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Review Article

AN INSIGHT INTO BIOFILM ECOLOGY AND ITS APPLIED ASPECTS

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ABSTRACT

Microbes are omnipresent in nature with different forms of shape, size, colony morphology and substrate interaction modes. Microbial biofilm is a unique assemblage of microbes on the surface of the substrate. Recently it has fascinated the scientific community owing to its special properties which makes it notorious as well as a worthy living system. The establishment of the biofilm ecosystem is a multi step process which involves quorum sensing communication mechanism. The interaction of the biofilm with its immediate environment, its applications and some of the strategies to remove the notorious biofilm have been described in this article.

Keywords*:* Quorum sensing, Petroleum recovery, Biofuel cells, Biological control, Medical transplants

INTRODUCTION

Some of the interesting phenomena of nature occur at the surface. Biofilm formation is one such phenomena which take place at the surface of the substrate. Group of microbial cells attached to a solid substrate form a biofilm [1]. These microbes produce an organic polymeric matrix in which they get embedded [2]. The first observation of biofilm can be traced back to the 17th century. In 1667, Antonie van Leeuwenhoek observed the microbes scraped from the human tooth surface under his microscope and described them as 'animalcules' [1] but the mechanism of biofilm process was not developed until 1978 [3].

Most microbial communities persists on the surface and often composed of multiple species that interact with each other and their environment [4]. Microbial biofilm can be considered as the association of a biologically active matrix of cells and extracellular substances with the surface[5].According to Dalton HM and March PE, about 99% of the world's population of bacteria occur in the form of a biofilm [6]. Microbes in biofilm differ from their free-living counterparts in their ability to show coordinated behavior due to communication by quorum sensing and produce an extracellular polymeric matrix containing polysaccharides, proteins and DNAs which help them attach firmly to the surface [7-10]. Biofilms are polyelectrolyte in nature and can absorb significant amount of silt, clay, heavy metals or other substances from their immediate environment [11]. Biofilms are crucial in a number of biotechnological applications; e.g. in the treatment of wastewater, for oil recovery in petroleum industries and as seeding source in batch bio-hydrogen production [12]. There is another aspect of microbial biofilm. Some of the microbial biofilm cause serious and costly disturbances, as in paper machines [13], on biomaterials, marine constructions [14] interferes with the signals of computer chip [15], water system [16] and they even cause several diseases [17].

PROCESS OF BIOFILM FORMATION

Biofilm can be considered to occur as the result of adhesive force between the microbes and substrate and cohesive force between the microbes [18]**.** It has been described as a three stage process by Kokare et al. [19]**: -** (1) adsorption of microbe on collector surface; (2) the consolidation of the interface between the microbes and collector. It often involves the formation of polymeric link between the organism and collector; (3) colonization and division of organisms on the collector's surface. However this description of biofilm formation mechanism is limited when considering the intimate processes of cell–substrate or cell–cell interaction [18].

The foundation layer

The foundation layer or conditioning layer consists of organic or inorganic materials which surrounds the substrate and modifies it to favor microbial accessibility to the substrate [18]. In fact, it provides the base for the microbial growth on the substrate. This conditioning process is regulated by factors like the nature of the fabricating materials, surface tension, electrophoretic mobility, roughness and wettability of the surfaces [11]. In 1971, it was suggested that bacterial sorption to surfaces involves an initial reversible sorption step, followed by slower surface dependent sorption processes leading to irreversible adsorption and could be explained in terms of the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloid stability [20].

Reversible adhesion of microbes:-

Initially the microbes are reversibly attached to the conditioning layer. This attachment process is driven either by physical forces [21] or by microbial appendages (flagella) [18]. At this stage Brownian motion can be observed in the microbes attached to the layer [21]. This attachment of microbes is attributed to surface charge on microbes, steric interaction, weak Van der Waals and electrostatic forces between the interacting molecules [11]. If the activation energy of absorption remains greater than desorption; microbes detaches from the surface [18]. This adhesion process can also be explained in terms of the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloid stability. It describes the interaction between a cell and a flat surface as a balance between the Van der Waals forces of attraction and repulsive force of interactions from the overlap between the electrical double layer of the cell and the substratum [17].

Irreversible adhesion of the microbes:-

With the passage of time, a number of reversibly attached microbes become immobilized and get permanently attached to the surface. This immobilization is the result of formation of a tangled and channelled network of exo-polysaccharides (EPS) produced by the microbes. The EPS network serves many functions, such as protection from sudden environmental shocks, absorbing nutrient from the medium, means of intercellular communication within the biofilm, short-term energy reservoir and as an enhancer of intercellular transfer of genetic material [21]. The microbial appendages overcome the physical repulsive forces of the electrical double layer [22]; allowing the microbes to interact with the matrix of the substrate.

Cell division to increase the microbial population:-

The microbes attached to the substrate starts dividing as they get the proper nutrients and favorable condition. Nutrients from the substrate are utilized for rapid growth of the microbes. Stronger bond between the cells develop as the result of interaction between excretion of polysaccharide intercellular adhesion (PIA) polymers and the divalent cations [23].

Quorum sensing and colonization of new substrate

Once the cells reach stationary phase; the rate of cell division equals the rate of cell death. At this stage the concentration of cell is highest. At high cell concentrations, a series of cell signalling mechanisms are employed by the biofilm, and this is collectively termed quorum sensing [24]. According to Steven T. Rutherford and Bonnie L. Bassler [25]; Quorum sensing is a process of cell–cell communication that allows bacteria to share information about cell density and adjust gene expression accordingly. This process enables bacteria to express energetically expensive processes as a collective only when the impact of those processes on the environment or on a host will be maximized. Followed by the stationary phase; death phase comes. This is marked by the breakdown of polysaccharides holding the biofilm together, in response to enzymes produced by the microbial community itself. The biofilm breaks releasing surface bacteria for colonization of fresh substrates. Simultaneously, the operons coding for microbial appendages are up regulated so that the organisms have the apparatus for motility, and the genes coding for a number of porins are down-regulated, thus completing a genetic cycle for biofilm adhesion and cohesion [18].

APPLICATIONS

Their unique physical, biological and chemical properties make them a very useful tool for environmental cleanliness, industrial purpose and for processes favored at the surface.

Waste water treatment

Biofilm reactors like Up flow Sludge Blanket, Biofilm Fluidized Bed, Expanded Granular Sludge Blanket, Biofilm Airlift Suspension and Internal Circulation reactors have been successfully used to treat municipal and industrial wastewater [26]. The property of biofilm to adsorb material on its surface is utilized in waste water treatment. Adsorbent property of biofilm is because of the presence of different functional groups secreted by microbes living in it [27]. It adsorbs heavy metals from waste water [28]. Trickling filters have been in use in wastewater treatment processes for over a century. The microbial biofilm in the trickling filter removes the pollutants from the water [29]. The research shows that the efficiency of the biofilm electrode in waste water treatment depends on the method of biofilm preparation. Further, COD removal also depends on the thickness of biofilm [30]. Another use of biofilm in wastewater treatment is in Rotating Biological Contactor (RBC) [31]. Biofilm is formed on a rotating cylindrical disc which is half submerged in water and removes organic matter because of biofilm formation on it. The immobilization of microbes on beads improves the degradation of organic matter [31]. Use of biofilm in waste water treatment removes organic matter and at same time hydrogen can be produced which is a clean energy [32].

In biofuel cell

For the last decade, extensive research is being carried out to understand and optimize the biofuel cell. In biofuel cell, electricity is produced via oxidation of fuel at anode and reduction of any reducible substance at the cathode. Biofilm is developed on anode and microbes present in it have the capability to use a vast variety of substances as fuel. Research shows that use of artificial redox mediator molecules such as osmium complexes [33, 34] and 2,2-azinobis (3-ethylbenzothiazoline) -6-sulfonic acid [35, 36] optimize the current produced in biofuel cell. Biofuel cells can be used as implants in living tissue which works under physiological condition [37, 38].

Biofuel cells are promising as power sources for long-term underwater or littoral distributed autonomous sensing (DAS) networks because of their self-sustainability [39]. But till now production of electricity for commercial use is not possible because of low efficiency of biofuel cell.

In petroleum industry

In biofilm, the microbes are sandwiched between impermeable layers of biomolecules produced by them [40]. The biofilm can be used to reduce the permeability of sediments. The biofilm is used to deliberately plug the oil reservoir to enhance the oil recovery [41]. It prevents the mixing of injection water to the oil production area. The biofilm can also be used to deliberately plug the pore channels between oil spills and water reservoirs [42].

Bioleaching

Bioleaching is an extraction of metal from ore using living organisms. In this, the microbial biofilm is allowed to grow on ore heap. Indigenously present microbes are often used for this purpose. Microbes present in the biofilm oxidize the ore; forming metal oxides [43]. The liquor obtained is sent for further purification. Bioleaching is mostly used in copper mines and getting attention of industries because of its eco-friendly nature and economic value [44].

As plant growth promoter or biological control

Microbes living in rhizosphere regions of root have been found to benefit the host plant. Bacteria like *Pseudomonas putida* [45], *Azospirillu brasilense* [46] and related species living in the root ecosystem; promotes the growth of their host plants. They grow as biofilms in and around the rhizosphere. Most plant–bacterial associations rely upon the physical interaction between bacteria and plant tissues [47].

Bacterial biofilm have been reported to act a biocontrol agent against the pathogenic microbes. It has been experimentally found that, the wild species of *B.subtilis* strain effectively removes the pathogenic *P.syringae* infecting the roots of Arabidopsis [48] and *B.subtilis* acts against *Ralstonia solanacearum* which causes wilting in tomatoes [49].

Some other applications of biofilm include biofilm reactors in various processes e.g. fermentation, production of enzymes, production of primary and secondary metabolites, production of antibiotics, have been reviewed by [50-52]. The unused potential of biofilm based processes and technologies for several industrial, medical and environmental processes is yet to be harnessed.

HARMFUL EFFECT OF BIOFILMS

Microbial biofilm is a problematic microbial association. It interrupts several industrial processes and badly affect living systems.

Plugging and corrosion of the water supply pipes

The formation of the microbial biofilms on wet surfaces of the water supply pipes and tubes increases the fluid frictional resistance, reduces the cross sectional area for flow, enhances the roughness of the surface and viscoelasticity of the fluids [11]. Due to reduction of sulfur to sulfur dioxide by anaerobic, and oxidation of metals by an aerobic microbial population of biofilms; plugging and corrosion of the water supply pipes takes place respectively [15].

Water contamination

Microbes form biofilm on the surface of water pipes and contaminate the water. Legionella, a pathogenic group of gram negative bacteria form biofilm in a model warm water system with pipes of copper, stainless steel and cross linked polyethylene [53]. Coliform bacteria can grow as a biofilm on rubber coated valves of the water pipelines and contaminate the water [54]. Water treatment lines in the hospital can hold a large number of bacteria due to the sloughing off of bacteria from the biofilm formed on the surface of the treatment pipes [55].

Airway biofilm disease

Pseudomonas aeruginosa help in bacterial biofilm formation which often can be observed on airway surfaces of patients with diffuse panbronchiolities, cystic fibrosis and bronchiectasis [56]. It forms a large mass on the affected portion of the airway surfaces and in this way it becomes resistant to attack by antimicrobial agents [57], and antibiotics are unable to penetrate into surrounding alginate layer and then bacteria present inside the biofilm do not come in contact with drugs [56].

Malfunctioning of computers

EPS part of the biofilms are polyelectrolyte in nature which makes them an electron sink at the cathode and serves as conductor for interrupting the electronic signals of the computer chips leads to malfunctioning of computer [15].

Dental problem

Organic acid produced by bacteria of dental Biofilm while fermenting carbohydrates from human diets, causes caries, the result of a chronic undermining demineralization of teeth [58]. Mostly mutants of streptococci are found to be primary etiologic agents for caries. Caries can be observed mostly in children and adults more than 55 years [59]. Dental plaque formation on teeth is also caused by Biofilm in healthy as well as diseased mouth [60].

Human microbial infection

Biofilm causes a significant amount of all human microbial infections. Microbes in the biofilm are more resistant to antibiotics than their free living counterparts [23]. All medical devices or tissue engineering constructs are susceptible to microbial colonization and infection [61, 62]. These infections are can lead to failure of medical transplants and even death of the patient. In chronic wounds bacterial biofilm are formed on the wound surface and in tissues in the wound [63]. Aggregation of bacterial biomass interferes with the defense system and weaken the wound healing process [64].

Plant microbial infection

Pathogenic microbial biofilm have been reported to infect the phyllosphere and vasculature of plants. Pierce's disease of grapes and citrus fruits, has been attributed to the ability to *Xylella fastidiosa* to form extensive biofilms, which blocks the plant's vasculature [65].

Some of the human pathogens have been reported to infect the plants through biofilm formation. For instance, *Pseudomonas aeruginosa* PA14, a human pathogen has been recognized to be a potent pathogen of plant; infecting the leaves [66]. This infection is promoted several factors including the ability of the microbes to form biofilm [67].

REMOVAL OF BIOFILM

Biofilm is a serious threat to public health and industrial process. It can be removed by physical, biological and chemical processes.

Sterile scrapping

Generally, Biofilm formation occurs at the polycarbonate coupons of annular reactor which can be removed by scrapping with sterile tools. Firstly, coupans are separated aseptically from the reactor and then dipped into de-ionized water followed by scrapping the biofilm five to ten times using a sterile utility knife [68].

Enzymatic removal of biofilm

Many fungal and bacterial enzymes are capable of removing the biofilm. These enzymes have been proven to be effective for the degradation of the EPS (Extracellular Polymeric Substance) of the biofilm [69-71]. Removal of biofilm involve the destruction of physical integrity of the EPS [72] through weakening the framework components; i.e., protein, carbohydrate and lipid. Bacterial proteases (everlase, savinase) have been found to be very effective in removing biofilm. Alphamylase is also capable of removing the biofilm [73]. In industries, the bacterial Biofilm is removed by applying fungal enzymes. For instance; fungal enzymes obtained from three fungal strains namely *Aspergillus niger, Trichoderma viride* and Penicillium species can be used to detach the Biofilm developed by *Pseudomonas fluorescens* [74, 75].

Viruses infecting bacteria may provide a natural, highly specific, non-toxic, feasible approach for controlling several microbes involved in biofilm formation [76]. But no such practical system have been designed so far for biofilm removal on large scale. Another bioregulatory method to control biofilm may include the use of microbes producing Siderophores. Siderophores is an iron chelating compound secreted by some microbes. It has strong affinity for iron. It chelates with iron making it unavailable to the microbes in the biofilm. It has been found that some iron binding proteins are able to hinder the growth of microbes like *E. coli* and *S. typhimurium* [77]. This approach of biofilm removal is bacteria specific. Further the bacteriostatic effect of these iron binding proteins is reversible; as with increase in iron availability bacteria resumes its growth [78].

Using a sterile vial

Biofilm formation can be observed on several medical devices like urinary catchers, central venous catchers, contact lens and dental syringes. Teflon and medical grade PVC tubing are also noticed with Escherichia coli Biofilm formation. These Biofilm can be removed by placing Biofilm segments into a sterile vial (containing detergent) for a certain time and at a specific temperature. Cleaning detergent used for cleaning endoscope may be with enzyme or without enzyme [79, 80].

Anti-plaque agents

Frequent biofilm formation occurs in our mouth which results in dental plaque [81]. Several anti-plaque agents like surfactants, essential oils and antimicrobial agents (metal ions, phenols, quaternary ammonium compounds, bisbiguanides) are being included in mouthwash and toothpastes to inhibit the dental plaque Biofilm formation. These agents are able to remove the Biofilm completely and can kill the bacteria associated with the disease [82].

Chemical treatment

Chemical treatments are available to remove the biofilm. Treatment formulations containing NaCl and CaCl2, two chelating agents (EDTA and Dequest 2006), surfactants (SDS, Tween 20, and Triton X-100), a pH increase, lysozyme, hypochlorite, mono chloramine and concentrated urea, can remove more than 25% of biomass (as total protein) and treatments containing the control, MgCl2, sucrose, nutrient upshifts and downshifts and a pH decrease, can remove less than 25% of biomass [83].

It has been reported that both free and chitosan coated extracts of Azadirachta indica, Vitex negundu, Tridax procumbens and Ocimum tenuiflorumi inhibits the biofilm formation [84]. Microbes in the biofilm are resistant to drugs and antibiotics; therefore synthesis, characterization and pharmacological testing for new chemicals which can destroy the harmful biofilms has became the major area of microbiological research [85].

CONCLUSION

The special aggregation mechanism makes the microbes in the biofilm more notorious and capable. The reason behind the special properties of microbial biofilm which makes it different from its free living counterpart needs to be more deeply investigated. Detail study will pave the way to prevent various diseases and ease the treatment process. Further it will also help in finding solutions to the problem caused by microbial biofilm in the industries .

At the same time, the importance of biofilm cannot be neglected. It has successfully been used in some of the industrial processes like water treatment, bioleaching and in petroleum recovery. The microbial biofilm fuel cell is one of the novel applications of biofilm. Further research work is needed to exploit the benefits on commercial scale from the microbial biofilms. The vast unused potential of biofilm is still waiting to be explored.

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