Aspects of Microbial Biofilms in Water Treatment

Riddha Dey and Richa Raghuwanshi

Abstract

Global water crisis created due to increasing population and its demands needs to ascertain proper water management practices. Biofilm, which is a conis the fundamental unit of atgregation of microbial cells that are irreversibly attached to the surface and confined within the matrix of polysaccharide material, plays a major role in water treatment and reuse technology. A biofilm structure has a specific architecture that consists of microbial cells and extracellular polymeric substance provided with an ideal environment for the exchange of genetic material between cells. The growth and attachment process depend on the growth medium, substratum, and the cell surface. Biofilms are the main component of membrane bioreactors. The microorganisms present in the biofilms participate actively in contaminant bioremediation and degrade the organic contaminants of the polluted water. The planktonic-biofilm transition is a highly regulated and complex process that depends on the phenotypic characteristics of the bacteria and environmental factors. The microorganisms that help in biofilm formation have specific regulatory genes and communicate through quorum sensing which in turn can initiate certain biofilm processes such as detachment. Recently, studies have identified the genes and regulatory circuits which are involved in initial cell surface interactions, biofilm maturation, detachment, and the conversion of biofilm microbial cells into planktonic mode of growth. This chapter explores the ways of formation and architecture of biofilms and deals with the ecology of the surface microbes, their growth control mechanisms, and their role in water reclamation system.

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1 Introduction

Modern civilizations have witnessed the process of rapid industrialization and urbanization which have not only affected the individual lifestyle and society but have also modulated the environment. Unlike the positive drive it has on the economical and social frames, the impact of both industrialization and urbanization has adverse effect on nature and its precious resources especially the air and water. Since water has guided the growth of various epical civilizations, its importance in structuring a stable society can never be overlooked. However, the discharge of toxic chemicals and untreated sewages from the rapidly growing industries and cities has contaminated a large portion of usable water, affecting the water quality and human heath at the same time. Contaminated water generally consists of industrial, agricultural, and domestic effluents which may be comprised of detergents, fats, oils, pesticides, and trace metals like lead, cadmium, mercury, nickel, zinc, etc., making it unfit for drinking. The lack of proper water treatment plants has also contributed to increased toxicity of water bodies to a large extent. According to the UNESCO (2017), over 80% of the effluents released worldwide in the water bodies are without sufficient treatment. Moreover, the WHO has quoted that 40% of the deaths and 30% of all the diseases have been resulted due to contaminated water (Kantawanichkul et al. 2009), causing it as a global concerned.

Deterioration of life and health quality due to polluted water and regular assessment of progressive contamination of water bodies by various researchers suggest the need of focusing toward a necessary cure for the existing situation. Treatment of contaminated water bodies in an efficient way to remove toxins has gained attention. The process of water purification starting with the filtration was appreciated due to its cost-effectiveness and practicability. However, the use of chemical filters or the contemporary physical methods had many drawbacks. The use of biological knowledge for wastewater treatment was introduced in the year 1893 in England as trickling filters (Metcalf and Eddy Inc 1991). The concept of utilizing microbial population for the metabolic degradation of organic wastes and chemicals and removal of trace metals from the effluents has gradually evolved and is being commercialized. The pioneering works in this area guided the development of biofilters and the successful application of biofilms for filtering both polluted air and water at industrial level.

Initially, the biofilters were developed on slag or rock as filter media; however, with the latest advancement, an array of filter media are purposefully used for obtaining requisite biomass preeminent toward metabolic degradation of pollutants. Progress in the knowledge of biofilms and their effective utilization using array of support media leads to the development of plastic-based media (bundled plastic transition to expanded polystyrene units) into various fixed film filtration units in the mid-twentieth century (Antonie and Aacken 1971; Antonie 1976). Subsequently, the advanced biofiltration unit was developed with high treatment efficacy by the purposeful implication of support media. Further, hybrid (suspended and fixed film system together) and upgraded fixed film systems were also introduced for better water treatment (Canler and Perret 1994). However, in biofiltration units, the optimum operational parameter, which includes pH, temperature, moisture, aeration, etc., should be properly maintained for better results. Govind (2009) percolated that biofiltration can effectively remove as high as 5000 ppmv concentration of contaminants if provided with structural and operational optima.

Utilization of biofilms for filtration has many advantages over the physical methods which include cost-effectiveness and filtration efficacy due to metabolic breakdown of biodegradable pollutants along with environmental benefits. In nature, microorganisms preferably form consortia for proper utilization of available nutrients and combat stress. This natural process gradually results in the form of biofilm which may consist of microbes belonging to different trophic levels. In a biofilm, complex organic contaminants can be utilized sequentially by various classes of microbes ultimately resulting into increased biomass and subsequent biodegradation of contaminants. Therefore, the utilization of biofilms in the membrane reactors and filtration units for the removal of pollutants has great scope. Today, modern biofilter technology is playing a paramount role over the quintessential physical methods for water reclamation and reuse processes. Nevertheless, the increasing demand of water due to rapid rise in the global population and following depletion of freshwater sources is urging for more efficient biofilters to increase the mass reuse of available water and prevention of water pollution.

2 Wastewater Treatment

Wastewater treatment is a process through which the contaminants present in the effluent are removed and converted into reusable form with minimum impact on the environment. Wastewater treatment can be broadly divided into three categories: physical treatment, chemical treatment, and biological treatment.

Physical Water Treatment: In this process, solid wastes are removed without involving any use of chemicals. Processes like screening, sedimentation, and skimming are used in which the insoluble heavy particles settle down at the bottom and the pure water is separated. Other effective methods are aeration (providing air through the water to provide oxygen) and filtration (wastewater is passed through the filters to separate the contaminants and insoluble particles). Sand filters are mostly used in this process. The major disadvantage is that not all the contaminants can be removed by using physical treatments. The particles having smaller size cannot be removed.

Chemical Water Treatment: This process involves the use of chemicals for water treatment. Chlorine is widely used for water treatment. Ozone is an excellent disinfectant and oxidizing agent and is used in water purification. Neutralization technique is also used where acid or bases are added to neutralize the pH of the water. Chemical treatments can check the growth of microorganisms, but its prolonged use can cause water hardness and make it turbid changing its taste and odor.

Biological Water Treatment: Biological treatments make use of microorganisms to break down organic wastes using normal cellular processes. Among the available treatment processes, application of biological processes is gradually gaining pace as it involves no chemical use and is ecofriendly, cost-effective, and efficient in lower level of contamination. It is mainly of two types: suspended and fixed film growth systems. Suspended growth systems have some definite disadvantages like washout and low biomass concentration (Metcalf and Eddy 2003). Fixed film systems have many advantages like:

1. They are closer to natural biofilm systems.
2. Prevent washout of biomass.
3. Increased mean cell retention time.
4. Enhanced biomass loading per unit reactor volume.
5. Easy solid and liquid separation.
6. Surface biodegradation facilitates providing resistance to shock loadings.
7. Higher biodegradation rates.
8. Higher active biomass.
9. Enhanced rates of genetic transfer resulting in stable gene pool.
10. Extensive microbial diversity.
11. Greater efficiency to degrade recalcitrant (Bisop et al. 1995).

The concept of biofilms was followed for the development of biofilters which are the most recent and promising technique possessing higher efficiency and better performance for biological wastewater treatment (Cohen 2001).

3 Biofiltration

Biofiltration system involves removal of pollutants through biological degradation unlike normal filtration technique where physical straining is used (Chaudhary et al. 2003). This breakdown of pollutants is carried out by microorganisms fixed to a porous medium. The microorganisms including (anaerobic, aerobic, and facultative) protozoa, bacteria, algae, and fungi suspended beside the medium particles gradually develop a slime layer known as biofilm over the surface of the filter media. To ensure large surface area for attachment and increase in nutrient supply, the filter bed medium consists of relatively inert substances. The potential of a biofilter depends on the properties and characteristics of the support medium including degree of compaction, porosity, ability to host microbial populations, and water retention capabilities. The parameters that determine the performance and successful operation of biofilters include the medium pH, microbial inoculation, moisture, temperature, and nutrient content (Devinny et al. 1999). Over the last few decades, fixed film systems like trickling filters and rotating biological contractors have been used for

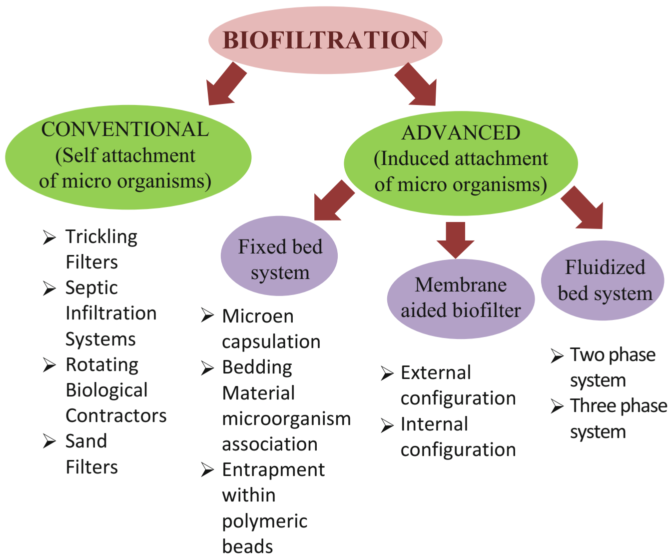

Fig. 1 Types of biofiltration processes

wastewater treatment (Antonie 1974; Pederson 1982; Hinton and Stensel 1994; Parker and Bratby 2001). The various abatement techniques related to biofiltration is illustrated in Fig. 1. As the performance of biofilter depends on the microbial activity, so availability of constant source of substrates is important to maintain and control a healthy biomass present on the surface of the filter.

4 Performance Parameters of Biofilters

The parameters that play a crucial role on the performance of a biofilter are briefly discussed below:

1. Physical factors
   (a) Filter media
   Filter media or packing is the fundamental unit of attached growth wastewater treating technology. It provides a surface on which the microbial growth takes place and forms a biofilm layer. The source and concentration of pollutants are the basis of selection of biofilter media which makes it crucial for efficient functioning of biofilters. Porosity, size, density, and resistance to erosion and chemicals are also important parameters for selection. The biofilter media should provide suitable and larger surface area for quick biomass growth and good quality surface texture to withstand shear and sloughing of biomass. The filter medium should be insoluble, durable, and resistant to chemicals (Christensson and Welander 2004). The hydrodynamic

conditions of the support material are affected by the geometry of the reactor, i.e., surface area and texture, affecting biofilm formation causing inappropriate wastewater treatment (Yu et al. 2008; Matos et al. 2011). A medium that has high void fraction, resistance to clogging, large passage diameter, high specific surface area, good mechanical strength, inert material of construction, flexibility in shape, wettability for better growth of biofilm, light attenuation, and ease of maintenance and is lightweight and cost-effective is considered as a good filter medium. Various researchers have reported the use of different synthetic and natural materials like polypropylene (Khatoon et al. 2014), polystyrene (Naz et al. 2013), and pebbles (Naz et al. 2015; Khan et al. 2015) as efficient media. For primary wastewater treatment, blast furnace slag, granite, and synthetic media can be used as filter media. The selection of filter media largely depends on the volume of wastewater. Moreover, for the tertiary wastewater treatment, granular activated carbon (GAC), filter coal, anthracite, and sand can be used. The use of GAC for the removal of organic substances from tertiary wastewater is better than using anthracite or sand as they are non-adsorptive media (Le Chevallier et al. 1992; Wang et al. 1995a, b). The biodegradable components are retained and adsorbed by GAC, leading to continuous bioregeneration additionally providing protection from shear loss of biomass. Certain spectroscopic techniques like X-ray photoelectron spectroscopy and energy-dispersive X-ray spectroscopy are utilized for the detection and quantification of the elemental composition of the filter medium.

(b) Filter backwash

Filter backwashing is a very crucial step for the maintenance of the biofilm, and the backwashing technique should be selected appropriately, or else it could damage the biomass attached to the surface (Ahmad et al. 1998; Bouwer and Crowe 1988; Bablon et al. 1988; Graese et al. 1987; Miltner et al. 1995). The hydrophobic biological particles are attached to filter media with greater force compared to that of nonbiological clay particle (Ahmad and Amirtharajah 1998). Backwashing in granular activated carbon biofilter showed no significant loss in vertical biomass profile (Servais et al. 1991).

(c) Empty bed contact time

The empty bed contact time (EBCT) is the main operating parameter of a biofilter. The concept dimensionless contact time incorporating EBCT was given by Zhang and Huck (1996). With the increase in contact time escalates the organic substance removal until it reaches its optimum value. The hydraulic loading and filter depth can be amended to enhance the EBCT. In a rapid filtration unit, organic removal is not affected by hydraulic loading for a given EBCT (Carlson and Amy 1995).

(d) Temperature

The effect of temperature on the bacterial activity is yet another parameter for biofiltration. The activities of bacterial community adapted at 10 °C and 20 °C but can increase with the rise in temperature by 10–30 °C.

2. Biological factors
   The formation of microbial biofilm is the fundamental principle of biofiltration technique. So it is required to understand the mechanism of the biological processes involved in the formation of biofilms. Microorganisms play a crucial role in removing anthropogenic contaminants from wastewater. The knowledge of composition of the microbial consortia, which form the biofilm, is preferably significant not only to construct highly efficient engineered biofilms but also to understand the basic mechanism of decomposition performed by them. Although microbial consortia are responsible for the biofilm formation, the methanogens are the key players in waste removal. The microbes recovered from anaerobic bioreactors mostly belong to the Euryarchaeota, Crenarchaeota, *Korarchaeota*, and Thaumarchaeota (Riviere et al. 2009; Leclerc et al. 2004; Collins et al. 2006; Dang et al. 2013). Along with Crenarchaeota, other potential ammonia-oxidizing organisms, including *Nitrosopumilus maritimus*, *Candidatus*, and *Nitrososphaera viennensis*, were also isolated from biofilms (Konneke et al. 2005; Tourna et al. 2011). Methanogenic archaea, dominantly consist of Methanobacteriaceae, Methanosarcinaceae, and Methanosaetaceae, get attached to the packing support materials carrying out methanogenesis efficiently (Zhang et al. 2011; Buzzini et al. 2006; Del Nery et al. 2008). Moreover, Leclerc et al. (2004) reported the consortia of *Methanobacterium* spp. and *Methanosaeta concilii* in the biofilm layer. However, in the stirred tanks and fixed film digesters, *Methanosarcina frisus* is the prevalent species, yet, in anaerobic sludge bed, *Methanosaeta* spp. is mostly found. Visser et al. (1991) revealed that even after a rise in temperature from 38 °C to 55 °C, several subpopulations including *Methanobrevibacter smithii*, *Methanospirillum hungatei*, *Methanobrevibacter arboriphilus*, *Methanobacterium thermoautotrophicum*, *Methanogenium cariaci*, and *Methanosarcina thermophila* continue to carry out their activity proficiently.

5 Microbial Biofilms

A biofilm is defined as an accumulation of microbial cells that are attached irreversibly with a surface enclosed in a matrix consisting primarily of polysaccharide. Biofilm matrix may also contain noncellular materials according to the environment in which the biofilm has developed. The water system biofilm contains clay material, corrosion products, filamentous bacteria, and freshwater diatoms making it more complex. There are multiple biofilm growth modes. They may grow in flat sheets, discontinuous patches, cluster shape, or columnar form. The mechanism of biofilm formation is illustrated in Fig. 2.

There are three main biological processes that can occur in a biofilter:

1. Attachment of microorganisms.
2. Growth of microorganisms.
3. Decay and detachment of microorganisms.

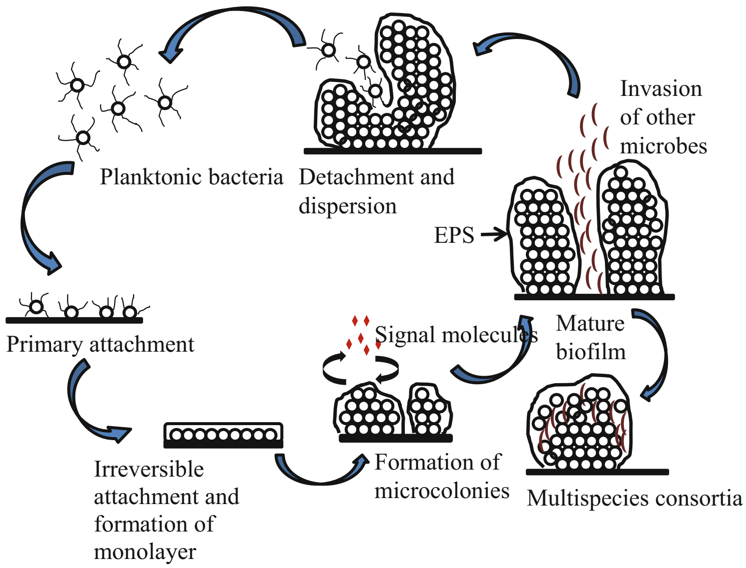

Fig. 2 Mechanism of biofilm formation

5.1 Attachment of Microorganisms

Attachment is a process in which the microorganisms attach and colonize over the filter media surface through transportation, initial adhesion, firm attachment, and colonization (Van Loosdrecht et al. 1990). The microorganisms then get transported toward the filter media surface further involving four processes: diffusion, convection, sedimentation due to gravity, and active mobility of the microorganisms. Depending upon the total interaction energy (sum of van der Waals forces and electrostatic force), the initial attachment can be reversible or irreversible. Irreversible adhesion of microorganisms occurs through production of extracellular polymeric substances. The polyhydroxyl groups in extracellular polymeric substances form hydrogen bonding through which bacterial colonization takes place (Kjelleberg et al. 2007). The process of adhesion of the microorganisms on the filter media surface stands by the DLVO (Derjaguin-Landau-Verwey-Overbeek) theory. The influent characteristics and surface properties of the filter media decide the processes of firm attachment and colonization. The method of surface attachment of microorganisms is greatly influenced by hydrophobicity of the microorganisms, steric effects, contact angle, and electrophoretic mobility values.

5.2 Growth of Microorganisms

Following the process of microbial attachment on the filter media surface, the formation of monolayer microcolonies takes place. The bulk and surface transport phenomenon governs the supply of organic substrate. Once the substrate is transported to the biofilms from the bulk liquid, it gradually diffuses into the biofilms initiating the process of metabolism. Transportation of mass substrate to the biofilm, rate of diffusion of the substrate into the biofilm, and kinetics of its utilization within the biofilm decide the rate of substrate utilization. A three-dimensional arrangement is formed by the attachment of the biofilms to debris from the adjacent environment. Growth yield of the substrate also influences the performance of a biofilm process. Invasion of new planktonic bacteria also takes place and forms multispecies consortia.

5.3 Detachment of Biomass

Detachment is a critical phenomenon where matrix-encased and sessile microbial cells are converted to free planktonic microorganisms that affect the maintenance of biomass. Some of the important detachment mechanisms are erosion of biomass (due to the fluid shear), abrasion of biomass (scraping the biocell off the surface by collision of external particle), sloughing (detachment of large patches of biomass), predation of protozoa (detachment of biomass on the outer surface of the biofilm), and filter backwashing. During backwashing, the biomass is affected by expansion of backwash bed, mode of backwash like filter effluent, chlorinated water, and air scour (Chaudhary et al. 2001). There is no loss of effective biomass during normal filter backwash (Ahmad and Amirtharajah 1998). However, it has been also reported that biomass loss occurs due to shear stress and is stimulated by cell-to-cell signaling mechanism through quorum sensing (Webb 2007).

6 Factors Promoting Biofilm Formation

The factors that promote the process of biofilm formation are as follows:

(a) Nutrients, pH, and temperature

Nutrient condition is the major factor that plays a pivotal role in microbial growth in the biofilms. It ranges from enriched media to non-detectable one. The conversion of microbial cells from planktonic to biofilm state needs dense nutrient-rich environment, whereas the depletion of nutrients promotes biofilm cell detachment from surfaces. The microbial community obtains nutrients through various prominent ways like utilization of waste products from secondary colonizers, accumulating trace organic materials through extracellular polymeric substances, and biochemical resource pool (Sehar and Naz 2016).

Alteration in pH affects the microbial growth and development. The bacteria present in the media modify the synthesis and activity of proteins associated with different cellular processes. Although the formation of polysaccharides and excretion of exopolymeric substances do not respond to pH variations, the optimum pH for the production of polysaccharide is around 7 (Oliveira et al. 1994).

Temperature is yet another factor which is very sensitive for microbial growth. A healthy growth of microbes needs optimum temperature, or else it will lead to reduction in bacterial enzyme reaction rates which will result in loss of bacterial growth efficiency. The optimum temperature for bacterial growth found in cooling water systems is about 40 °C (Ells and Hansen 2006).

(b) Surface topography

Bacterial adhesion to the surface is greatly governed by surface topography. The aqueous media with higher flow rate and rough surface reduce the shear force on microbial cells.

In the initial steps of attachment, a limited surface roughness enhances the adhesion of microbes by providing it increased surface area for attachment of cells. The surface exposed to the aqueous medium is coated with polymers resulting in chemical modifications that affect the rate of microbial attachment. Furthermore, the process of adhesion is influenced by factors like hydrophobicity, charge, and elasticity (Prakash et al. 2003)

(c) Hydrodynamics

The zone adjacent to the substratum which experiences negligible turbulent flow is known as the hydrodynamic boundary layer. The flow velocity in this zone is insufficient to remove the biofilm layer. The region outside this layer shows high level of turbulent flow and influences the attachment of the microbes on the surface. The microbial cells act as particles in a liquid, and the linear velocity of the liquid is the deciding factor for the rate of association of the cells with the submerged surface. Association of the cells largely depends on cell motility and cell size. The thickness of the boundary layer decreases with the increase in linear velocity. When the magnitude of the linear velocity is less, the cells travel through the hydrodynamic boundary layer, but as it increases, the boundary layer decreases. As a result, the microbial cells experience high turbulence level and mixing (Characklis 1990). Higher linear velocities can be considered the same as rapid association with the surface until the detachment of the cells occurs due to shear force caused by high velocities (Rijnaarts et al. 1993; Zheng et al. 1994). Simoes et al. (2007) showed that physical properties such as structure, thickness, and mass along with extra polymeric substance production and metabolic activities of biofilms are influenced by hydrodynamic conditions.

(d) Gene regulation

Initial attachment of the cells with the solid media involves upregulation and downregulation of a number of genes. *Pseudomonas aeruginosa* is an important bacterium which is involved in biofilm formation. Approximately 22% of its genes are upregulated, and 16% are downregulated during the process (Steyn et al. 2001). Bacterial alginate functions as extracellular matrix material which initiates differentiated biofilm formation. Genes like *algD*, *algU*, and *rpoS* which encode bacterial alginate formation and genes regulating polyphosphokinase synthesis are also upregulated during the process of biofilm formation by *Pseudomonas aeruginosa* (Prakash et al. 2003). In case of

Staphylococcus aureus, genes responsible for the synthesis of enzymes like phosphoglycerate mutase, triphosphate, and alcohol dehydrogenase used in glycolysis or fermentation are also upregulated (Becker et al. 2001)

(e) Extracellular polymeric substances

Extracellular polymeric substances are voluntarily secreted by microbial biofilms in nature which are, generally, composed of high-molecular-weight polymers, like polysaccharides, proteins, glycoproteins, DNA oligomers, phospholipids, and humic acids, comprising of 50% to 90% of the total organic carbon of the biofilms ((Flemming and Wingender 2010; Flemming et al. 2000). The uronic acids (D-galacturonic, D-glucuronic, and D-mannuronic acids) or ketal-linked pryruvates (Sutherland 2001) provide it the negative charge that facilitated their association with divalent cations causing cross-linking of the polymeric strands that provides greater binding force in the biofilm (Flemming et al. 2000). The extracellular polymeric substances are also highly hydrated by large amounts of water into their structure due to hydrogen bonding. The extensive hydrophobic interactions along with its bridging with multivalent cations aggregate the bacterial cell in the gel-like network. However, extracellular polymeric substances are not essentially required for the sustainability of microbial life, yet their provenance has significantly influenced the increased survival, plasticity, adaptability. and their metabolic efficacy. Although extracellular polymeric substances are considered as the secreted metabolic wastes, they crucially secure the biofilm attachment and facilitate organization of each microbial cell in the consortium. The extracellular polymeric substances, thus, not only enable the increase in the efficiency of biofilm for entrapping and gradual degradation of organics and nutrients from the surrounding but also increase cell-to-cell exchanges and signaling (Zhao et al. 2013; Decho and Gutierrez 2017). Moreover, it causes flocculation and granulation and further protects bacteria against environmental stresses (Zhao et al. 2013). The properties and primary characteristics of biofilm can be determined by the compositional and structural variations of the extracellular polymeric substances, since it is not uniform and rather alters on the basis of microbial composition along the spatial and temporal scales. Nevertheless, the extracellular polymeric substance production in microbial community is affected by nutrient status, where excess availability of carbon and limitation of nitrogen, potassium, or phosphate promote extracellular polymeric substance synthesis (Sutherland 2001). Retardation in bacterial growth also promotes extracellular polymeric substance production, likely, as a mechanism of tolerance. Further, it also contributes toward antimicrobial resistance of biofilms by slowing down the bulk transfer and exposure of antibiotics through the biofilm by binding directly to these agents (Donlan 2000). Notably, the increasing age of biofilms promotes the biosynthesis and secretion of extracellular polymeric substances significantly (O'Toole 2011).

(f) Extracellular DNA

Extracellular DNA serves very crucial role in various stages of biofilm formation including initial adhesion of bacterial cells followed by aggregation and microcolony formation promoting wastewater treatment. It is an integral part in production of extracellular polymeric substances (Bockelmann et al. 2006). It also provides strength to biofilms along with protecting the biofilms from antibiotics, physical stress, and detergents (Das et al. 2013). Currently, it is also prominently used in engineered biofilms for environmental pollutant remediation and production of electricity or fuel in bioelectrochemical systems or bioreactors.

(g) Divalent cations

Divalent cations are abundant in aquatic environments and are involved in bacterial growth in biofilms. The extracellular DNA chelates divalent cations which results in amendment of surface properties of microbial cell providing resistance against detergents and antimicrobial agents (Mulcahy et al. 2008). Calcium which is a divalent cation contributes in initial attachment by chelating anionic sites on extracellular polymers between microbial aggregates of anaerobic sludge granules, activated sludge flocs, and biofilms (Kerchove and Elimelech 2008). Calcium also influences cellular and extracellular product formation, cell signaling, alginate regulation, and biofilm virulence (Sarkisova et al. 2005). Cations like sodium, calcium, lanthanum, and ferric ions cause reduction in the repulsive forces created between the negatively charged microbial cells and the surfaces of attachment (Fletcher 1988). Introduction of divalent cations enhances the thickness of the biofilms making it denser and mechanically more stable (Das et al. 2014).

7 Biofilm Ecology

The microcolonies are the fundamental unit of biofilm structure. The compaction and proximity of the microcolonies provide an ideal condition for exchange of genes, quorum sensing, and creation of nutrient gradients. The cycling of various nutrients like nitrogen, carbon, and sulfur occurs through redox reactions. Microbial interactions like predation and competition also take place in the biofilm.

(a) Gene transfer

Gene transfer among the bacterial communities occurs through horizontal gene transfer (HGT) events which generally includes the exchange of plasmid DNA. HGT events confer the transfer of novel properties like antibiotic resistance, hydrocarbon degradation, or stress tolerance among bacterial communities; however, the lower rate of such interactions due to spatial separation of planktonic cells is a prime barrier. In contrast, the biofilm provides an ideal niche for such genetic exchanges as cells are more or less spatially fixed in the space (Ehlers and Bouwer 1999; Roberts et al. 1999; Hausner and Wuertz 1999). The consensus established that the HGT in the biofilm mostly proceeds with the phenomenon of conjugation as the biofilm structure allows cell-to-cell contact requisite for it. Thus, biofilms are considered as HGT "hot spots" due to high frequency of plasmid exchanges (Van Elsas and Bailey 2002; Aminov 2011;

Fux et al. 2005; Madsen et al. 2012). Nevertheless, the several abiotic factors, nutrient status, and biofilm compositions influence the frequencies of conjugation. Conjugation takes place with conjugative pilus, encoded by the *tra* operon of the F plasmid that facilitates as adhesion factor between donor and recipient cells, forming three-dimensional biofilm (Ghigo 2001). Additionally, effective and enhanced conjugation may be obtained in the biofilm as a consequence of closer cell-to-cell contact facilitating minimal DNA shearing. The recipient organisms which lack plasmid only produce microcolonies without any further development, but plasmid-carrying donor strains convert them into biofilm forming organisms through plasmid transfer.

(b) Quorum sensing
Among all the chemical signals required for the formation and regulation of biofilms, quorum sensing is the most common one that facilitates biofilm homeostasis through regulated cell attachments and detachments. This mechanism of quorum sensing not only maintains the microfloral density but also modulates toxin productions, bioluminescence, secondary metabolite secretion, etc. (Harmsen et al. 2010). Microbiota densely packed in extracellular polymeric substances matrix release a density-dependent chemical signal that mediates the growth and development of biofilms on different surfaces via quorum sensing. Quorum sensing uses the transcriptional activator protein which acts with small autoinducer signaling molecules to stimulate expression of target genes, only after its significant accumulation, resulting in changes in physiochemical behavior (Xiong and Liu 2010). The cell-to-cell signaling systems in *Pseudomonas aeruginosa*, namely, *lasR-lasI* and *rhlR-rhlI*, are involved in biofilm formation (Davies et al. 1998). Biofilm differentiation is initiated when these signals reach their optimum concentrations required for the activation of genes. The microbes with double mutant although produce a biofilm lack typical biofilm architecture with much thinner cell layer and more densely packed cells. Moreover, the mutant biofilms are easily removed from surfaces. Quorum sensing mediates induction of genetic competence, i.e., enabling the uptake and incorporation of exogenous DNA by transformation in *S. mutans*, and increases it up to 10–600-fold than planktonic cells (Yung-Hua et al. 2001). The mechanism by which the bacterial communication is interrupted is known as quorum quenching. It enhances the bacterial dispersal across membrane during the early development. It suppresses the growth of specific microbial species (e.g., *Acinetobacter*). It also influences the community dynamics affecting correspondingly the ecological selection and dispersal processes (Jeong et al. 2020).

(c) Predation and competition
The microbial biofilms due to localized cell concentration are subjected to predation by protozoa, bacteriophage, *Bdellovibrio* sp., and polymorphonuclear leukocytes (PMNs). Murga et al. (2001) reported colonization of heterotrophic biofilms leading to predation by a free-living protozoon, *Hartmannella vermiformis*. McLaughlin-Borlace et al. (1998) demonstrated *Acanthamoeba* sp. as a predatory organism in contact lens storage case biofilms.

Competition is also a regular phenomenon observed within biofilms. James et al. (1995) noted that in spite of the notable count of *Hyphomicrobium* sp. in *Hyphomicrobium* biofilm, the invasion of *Pseudomonas putida* always results in the dominance of *Pseudomonas putida*. Biofilms containing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* show consortial growth in stable community, but when it comes to mixed culture biofilms, the growth of *Pseudomonas aeruginosa* is dominated by *Klebsiella pneumonia* (Stewart et al. 1997). *Pseudomonas aeruginosa* grow primarily as a base biofilm, but *Klebsiella pneumoniae* form localized microcolonies that are advantageous as they may have greater access to nutrients and oxygen. *Pseudomonas aeruginosa* compete by rapidly colonizing the surface, thereby establishing a long-term competitive advantage, but they are outcompeted by *Klebsiella pneumoniae* as they grow more rapidly by attaching themselves to the surface layer of *Pseudomonas aeruginosa* biofilm.

8 Use of Biofilms in Biofiltration

Biofilm system is an advanced technology that is used in biofiltration for the wastewater treatment. Here, solid media is provided to the suspended growth reactors which act as attachment surface for biofilms. The solid media increase microbial concentration which leads to enhancement in contaminant degradation. Besides from that, biofilm system also involves many processes like biodegradation, bioaccumulation, biosorption, and biomineralization (Pal et al. 2010). Along with the degradation of different pollutants, the microorganisms present in the biofilm break down the trapped pathogens present in the wastewater. The treated water after biofiltration is then reused for other recreational purposes. The mechanism of water treatment using microbial biofilm technology is schematically shown in Fig. 3.

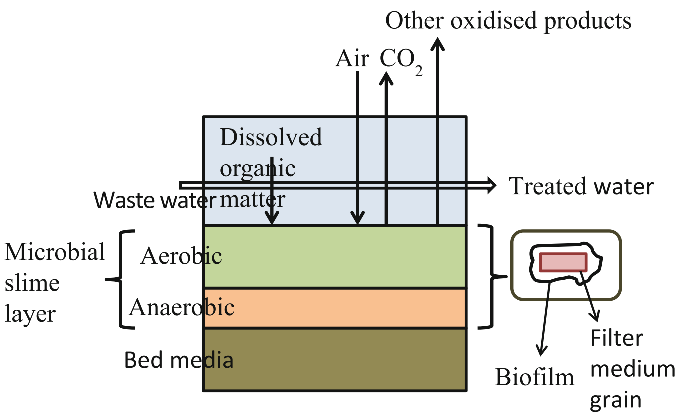

Fig. 3 The mechanism of water treatment using microbial biofilm technology

Membrane filtration technology is used in membrane bioreactors (MBR) in pilot plants. Its working principle is based on the combination of two basic processes, i.e., biological degradation and membrane separation. The biodegradation is done by the suspended solids and microorganisms, and the treated water is separated by a membrane filtration unit (Manem and Sanderson 1997). As a result of this, the slow-growing microorganisms and the particulate matters get accumulated, leading to the enhancement of recalcitrant organic treatment. Moreover, it causes retention of most pathogens which act like disinfectant. All biological solid wastes are trapped in the system, and the excess growth is removed as waste activated sludge reducing the turbidity as well as the suspended solids. Subsequently, the inorganic nutrients like nitrogen and phosphorous are also reduced (Di Giano et al. 2004).

Traditionally, membrane filtration is used in reverse osmosis process for water treatment. Reverse osmosis membrane filtration is more popular because it reduces the cost of desalination process using various improvements in the technology which decreases the energy consumption making the process more robust and cost-effective (Veerapaneni et al. 2007). Besides this, it can be used in water softening process also (Conlon et al. 1990). Instead of other traditional processes in water treatment like coagulation, sand and activated carbon filtration, or ion exchange, membrane filtration can be used without addition of chemicals to the water preventing the formation of harmful by-products. The main limitation of membrane applications is excessive biofouling which is prevailed by the use of reverse osmosis and nano-filtration (Vrouwenveldera et al. 2009). The advantages of using biofilm systems include low-space requirements, resilience to changes in the environment, operational flexibility, reduced hydraulic retention time, high active biomass concentration, increased biomass residence time, enhanced ability to degrade recalcitrant compounds, slower microbial growth rate, and lower sludge production. Over the year, biological membrane-based system has shown a great success in converting alternative water sources into potable water.

9 Research Status

Recent research works focus on exploiting the natural theory behind the microbial biofilm formation for wastewater treatment technology in pilot plants. Odegaard et al. (1994) reported the use of moving bed biofilm reactor for the first time in Norway during late 1980s and early 1990s. Later on, he also added that designing the biofilm carriers with increased specific surface area to escalate the future treatment capacity excludes the requirements of additional reactors (Odegaard 2000). Further, Maurer et al. (2001) used two types of biofilm carriers, sponge cubes and plastic tubes, while studying denitrification in a full-scale pilot plant. Laboratory models were also built with both attached growth biological reactor and suspended growth biological reactor to study the effect of combined reactors. It was deduced that combined reactors showed better oxygen transfer rate (Karamany Hesham 2001). Borghei and Hosseini (2004) pioneered the use of moving bed biofilm reactors in domestic and industrial wastewater treatment. The role of aeration system in rapid biodegradation was justified by Ahl et al. (2006). Biofilm reactors with freely moving carrier media were also operated on activated sludge treatment process (Odegaard 2006). Studies on organic phosphorus and nitrogen removal process have been examined in synthetic wastewater on a laboratory scale (Kermani et al. 2009). Sombatsompop et al. (2011) deduced the fact that when organic load is increased, the moving bed batch reactor showed more efficiency than conventional batch reactors. Yang et al. (2012) studied biosolids dynamics and explained biofilm growth and detachment, suggesting innovative design of reactors in membrane bioreactors which drew attention of researchers.

10 Future Research Prospects

Progressive application of microbial biofilm for enhancing not only water quality but also various fields has gained significant attention among the researches and industries. However, the lack of knowledge in understanding the molecular aspects and gene-dependent phenomenon of biofilm-associated organisms and their ecological interactions has somehow remained unappreciated. Microbiologist should focus on the dynamics of microbial interactions and also elucidate the genetic factor influencing such versatile phenotypes persisting among different biofilms. Recent researchers are escalating toward understanding the intrinsic and extrinsic factors that have driven versatility among the biofilm phenotypes. Nevertheless, it is crucial to explore the scope of utilizing biofilms in pathogenic resistance and treatment of chronic diseases as well. It is a matter of acknowledgment for the microbiologist as the pharmaceutical industries are embracing this novel biofilm-based approaches that may have potential benefits for the mankind.

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