organic pollutants. SRBs use sulphate as electron acceptor and are found in biofilms that results in bio corrosion on the surfaces of waste water pipelines, ship hulls and heat exchangers. MIC by electro active microbes occurs as part of an extracellular electron transfer (EET) (Lie *et al*., 2018) and there are still research gaps in the field of MIC (Little *et al*., 2020).

Biofilms in Microbial Fuel Cells

The ability of microorganisms to generate electrical power through extracellular electron transfer by converting the energy present in organic compounds has been shown since 1900's, but the Microbial Fuel Cells (MFC) has gathered attention only recently. The applications of MFCs in various fields include waste water treatment (Yakar *et al*., 2018), bioremediation (Rosenbaum and Franks, 2014) biosensors (EIMekawy *et al*., 2018) desalination (Zhang *et al*., 2018) as an alternative energy source in remote areas (Castro *et al*., 2014). In the recent years, the ever growing population, their increasing energy demand along with the inadequate supply of fossil fuels has become one of the biggest threat to human survival and economy (Panwar *et al*., 2011). This scenario demanded the researchers to explore alternative energy sources which are sustainable, renewable and economical. These explorations came with the idea of colonizing on electrodes with biofilms serving as Microbial Fuel cells (Aelterman *et al*., 2006; Li *et al*., 2013; Chaturvedi and Verma, 2016). The principle behind is that the microbes employed in MFC convert the chemical energy present in the organic compounds to electrical energy through catalysts (Chaudhuri and Lovely, 2003). Microbial Fuel Cells are generally made of a cathode, an anode, a PEM (Proton Exchange Membrane) and a resistor through which the electrons travel to the anode. Generally the anode is entrapped with the bacterial consortium (Gouveia *et al*., 2014) or organic material (Zhao *et al*., 2005) where oxidation occurs. The cathode is provided with desired source microbe. After oxidation at the anode protons pass through the PEM to the cathode where they get reduced into water (He *et al*., 2014).

Biofilms in Waste water treatment

Water contamination caused by industrialization and urbanization has become a major public concern. Waste water is composed of a broad range of organic and inorganic contaminants originating from agriculture, industry, domestic and commercial sewage (Naidoo and Olaniran, 2013). Biofilm systems have been successfully practiced for the treatment of waste water besides the conventional primary, secondary and tertiary treatment. The use of biofilm systems serves a number of removal mechanisms such as bioaccumulation, bio sorption, bio mineralization and biological degradation (Singh *et al*., 2006). The process attempts to improve the waste water treatment either by increasing the diversity or activity through direct introduction of selected naturally occurring or genetically altered microorganisms to the system (Stephenson and Stephenson, 1992). Studies showed the efficient removal of heavy metals and organic solvents from the waste water by biofilm matrix components through the process of bio sorption (Spath *et al*., 1998; Guibaud *et al*., 2006). Specific bacterial strain have been used to improve the performance of waste water treatment which is termed as bio augmentation in which the bacterial communities neutralize and degrade organic and inorganic compounds in wastewater through the use of biofilm-based water treatment technology. The basic nutrients present in wastewater are mostly nitrogen and phosphorus (Yamashita and Yamamoto-Ikemoto, 2014), hence among the bacterial species used in waste water treatment are often denitrifying species or those capable of neutralizing phosphorus (Zielinska *et al*., 2016).

Biofilms in bioreactor

Biofilm bioreactors are reactors that use immobilized micro-organisms on solid support for various purposes. Biofilm bioreactors have been commercially used for treating industrial waste water for over two decades (Qureshi *et al*., 2005). According to Boon *et al*. (2002), biofilm reactors have been used for biochemical conversion and the sorption of pollutants, particularly heavy metals and hydrocarbons from municipal and industrial waste water. The features like enhanced metabolic activity, increased flow rates, large mass transfer areas and optimum physicochemical control made the biofilm bioreactors more advantageous over conventional treatment of waste water. Researchers use different types of biofilm reactors for the production of value added products or for waste water treatment. Stirred tank reactor, Packed bed reactor, pulsed plate reactor, spouted bed reactor, airlift reactor, rotating disc reactor, membrane biofilm reactor etc. are the various types of biofilm reactors. Selection of a proper reactor system is important to reflect the microbial traits and the properties of biomass support particles. The effect of biofilm thickness on biofilm density and substrate consumption rate in a differential fluidized bed biofilm reactor was studied by Sekar *et al*., (1995) which showed that the consumption rate increased parabolically with biofilm thickness up to some critical value. A wide variety of reactors have been developed by scientists that exploited biofilm process for waste water treatment (Van Loosdrecht *et al*., 1993) and observed that the reactors are suitable for large dilute streams where the productivity is enhanced by using large surface area of biofilms instead of biomass concentration. The formation, growth and biofilm characteristics of *Anthrobacteroxydans* on different kinds of polymer matrices (copolymer of acrylonitrile with acrylamide dissolved in DMF, polymethyl methaacrylate (PMMA), mixture of copolymer of acrylonitrile with acrylamide and cellulose acetate butyrate) and quantity of EPS production have been investigated by (Yotova *et al*., 2009) and found that the best polymer matrices that can be used for biofilm formation is PAN (Polyacrylonitrile) with PAA (Polyacrylamide).

CONCLUSIONS

Biofilm represents the predominant life style adopted by bacteria in most of the natural as well as manmade environment. Biofilm investigations have vast potential in terms of its formation, adhesion, detachment and characteristic features. Its phenomenal use and adaptability has great scope in biotechnological as well as environmental studies. There is a lot of scope to study the characteristics of biofilms in different application mentioned in the review and also to compare their characteristics. Moreover, the review provides a clear understanding of the complexity of biofilms and the harmful as well as beneficial aspects of biofilms that are ubiquitous in nature. Therapies existing now will not be effective for controlling diseases due to the emergence of highly resistant strains and also they are targeting the planktonic phase of bacteria. So the future course of action would be directed to find novel and effective treatments that target the biofilm mode of life of bacteria. For this we should have a better understanding of the genes and proteins that are differentially expressed under biofilm and planktonic growth conditions. Moreover, beneficial biofilm formation can be encouraged in many industrial and environmental areas through modifications.

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