

Review Article

CONTEMPLATION OF SYMBIOTIC MICROBIAL BIOFILMS IN WASTEWATER TREATMENT

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ABSTRACT

State of symbiosis is created among the species that are found in naturally existing biofilms. Biofilm formation provides protection against toxic shocks, mechanical stress, and predation. Biofilm can play an important role in wastewater treatment technologies and on the other hand could also lead to plague water. Biofilm-based treatments have been traditionally used for the treatment of water but the recent development in the stream has boosted the use of biofilm in various strategies of waste water treatment especially for strategies related to BOD and nutrients. However, the blueprint and execution of this idea is still being worked on due to the problems which arise in the implementation such as corroding pipes, increasing head loss, allowing pathogens to persist in distribution systems, and fouling membrane processes. Design for choice of species for biofilm processes in particular techniques is important wastewater treatment. All these data are essential to develop the performance, effectiveness and constancy of biofilm-based wastewater treatment strategies.

Keywords: Bacteria, Biofilm formation, Wastewater treatment, Fouling membrane, Bioreactor

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INTRODUCTION

A congregation of microbial cells that is irreversibly linked with a surface is called a biofilm. The process of formation of biofilm occurs over a series of events leading to adaptation under diverse environmental and nutritional conditions [1, 2] A mature biofilm is organized by a hydrated polymeric matrix, has a highly differentiated structure, being called mushroom and pillar-like assembly [3]. Biofilm formation is regulated by various genetic and environmental factors. Bacterial mobility, extracellular polysaccharides, cell membrane proteins and signalling molecules play significant roles in biofilm formation.

Biofilm development: structure and function

Biofilm matrix may be composed of acellular resources such as corrosion particles, mineral crystals, clay or silt particles, or blood components. Biofilms may cast on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems. Breakdown of different nutrients, such as phosphorus and nitrogen-containing compounds, carbonaceous materials as well as trapped pathogens are done by the microbial communities of biofilm from the wastewater. Biofilm development has following important steps (a) attachment (b) maturation and (c) detachment (dispersal)

A. Attachment

Primary adhesion of bacteria to the surface begins the formation of a mature biofilm and involves the reversible attachment of planktonic bacteria [4]. Cell surfaces have locomotor structures such as flagella, pili, fimbriae providing an advantage in biofilm formation. The direct primary adhesion to abiotic surfaces is mediated by non-specific physicochemical interactions (hydrodynamic forces, electrostatic interactions, Van der Waals forces and hydrophobic interactions) and the planktonic cells adhere to a surface randomly (Brownian motion and gravitational force) or in a directed way via chemotaxis, flagella motility and pili [5, 6]. Motile bacteria can utilize flagella to overcome hydrodynamic and repulsive forces, which gives it a competitive advantage. The flagellar motility is important for initial attachment, as has been reported for many bacteria [7]. At this stage, the bacteria can bind to the biofilm lifestyle or vacate the surface and return to the planktonic lifestyle. After primary adhesion the next step is secondary adhesion, which links to an irreversible binding to the surface. The microorganisms begin to produce the EPS in this stage, forming micro

colonies as it complexes with materials present on the surface firming up the links between the cells and the surface [8, 4]. At this instant, the cells start to communicate through QS signals as there is no motility [9]. Surfaces which are rough, hydrophilic and coated deliver a better environment for most recurrent attachment and biofilm formation [10]. Microorganisms multiply into micro colonies encapsulating themselves in the EPSs.

B. Maturation

The maturation of biofilm occurs in response to increasing population density and high EPS production, this increases the biofilm thickness and the stability of the colony; cell division and adhesion of new planktonic cells are the two means by which the population growth takes place [8]. QS Signal and EPS build-up through continued cell division, are two factors essential for the maturation of biofilm. During biofilm formation many species of bacteria are able to communicate with one another through this mechanism called quorum sensing [11]. More than 90% of the dry mass in mature biofilms is characterized by EPS [12]. EPS components take account of polysaccharides, nucleic acids, proteins, lipids, and other biopolymers. EPS is responsible for scaffolding cells together, adhesion to surfaces and maintaining the three-dimensional architecture of the biofilm. Moreover, the bacterial cell surrounded by EPS is protected against various stresses such as antimicrobials, host immune systems, oxidation and metallic cations [12]. Inside the biofilm, EPS retains quorum sensing (QS) signaling molecules, extracellular enzymes, and metabolic products. Therefore, EPS supports cell to cell communication and degrading substances [12, 13]. Cells or small portions of the biofilm may detach and disperse after the maturation of biofilm, as a result of nutrient depletion, QS signaling or shearing of biofilm aggregates because of flow effects [14, 4].

C. Dispersal

After biofilm formation, the bacteria leave the biofilms itself on a regular basis so it can undergo rapid multiplication and dispersal. Detachment of planktonic bacterial cells from the biofilm is programmed and has a natural pattern. Sometimes bacteria are detached from the colony into the surrounding due to some mechanical stress, but in most cases some bacteria stop EPS production and are detached into the environment. Dispersing of biofilm cells occurs either by detachment of newly formed cells from growing cells or due to flowing effects or due to quorum-sensing [15]. The mode of biofilm dispersion affects the phenotypic

character of organisms. Dispersed cells from the biofilm have the ability to hold its certain properties, like antibiotic in-sensitivity. The cells which are dispersed from biofilm as result of growth may return quickly to their normal planktonic phenotype. Alterations in nutrient availability, oxygen fluctuations, and increase in the toxic products or other stress-inducing conditions may also result in biofilm dispersion [16].

Acellular materials such as mineral crystals, corrosion particles, clay or silt particles as well as blood components (in case of bacterial biofilms present within the human body) might also be found in the matrix of biofilm [14]. There are channels for the circulation of nutrient and water within the matrix [12]; they also provide interspecies bacterial exchange or sharing of different metabolic substrates in biofilm. Under rigorously low nutrient conditions, bacteria can produce amino acids that cleave the structural polysaccharides allowing for detachment of cells from the biofilm hence modulating the structure of the biofilm [17]. EPS can incorporate large amounts of water into its structure by hydrogen bonding and hence is highly hydrated; it prevents desiccation in some natural biofilms. Nutrient availability, temperature, light, pH, ionic strength, carbon source, and water content can alter the structure of biofilms [12]. By increasing cross-linkage between polysaccharides the structural veracity of the biofilm can be improved by cations [18]. Biofilm thickness could be affected by the number of component organisms. Biofilm architecture is constantly changing because of external and internal processes, and heterogeneous both in space and time. Structure may also be influenced from the host or environment by the interface of particles of non-microbial constituents. Another example of particle interactions with biofilms are minerals such as calcium carbonate, corrosion products such as iron oxides and soil particles collecting in biofilms of potable and industrial water systems.

D. Factors affecting biofilm formation

Surface

Surface geography greatly affects the ability of bacteria to adhere to a surface. Surface roughness reduces the shear force on bacterial cells and communities present in fluid at high flow rates, such as water pipes in industrial plants. A material surface will inevitably become conditioned or coated by polymers from the medium when exposed in an aqueous medium, and the consequential chemical modification will affect the rate and extent of microbial attachment. Furthermore, other factors influencing microbial attachment are such as charge, hydrophobicity and elasticity [19].

pH

The growth and development of bacteria and biofilm formation are greatly affected by the change in pH as it can overwhelm different mechanisms and have negative or killing effects on the microorganisms. For the majority of bacteria, the optimal pH for polysaccharide production is around 7, but it varies among different species.

Salinity

Salt tolerance in plants depends mainly on the capability of roots for (i) restricted or controlled uptake of Na^+ and Cl^- (ii) continued uptake of essential elements, particularly K^+ and NO_3^- .

Temperature and moisture content

Limited water availability is typically the most critical factor to which terrestrial bacterial communities are exposed to including other environmental stress factors which exhibit the greatest effect on survival and activity of these communities.

Nutrient availability

Biofilm bacteria acquire nutrients by concentrating trace organics on surfaces by the extracellular polymers, using the waste products from their neighbours and secondary colonizers, and by using different enzymes to break down food supplies nutrients such as sucrose, phosphate, and calcium enhance biofilm formation as their concentrations increase

Velocity, Turbulence and hydrodynamics

The boundary layer is the area from the surface where no turbulent flow is experienced. Contained by this area, the flow velocity has

been shown to be insufficient to remove biofilms. The area outside this layer is regarded as high levels of turbulent flow and has an influence on the attachment of cells to the surface. The size of the boundary layer is dependent on the flow velocity of water. The boundary layer decreases in size at high velocities, and the cells are exposed to a high turbulence level. Hydrodynamic conditions can power the formation, structure, thickness, mass, EPS production and metabolic activities of biofilms [20].

Gene regulation and quorum sensing (QS)

For cell attachment and detachment from biofilms cell-to-cell signalling, also termed QS signalling, has been proven to play an important role. Bacterial cells release a density-dependent chemical signal densely packed with an EPS matrix to mediate growth and development of biofilms on different surfaces. A transcriptional activator protein is used by QS that acts in concert with small auto inducers (AIs) signaling molecules to stimulate expression of target genes, resulting in changes in chemical behaviour. After amassing sufficient AIs, this form of intercellular communication serves to coordinate gene expression, morphological differentiation and the development responses of bacterial cells [21].

Production of extracellular polymeric substances (EPSs)

By bridging with multivalent cations and hydrophobic interactions EPSs aid in the formation of a gel-like network that keeps bacteria together in biofilms. In addition, EPSs also cause the adherence of biofilms to surfaces, flocculation and granulation, protect bacteria against harmful environmental conditions and enable bacteria to capture nutrients from the surroundings [22].

Extracellular DNA (eDNA)

A number of single and multispecies biofilms has extracellular DNA as a major constituent. Its role is very important in numerous stages of biofilm formation, such as initial bacterial adhesion, aggregation and microcolony formation that favors wastewater treatment. eDNA also helps strengthen biofilms, be responsible for protection to biofilms from physical stress, antibiotics and detergents as well as aids as an exceptional source of nutrients for biofilm growth [23].

Divalent cations

Latest studies showed that eDNA chelates divalent cations that help in the modification of bacterial cell surface properties and thus favor resistance of biofilms to detergents and antimicrobial agents [24]. In terrestrial and aquatic environments divalent cations such as Ca^{2+} are present in abundance; therefore, calcium may be one of the factors that bacteria sense during biofilm-associated growth. By associating negatively charged sites on extracellular polymers, divalent cations, such as those of calcium, play a critical role in the initial attachment of microbial aggregates of activated sludge flocs, anaerobic sludge granules and biofilms [25]. Biofilm can become denser and mechanically more stable by introducing more divalent cations which enhances the thickness of biofilm, as shown in current studies [26]. Calcium has been found acting as a cofactor for certain proteins and is also active in cell signaling, cellular and extracellular product formation, biofilm virulence, and alginate regulation [27].

Bacterial, fungal and microalgal biofilms

Biofilms are intricate surface-associated cell inhabitants embedded in an ECM and are capable of adhering to a sweeping diversity of surfaces with distinct biotic and abiotic compositions, including human tissue and medical expedients. Present-day applications of biofilms take account of the humiliation of toxic effluents in soil and water, the viable production of chemicals, and the generation of electricity. Bacterial biofilm is infectious in nature and can result in nosocomial infections. Lots of species of bacteria are able to communicate with one another throughout the biofilm development over a specific mechanism called quorum sensing. Bacterial biofilm construction is considered to be an up-and-coming microbial lifestyle in usual and artificial atmospheres and befalls on all surface types [28, 29]. Some biofilm forming bacteria are, *P. aeruginosa*, *Vibrio cholerae*, *Listeria monocytogenes*, and *E. coli*. Temperature, pH differences, ultraviolet radiation, oxidation, metal ions and desiccation are some exterior issues against which biofilm provides protection to the bacteria.

Additionally, biofilms are able to evade inherent and/or adaptive immune defences and avoid antimicrobial treatments by several appliances [30-32]. Fungi habitually flourish as biofilms, which are aggregated communities wrapped in a protective extracellular matrix. Fungal biofilms are communities of adherent cells bounded by an extracellular matrix. Fungi are also used for pollution removal, besides bacteria. Fungal biofilms help in the deprivation of environmental organic chemicals, from proteins to complex carbohydrates, lipids, aromatic hydrocarbons, pharmaceutical compounds, heavy metals, endocrine disrupting chemicals by means of wide array of intra- and extracellular enzymes [33, 35] and therefore it forms a significant group of microscopic communities in wastewater treatment plants [36]. Many medically important fungi produce biofilms, including *Candida* [37], *Aspergillus* [38], *Cryptococcus* [39], *Trichosporon* [40], *Coccidioides* [41], and *Pneumocystis* [42].

Mixed culture biofilm

Single microbial species or a combination of different microbial species which includes bacteria, algae, fungi etc, that attach tightly to one another and to biotic or abiotic surfaces to form biofilm [43-47]. Due to coexistence of multiple microbial species close proximity is formed which promotes interaction among its members. By the synergistic interactions between algae and prokaryotic microbial communities biological wastewater treatment processes can be improved. Increases in biomass activity, growth efficiency, and enzyme production is achieved by the effect of mixture of microorganisms. In mixed culture to overcome feedback regulation and catabolic repression the products of one microorganism act as substrate for the other. In an example of biofilms in sewage treatment, the association of *Nitrosococcus* sp and *Nitrospira* sp is proved beneficial [48]. By pure culture, there are several microbial processes that cannot be achieved. Also, in tempheh wastewater contains diverse gram-positive and gram-negative bacterial species, such as *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klozaena*, *Enterobacter agglomerans*, *Streptococcus Dysgalactiae*, *Lactobacillus casei*, *Enterococcus faecium*, *Staphylococcus epidermidis* [49]. By providing additional oxygen from photosynthesis, microalgae help in improving the purification performance of bacterial systems and also decreases the total energy costs of direct or indirect oxygen supply [50].

Undesirable biofilm

In the treatment process biofilms can have both positive and negative treatment. For membrane filtration Membrane bioreactors (MBRs) and Membrane biofilm reactors (MBfRs) are used. Membrane biofouling in a moving bed biofilm reactor (MBBR) reduces permeate flow and can cause problems in membrane bioreactor (MBR) [51]. For large scale operations the irreversible fouling cannot be removed by cleaning therefore it is very difficult to manage [52]. The major reasons for the occurrence of biofouling is the production of membrane foulants by microorganisms present in the wastewater and colonization of membrane surfaces with microorganisms. For membrane fouling problem interactions and activities of microbial community in wastewater and on membrane surface should be well understood for developing novel solutions. Microorganisms and their organic products are the main reason for membrane fouling. By this fouling and the formation of biofilm the flux and permeability of the membrane is decreased [53]. Therefore, fouling needs to be kept in control, to decrease operational costs and increase membrane lifetime.

Biofilm characterization approach both traditional and modern

A complex, three-dimensional microbial community that grows at an interface and interacts with the surrounding environment is known as biofilm [54, 55]. By sequestration and alteration of potentially toxic compounds, as a renewable aid in applications of waste, soil and water remediation is done potentially by biofilms [56-58]. With respect to its compatibility with the emerging biofilms the chemical composition of the filter media is very critical; its elemental composition should be assessed. To analyse the surface chemistry of a material, different techniques can be applied, for the detection and quantification of the elements in a filter medium. The elemental composition is measured at the parts per thousand range, empirical formulas, electronic state and chemical state of the elements that exist contained by a material [59].

Traditional methods

Determination of viable cell numbers by plate count (Colony Forming Units/ml or CFU)

A standard quantification method is used to determine the number of viable cells called viable cell enumeration of CFU/ml assay [60, 61]. Living cells are differentiated from dead cells and their enumeration without dyes or instrumentation hence separating the individual cells on an agar plate and growing colonies from cells, is the basic concept of this assay. It is noted that in a mixed culture bacteria are replicated at different rates. Consequently, the culture expansion may not be suitable as it will disrupt the ratio of cells from the original biofilm. To accommodate for slow colony forming bacteria the colony forming incubation time may need to be extended [62] (table 1).

Determination of biofilm weight (Wet weight and dry weight)

A digital weighing balance is used to determine the weight of biofilm in terms of dry weight and wet weight. The wet weight is measured after soft rinsing with distilled water; however, the dry weight is estimated by allowing it to dry under aseptic conditions in laminar flow until the execution of the constant weight of polypropylene and polystyrene filter media [63, 64]. Contrariwise, natural filter media such as rock, granite or stone media, should be dried in the oven at 60 °C to attain constant weight [65]. The difference between the weight of medium with biofilm and that of medium without biofilm gives the weight of biofilm.

Determination of the biofilm Optical Density (OD)

The biofilm is also measured by the OD method. To ensure the removal of any material on their surface the filter media supporting biofilm are first rinsed with sterilized water. Then the biofilm is removed from the filter media in 0.9% saline by sonication for 15 min. Finally, at 550 nm wavelength (OD550) using saline as blank the spectrophotometric absorbance of dissolved biofilms is recorded [63-65].

Determination of heterotrophic plate count (HPC)

The HPC concentration is determined by the conventional serial dilution method. The biofilm dissolved in 0.9% saline is serially diluted (up to 10⁻⁵) and then spread on the selective growth media plates and incubated at 37 °C for a specific time period (24-48 h). The microbial growth giving the impression on specific media is enumerated in terms of HPC/ml (pathogen indicators). Further identification of pure cultures from these plates are done by observing colony morphology as well as microscopic and biochemical tests.

Microscopic analysis of biofilms

The truthful way of visualizing biofilms without disturbing their structure is provided by non-invasive microscopic technique. The traditional microscopic techniques used for imaging analysis of biofilm samples involve light microscopy (LM) and electron microscopy (SM). The most commonly used method for structural analysis is Scanning Electron Microscopy (SEM). Overall magnification of SEM can range from about 10-500,000 times, and can be used to develop a high resolution, magnified image of surface topography making this technique vital in the analysis of microscopic structures, including those of biofilms [66]. To understand formation and persistence, high resolution images can be gathered by SEM useful in evaluation of bacterial interaction, EPS organization and biofilm morphology [67-69].

B. Advanced methods

Clone library technique

Since the beginning of the 1990s cloning and sequencing of the 16S rRNA gene have been comprehensively and effectively employed for the study of microbial biofilms, and this is still the most widely used technique [70]. The clone library method allows complete 16S rRNA sequencing and identification with very precise taxonomic studies of both cultured and uncultured microorganisms in biofilms, design of primers for PCR and probes for fluorescence in situ hybridization (FISH) [71]. In combination with other advanced techniques, cloning

and rRNA gene library construction have also been applied in wastewater treatment for the exploration of biofilm communities.

Microbial fingerprinting methods

Microbial fingerprinting methods marks a distinction between microorganisms and groups of microorganisms on the basis of their distinctive characteristics of a universal component of a biomolecule, such as phospholipids, DNA or RNA, providing the overall profile of a biofilm [72, 73]. Phospholipid fatty acid analysis (PLFA), denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) are included in this method. The mass of PLFAs in a biofilm sample is directly proportional to viable biomass as type and proportion of phospholipid are distinctive to different microorganisms and break down rapidly upon cell death; still they are structural components of all cell membranes. Some sets of organisms have unique or "signature" types of PLFA [74]. DGGE is a nucleic acid-based technique and is engaged to generate a genetic fingerprint of a complex microbial community [70]. T-RFLP is a nucleic acid-based method and delivers the profile of a microbial community, which is used to detect specific microbial populations [75].

Fluorescence in situ hybridization (FISH)

FISH is an excellent method for the identification, localization, visualization and quantification of non-cultured microorganisms in their microcosm. The most commonly used target molecules for FISH are 16S rRNA, 18S rRNA, 23S rRNA and mRNA. Detection/Identification on any desired taxonomic level is enabled by the specificity of the fluorescent probe, from domain down to a

resolution suitable for differentiating between individual species [76]. Digitalization/Manipulation of images can be achieved by a charged coupled device (CCD) and appropriate image analysis software, quantifying rRNA content can help to maintain record of microorganisms, and measurement of the activity of single cells in biofilms. While obtaining three-dimensional images with thick samples with a high background (sludge flocs, biofilms) CLSM is used with FISH analysis. In order to overcome some of its pitfalls like increase its sensitivity and upgradation, FISH can be combined with other techniques. Enabling bacteria to be mapped, FISH-based methods have revolutionized investigations into the morphology and microbial composition of biofilms [77].

Next-generation sequencing (NGS) technology

A unique DNA sequencing technology which transforms microbial ecology, explores deeper layers of microbial communities and is vital in presenting an unbiased view of the composition and diversity of communities [78] is developed at the Royal Institute of Technology called pyrosequencing, based on the sequencing-by-synthesis principle [79] and on the recognition of released pyrophosphate (PPi) during DNA synthesis [80]. In comparison to the first-generation Sanger sequencing technology this technology NGS platform such as Roche/454, Illumina/Solexa, Life/APG and HeliScope/HelicBioSciences are much faster and less expensive [81]. The technique of pyrosequencing has no need for labelled primers, labelled nucleotides and gel electrophoresis. It has the potential advantages of accuracy, flexibility, parallel processing and easy automation. It has been effective for both confirmatory sequencing and *de novo* sequencing [80].

Table 1: Summary of methods for characterization of biofilms

Methods	Specialized equipment required	Biofilm preparation	Notes
Plate Count, Viable cell enumeration	Incubator-Consumables: disposable petri plates, culture flasks, suitable agar and medium	Cells are removed from the substrate, homogenized, resuspended in liquid medium, diluted, and aliquots are plated, incubated and counted.	Most readily adaptable to liquid/planktonic cultures. This method only quantifies live cells, and an assumption is made that each colony derives from one original cell. The differing metabolic states of living cells in the biofilm may complicate determination of accurate number of cells in the biofilm. Must be confirmed by cell mass or surface area.
Light Microscopy	Compound, bright field microscope	Biofilm can be grown directly on a transparent substrate, such as a slide or cover slip, stained and observed directly.	Counting or observing mature biofilms is limited, as accumulation of extensive biofilm mass prevents observation of individual cells. Can be used in conjunction with dry mass measurements to acquire biofilm thickness and quantifying specific visual characteristics of the biofilm.
Fluorescent Microscopy and Staining	Bright field/Fluorescent Microscope: Fluorescent stains, antibodies or endogenous fluorescent proteins	Biofilm can be grown directly on a slide or coverslip, stained, and observed in situ. Biofilm is stained with view of the desired outcome.	Some stains are potential mutagens. Stain should be chosen carefully--not all stains penetrate the cell membrane, and not all are compatible with maintaining a living biofilm.
Confocal Fluorescent Microscopy	Confocal Fluorescent Microscope: Fluorescent stains, antibodies or endogenous fluorescent proteins	Can image in place biofilm (on a coverslip, e. g.). Cells must be labelled. Fluorophores can be selected according to a variety of purposes, such as distinguishing live and dead cells, staining nuclei/DNA, etc.	Bio volume can be calculated with appropriate software and computing capability. Usually requires a dedicated technician to run and maintain the instrument. Can image any cell or particle that has a fluorescent label that can be detected by the microscope. It is better used for structures and 3D architecture than counting cells. Can image within the thickness of the biofilm and assemble z-stacks.
Determination of Dry Mass	Analytical Balance: Lab oven capable of reaching 100 °C	Film on substrate is dried, massed, then cleaned. Substrate is massed again.	Film area should be measured; thickness can be measured to give dry mass per unit of wet volume.
Optical Density	spectrophotometer	ensure the removal of any material on their surface the filter media supporting biofilm are first rinsed with sterilized water. Then the biofilm is removed from the filter media in 0.9% saline by sonication for 15 min	The biofilm is also measured by the OD method. at 550 nm wavelength (OD550) using saline as blank the spectrophotometric absorbance of dissolved biofilms is recorded
Atomic Force Microscopy (AFM)	Flow Chambers, Micropipette Aspiration and Centrifugation Devices	Bacterial adhesion, Mature Biofilm Morphology	AFM is suitable for a quantification of the interaction forces and can be provide a 3D surface profile
SEM	Scanning electron microscope: Sputtering Coater	Biofilm can be grown on a coverslip (or other substrate) and directly imaged on the microscope. Samples must be fixed, dried, and coated with metal (Pt-Pd).	Toxic chemicals may be involved in some fixation techniques. Usually requires a maintenance contract and special housing conditions.

Alternative qualitative characterization methods

By using scanning electrochemical microscopy (SECM) the topological structure and chemical properties of biofilm surfaces can be assessed [82,83]. Based on the distribution of reactive groups used to determine the distribution of extracellular polymeric substance (EPS) components at the biofilm surface this versatile technique can provide an extra dimension to 3D models of biofilms. Literature precedence exists to analyse biofilms with atomic force microscopy (AFM), although it is not commonly utilized currently. AFM would be useful in understanding biofilm characteristics such as roughness, topography, and stiffness; it can characterize the components on the underlying substratum as well as the substratum interactions [84]. But, requires specialized equipment costing more than \$100K and trained operators, like similar techniques. For effectiveness as a non-destructive method for greater understanding of biofilm aggregation, adhesion and EPS composition spectroscopic analyses of biofilm are becoming increasingly recognized. For providing similar data, Infrared spectroscopy delivers the vibrational information through the use of IR light, whereas Raman typically uses more energetic light, usually supplied by a near IR, visible, or ultraviolet laser. Despite some complications, with confocal scanning light microscopy (CSLM), or with specialized IR well-suited surfaces IR and Raman are good methods to use in aggregation with one another, [85-88].

Biofilm reactors

In modern water sanitation Biofilm reactors can be traced as its origination. Biofilms have led to the development of new and emerging biofilm reactors conducive to fundamentally based design approaches by making it significant by academic understanding, advances in the design, and mathematical modelling and its applications are fundamentally design and operation procedure for traditional biofilm reactors. All biofilm reactors have two characteristic processes (1) mass transfer and (2) biochemical conversion which influence biofilm structure and function. For these processes every biofilm reactor has common Compartments for optimisation.

Moving bed biofilm reactors

The MBBR has a two-(anoxic) or three-(aerobic) phase system with free floating plastic biofilm carrier which requires mechanical mixing for distribution of carriers throughout the tank. The process has submerged and completely mixed biofilm reactor and unit for separation of liquid-solids [89]. A series of pollutant loading and bulk phase external carbon sources in denitrification and dissolved oxygen concentrations in carbon-oxidation or nitrification MBBRs have been applied, and response of the system is evaluated. As the activated sludge process MBBR process is also capable of meeting similar treatment objectives for carbon oxidation, nitrification, and denitrification but the MBBR makes use of a smaller tank volume. For biofilm thickness control it does not require a special operational cycle because MBBR is a continuously flowing process. In the existing municipal wastewater treatment plant, the MBBR is well suited for retrofit installation. The plan's ratio with (length to width) L: W greater than 1.5:1 results in nonuniform distribution of the biofilm carriers. MBBRs contain a plastic biofilm carrier which gives up to 67% of the liquid volume. To allow treated effluent to flow to the next treatment step, screens are typically installed with one MBBR wall while retaining the free-moving plastic biofilm carriers. To evenly distribute the plastic biofilm carriers and meet process oxygen requirements Aerobic MBBRs use a diffused aeration system. On the other hand, in anoxic MBBRs there are no process oxygen requirements so it has mechanical mixers to evenly distribute the plastic biofilm carriers. For meeting basic secondary treatment standards medium-rare MBBRs are designed, typically designed for a loading of 5–10 g BOD₅m⁻² d⁻¹ at 10 degrees Celsius depending on the type of liquid-solid separation.

Biologically active filters

BAFs have a structure of natural mineral or random plastic media which supports biofilm growth and also serves as a filtration medium. Backwashing helps in removing solids accumulated from filtration and biochemical transformation. BAF configuration and

backwash regimes influenced by media density. Preliminary and primary treatment are required by BAF influent. For secondary and tertiary treatment, downflow BAFs with media heavier than water include the Biocarbone process and packed-bed tertiary denitrification filters such as the Tetra Denites process. Using an intermittent counter-current flow these BAFs are backwashed. InfilcoDegremontBiofor process in upflow BAFs with media heavier than water have been used for secondary and tertiary treatment. To provide area for biofilm development and filtration, these processes use a floating bed of media. To meet treatment objectives, flow and backwashing regimes, media selection is integral. Media can be categorized as mineral media and plastic media. In most cases, plastic media is buoyant and mineral media is denser than water. During backwashing and chemical degradation by constituents in municipal wastewater, the media needs to resist breakdown from abrasion caused by them. BAFs designed for removal of carbon oxidation and suspended solids in secondary treatment typically have volumetric BOD loading rates in the range of 1.5–6 kg m⁻³ d⁻¹.

Expanded and fluidized bed biofilm reactors

Expanded bed biofilm reactors (EBBRs) and FBBRs use small media particles that are suspended in vertically flowing wastewater, so that the media becomes fluidized and the bed expands. Individual particles become suspended once the drag force of the relatively fast flowing wastewater (30–50mh⁻¹) overcomes gravity and they are separated. In municipal applications, fluidized beds are typically used for tertiary denitrification. When treating groundwater or industrial wastewater, FBBRs are used for the removal of oxidized contaminants such as nitrate and perchlorate. Suspension of the media maximizes the contact surface between microorganisms and wastewater. It also increases treatment efficiency by improving mass transfer because there is significant relative motion between the biofilm and flowing wastewater. Silica sand (0.3–0.7 mm diameter) and granular activated carbon (GAC; 0.6–1.4 mm) are typically used. Other materials, however, have been used at pilot scale, such as 0.7–1.0 mm glassy coke [90], which is one of the key advantages of this process technology. In a study of tertiary nitrification of activated sludge-settled effluent using a pilot-scale EBBR, [91] found that the process also removed up to 56% CBOD and 62% TSS from the influent stream. Removal of these materials was attributed to the activities of protozoa (free-living and stalked) and metazoa (rotifers, nematodes, and oligochaetes)

Rotating biological contactors

RBC is an efficient attached growth system that purifies wastewater from different industries, namely food and beverage, refinery and petrochemical. In addition, it is efficient in purifying municipal wastewater, landfill leachate and lagoon effluent. When an average effluent waste water quality standard is less than or equal to 30 mg l⁻¹ BOD the RBC process has been applied. The RBC contains a horizontal shaft, in which a cylindrical, synthetic media bundle is mounted. The bundled media is partially submerged and slowly rotates to expose the biofilm to air (when not submerged) and to substrate in the bulk of the liquid (when submerged). The RBC effluent stream is removed by liquid–solids separation units to detach biofilm fragments suspended. By reduced life cycle costs, less sludge production, less space requirement, ease of operation and high process stability with load variations as well as high effluent quality with regard to both biological oxygen demand (BOD) and nutrients, the RBC system has an edge over suspended growth systems.

Trickling filters

A three-phase biofilm reactor with secure carriers is called a Trickling Filter. Past a distribution system wastewater enters the bioreactor, trickles downhill over the biofilm surface, and in the third phase air circulates where it diffuses through the flowing liquid and into the biofilm. An influent water distribution system, containment structure, rock or plastic media, an underdrain and ventilation system are the components of Trickling Filter. A net production of total suspended solids is the result of treatment of wastewater using a trickling filter. And so, liquid–solids separation is required, this is achieved with circular or rectangular secondary clarifiers. The Trickling Filter process generally includes an influent/recirculation pump station, the

Trickling Filter(s), and liquid–solids separation unit(s). Ideal Trickling Filter media encourage ventilation and be responsible for a high specific surface area, low cost, high permanency, and high enough porosity to avoid clogging [92]. Trickling Filter media types include rock, random (synthetic), vertical flow, and cross-flow (synthetic). Fixed-nozzle and rotary distributors are the two types of Trickling Filter distribution systems. Microorganisms forming on the packing material surface grow in biofilm and degrade the pollutants from the effluent.

CONCLUSION

Nutrients for growth are anchorage by Biofilm. Complex organics can be easily broken-down into metabolized substrates using enzymes beneath the biofilm matrix and also facilitate horizontal gene transfer. At one front where this biofilm strategies help in the treatment of waste water on the other hand it is difficult to remove these from environment as well as their growth is not only affected by the environment surrounding but is also affected by the native microflora. This study presents comparative data screening the benefits of biofilm treatment processes, describing their use in several stages of the wastewater treatment process. This study is important because, for improved scheming of these biofilm-based wastewater treatment strategies, knowledge about the microorganism involved, stages of treatment and factors affecting the treatment process are vital.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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