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Review Article

ANTIBIOFOULING BIOMATERIALS

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ABSTRACT

Antifouling refers to the process of control of fouling which occurs on liquid-solid surfaces. The term 'fouling' indicates an undesirable natural succession process during biofilm formation, in which a submerged surface or membranes becomes encrusted with material from the surrounding environment. It mainly involves microorganisms and their by-products developed on the surface by conditioning, attachment, biofilm formation followed by colonization. The accumulation of micro and macrofoulers on immersed structures results in economic as well as environmental losses. It is one of the major vulnerable problems currently disturbing many ecological niches as well as in shipping and other industrial aquatic processes. The existence of natural antifouling agents or biomaterials provides sustainable eco-friendly control and hence remains a challenge for future researchers. The use of biological tools for control of fouling is gaining importance day by day.

INTRODUCTION

Biofilms and biofouling originated on earth nearly 3.25 billion years ago in oligotrophic systems (Wimpenny *et al.*, 2000) where roughness of the surface provides more suitable environment for their growth. Microbes form biofilms in response to various factors, such as recognition to specific or non-specific attachment sites on a surface, nutritional factors, detergents and sub-inhibitory concentration of antibiotics (Hoffman *et al.*, 2005). Microbial biofilms leading to biofouling consists of organisms and their by-products. It occurs on any surface by colonization and accumulation of micro and macrofoulers on immersed structures. Adhesive property, biofilm formation as well as quorum sensing associated features like exopolysaccharide secretion, virulence factor all these together lead to biofouling. Majority of studies have been carried on pathogen treatment or in medical fields. However, literature about biofilm and quorum sensing of microorganism for environmental isolates are still in infancy. Biofouling study appears as unsaturated as well as interesting ground to be exploited by environmental scientists. For example quorum sensing in fungi was first reported in 2001 and studies were generally encircled till now with pathogenic strains of *Candida* sp (Burke *et al.*, 2007).

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Biofilm formation is key step towards biofouling process. The biofilm concept was coined by Bill Costerton in 1978 and now it is widely embraced by microbiologists, engineers and computer scientists (Jenkinson and Lappin-Scott 2001). It is a microbial development concept that was first proposed by O'Toole and his colleagues (O'Toole *et al.*, 2000). According to Martin Dworkin, 'development' refers to a series of stable and metastable changes occurring in a cell, in response to certain environmental stimuli that become a part of the normal life cycle of cells. It helps the cells to adapt and survive in their dynamic environment (Dworkin and Kaiser 1985). Initially microbiologists ignored the socio-biology concept but the studies on co-operative behaviour in *Myxobacteria* and quorum sensing/biofilm formation in *Pseudomonas aeruginosa* sparked biofilm research (Kalia and Purohit 2011). They are found nearly on every surface and interfaces exposed to oil, water or air (Halan *et al.*, 2012). They can colonize on both biotic and abiotic surfaces such as industrial and hospital settings (Stoodley *et al.*, 2004, Lear and Lewis 2012) and on human host (Jefferson 2004).

Why microbes form biofilm?

According to the Darwin's theory of evolution, the only true driving force behind the course of action of any organism is reproductive fitness that is any action that increases proliferation.

Since it remains an inherent action it almost seems contradictory that a biofilm mode of growth would impart reproductive fitness over planktonic mode. Outside the laboratory, bacteria would rarely find themselves in an environment as rich as the culture media and hence the biofilm offers a more protective mode of bacterial growth in nature. (Jefferson *et al.*, 2004). The biofilm matrix provides its members resistance to many environmental stresses such as fluctuations in pH, temperature, osmolarity, UV damage (Elasri and Miller 1999), desiccation (Chang *et al.*, 2007), predation (Matz 2005) and specific secondary metabolites such as antibiotics (Stewart and Costerton 2001), an advantage not available to their planktonic counterparts.

Microbial Biofilm Development in Environment

Biofilm formation in the environmental biofilms begins with a transition of bacteria from the planktonic (free swimming) to its genetically distinct attached state (Singh *et al.*, 2006, Parsek and Tolker-Nielsen 2008, Stoodley *et al.*, 2004). The physiological and genetical transition occurs across the life cycle of the biofilm as shown in Fig. 1.

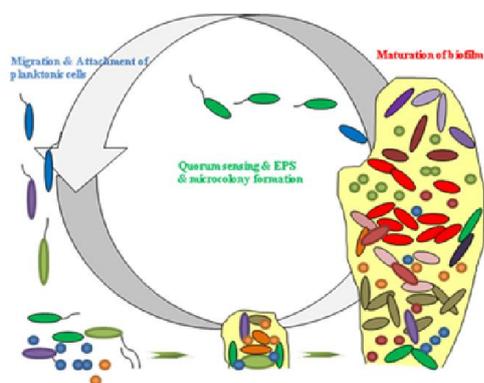


Fig.1 Biofilm developmental stages 1. Quorum sensing, EPS and microcolony formation 2. Maturation of biofilms 3. Migration and reattachment of planktonic cells

- Quorum sensing, EPS and microcolony formation
- Maturation of biofilms
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Quorum sensing, EPS and microcolony formation

During biofilm formation, cooperative communication — known as quorum sensing — occurs to coordinate bacteria during different stages of development. By releasing some intercellular signalling molecules bacteria can sense each other presence and they continue to secrete these molecules until a sufficient population is reached thereby forming a ‘quorum’. In this way bacteria can sense their quorum and start forming micro colony (Fig 1). This is called quorum sensing in bacteria. What is the relation between quorum sensing and biofilm formation? There is an acute relation between quorum sensing and biofilm formation because biofilms generally consists of clusters of cells. One can predict that the aggregates might be a product of quorum sensing. The prediction has truly provided evidence that quorum sensing is important for biofilm formation and dispersal.

Three most common pathways used by bacteria are Acyl Homoserine Lactone (AHL) system in Gram positives, peptide based signalling in Gram negatives (Waters and Bassler 2002, Fuqua *et al.*, 2001) and AI-2 signalling in both Gram positives and Gram negatives. In acyl homoserine lactone signalling system, a single enzyme synthesizes the signal i.e. acyl homoserine lactone from cellular metabolites for communication (Parsek *et al.*, 1999). It can diffuse across the cell membrane, interacts with a cytoplasmic DNA binding receptor protein which belongs to Lux-R family of genes which then activates the expression of quorum sensing genes. These AHLs are derived from S-adenosylmethionine which consists of a hydrophilic homoserine lactone head and hydrophilic acyl side chain that varies from species to species. The side chain consists of 4 to 18 carbon atoms, the variation occurs in length from the 3rd carbon. These alterations are the main source of specificity in QS signals and facilitates cell to cell communication in bacteria.

The chemical structures of Quorum sensing peptides in Gram positive bacteria also varies in the number and type of amino acid residues (Dusane *et al.*, 2010) and controls diverse physiological processes. Their biosynthesis processes are more complex than Gram negative bacteria on account of their post translational modifications in the peptides and their inability to diffuse through the membranes. Largest quorum sensing peptides in Gram positive bacteria are antibiotics which are having antimicrobial activity such as nicin produced by *Lactobacoccus lactis* (Lubelski *et al.*, 2008). Some other autoinducing peptides such as Type-1 autoinducing peptide produced by *Staphylococcus aureus*, *Enterobacter faecalis*, *Listeria monocytogenes* plays important role in quorum sensing process (Miller & Bassler 2001, Water and Bassler 2005). Some other non AHLs such as indole, small RNAs and secondary messengers are also involved in quorum sensing. Indole produces quorum in *Escherichia coli*. (Wang *et al.*, 2001). Quorum sensing process also depends on the nutritional conditions (Shrout *et al.*, 2006).

Many groups have demonstrated the link between quorum sensing and biofilm formation. In some species link was found while in some no relation was found between the two. Species in which quorum sensing mediates biofilm formation are through different stages initiated by attachment. Attachment or adherence of a bacteria to a surface or substratum is the initial step in biofilm formation (O’Toole, 2000). ‘Attachment’ in deeper sense means bacteria forms bonds with the surface by their adherence factors. Bacteria employ many adherence factors for this purpose such as pili, fimbriae, carbohydrate binding proteins etc. Intestinal pathogen *Helicobacter pylori* has luxX genes homologues involved in attachment (Kirisits and Parsek 2005). Firstly when a bacteria adheres, it forms reversible attachment that is it can also come out of the surface if loosely attached then irreversible attachment occurs that is strongly bound to the surface and cannot come out. Then attached bacteria secrete extracellular polymers (EPS) and begins to multiply by microcolony formation and thus biofilm matures. By further growth and secretion of EPS, 3D structure in between the cell cluster channels are also formed to deliver water, nutrients and waste removal. After irreversible attachment bacteria begin to secrete EPS and through quorum sensing bacteria multiply themselves until a specific cell density is reached and forms microcolony.

Further EPS secretion occurs and the biofilm matures by forming different shapes such as 3D mushroom like or flat microbial mats (O'Toole 2000). EPS or extracellular polymeric substances are the building blocks of the biofilm community. EPS serves variety of functions to support the biofilm mode of life by enabling cells to come in close proximity, facilitating cell to cell communication, store various extracellular enzymes which sequesters and digest colloidal, dissolved and solid substances leading to enhanced metabolism and growth. It also acts as a recycle centre which keeps and allows components of lysed cells to be used by live cells, which may also results in horizontal gene transfer by transformation, conjugation as well as transduction i.e involving viruses too. Although EPS represents external digestive system but complete digestion requires a large variety of enzymes on account of heterogeneity. It is also called 'dark matter of biofilms' because of heterogeneity of polymers and difficulty in analysing them (Flemming *et al.*, 2002, Chelsea and Brennan 2010, Carsten Matz *et al.*, 2011). This heterogeneity also varies from biofilms to biofilms depending upon the type of microbes, mechanical shear, temperature and availability of nutrients, Bacterial extracellular structures also stabilizes the matrix (Zogag *et al.*, 2001). The forces that stabilizes EPS are not covalent bonds but weak interactions such as hydrogen bonds, Vanderwaals interactions, electrostatic and ionic interactions, entanglement of long molecules. They behave like elastic bodies until a threshold pressure is reached. Beyond that threshold pressure it liquefies to highly viscous liquids (Korstgens *et al.*, 2001). During viscoelastic phase same partners react causing breakage and formation of bonds. After that equilibrium shifts in the breakage of bonds and EPS liquefies. This is the point which exceeded when we get slipped while walking in the streams containing biofilm coated rocks. The porous architecture of EPS allows convection of flow of fluid through the depth of the biofilm. Within EPS, substances flows by diffusion. So at the bottom, organisms get excess nutrients while those at top of the matrix competes among themselves to get nutrients from the bulk water phase.

Oxygen gradient also get formed in biofilms by a actively respiring heterotrophic organisms which consumes oxygen before it diffuses through the matrix thereby creating an anaerobic environment below the aerobic organisms allowing the growth of anaerobic organisms (Flemming 2010). Other gradients such as pH, redox and ionic gradients are also formed (Stewart 2001). Cells in a biofilm remain surrounded by EPS or extracellular polymeric substances and those cells forms capsules, are associated more closely to the surfaces than others (Flemming 2010). Multispecies biofilm community structure depends upon production and quantity of EPS (Sutherland 2001), concentration, cohesion, charge, sorption capacity, specificity, nature of components of EPS. Pores and channels determine mode of life in biofilms. CLSM examination revealed that EPS matrix provides physical structure which segregates different organisms in the biofilm community. These segregated regions contain different biochemical environments that are enzymatically modified in response to dynamic environment (Sutherland 2001).

Biofilm architecture depends upon nature and amount of EPS produced. EPS of *Escherichia coli* and colanic acid of *Bacillus subtilis* are essential for the formation of 3D structure.

Alginate is required for biofilm development but it is not essential in *Pseudomonas aeruginosa* (Flemming 2007). Acetyl groups also affect biofilm structures by modifying alginate with acetyl groups which increases cohesive and adhesive properties of EPS (Flemming 2007). Since the matrix is negatively charged and if it encounters multivalent cations it alters the structure. For example Ca^{2+} forms a bridge of polyanionic alginate molecules resulting in a thick and compact structure with increasing mechanical stability (Kortgens *et al.*, 2001). EPS also possess optical properties. EPS have slightly different refractive index than water hence light tend to enter. Actually it enters the biofilm rather that reflect on at the surface. This is called 'forward scatters'. EPS function as a light conductor. EPS gel allows 'recapture' of scattered photons from an underlying surface and increases the absorption potential of the underlying cells by cellular chromophores (Decho 2010). EPS are formed by nucleic acids, polysaccharides, lipids, water proteins, ketal-linked pyruvates, detritus etc. Each of the components have indispensable roles which cannot be ignored (Decho 2010).

Maturation of biofilms

Maturation of the biofilm involves growing of microcolonies and production of extracellular polymeric substances to form a spatial structure which stabilizes the biofilm community (Kim *et al.*, 2008, Marcato *et al.*, 2012)). Building of this spatial structure is determined by three distinct layers of organisms. The inner layer is inhabited by early or pioneer colonizers which attaches to the surface which are generally facultative anaerobes. They serve as a foundation of the biofilm structure by remaining attached to the surface. In a developing biofilm strong oxygen gradients are formed by actively respiring aerobes at the upper stratas whose oxygen consumption rate is faster than oxygen diffusion rate. An anaerobic environment gets created at inner layer due to little or no oxygen diffusion (Stewart & Franklin 2008). The basis of genetic inheritance for the biofilm community remains in the inner layer. The middle layer consists of organisms which are arranged in close proximity to each other, which allows them to exchange nutrients and genetic information (e.g., for antimicrobial resistance). The process of cell-to-cell communication and genetic interaction between the cells occurs in middle layer of the biofilms, which allows the members to coaggregate with each other (Otami *et al.*, 2007; Otzen *et al.*, 2007). Reports suggests that in domestic showerheads (Vornhagen *et al.*, 2013), biofilms of drinking water distribution systems (Simoes *et al.*, 2008) and dental plaques (Kolenbrander *et al.* 1989), the middle bacteria acts as an adaptor or bridge which connects the inner and outer most layers of bacteria in a biofilm. The outer layer comprises of actively respiring bacteria which behave in a manner similar to individual planktonic (non-biofilm-associated) bacteria (Fig 1). Planktonic bacteria migrates and reattaches to new surface for proliferation.

Migration and reattachment of planktonic cells

It is very significant stage in the biofilm life cycle which allows the cells to inhabit new surfaces (Flemming, 2011). It is influenced by nutrient starvation and secretion of EPS hydrolyzing enzymes like hexosaminidase which breaks off EPS to release planktonic cells in the fluid-surface interface (Kaplan *et al.*, 2003).

The dispersal is also affected by shear forces in its surroundings. Specifically, shear forces present in the microenvironment are high enough to cause detachment of a portion of the biofilm and formation of projections called streamers that migrates to new locations and environments. Consequently, more support are required for reattachment of planktonic cells to carry out their surface associated stages of life cycle (Leck 2005; Russel *et al.*, 2009). The association of bacterial layers in biofilms leads to biofouling at later stages.

Problems regarding biofouling

As learnt from literature survey, biofouling creates problems on any liquid-solid surfaces, for example on ship hulls. Roughness created by biofouling by bacteria results in high frictional resistance which leads to increasing weight and subsequent speed reduction of liquids with high power consumption. Relatively light biofouling that is made up diatom slimes results in increased power backing of 10–16%, whereas heavy calcareous fouling at full cruising speed results up to 86%. In the case of fuel consumption the loss rises can be up to 40% (Schultz 2007) involvement of higher efficiency machinery to overcome this problem leads to voyage overall costs as much as 77% higher. Biofouling debris clean up entails huge man power, machineries and high chances of time loss and wastage of resources. Toxic waste products and “alien species” get introduced to native ecosystems. One report showed introduction of new 16 species of barnacles at the port in Osaka Bay, Japan due to Biofouling (Oumi *et al.*, 2007, Chelsea and Brennan 2012).

In many industries like water treatment, food processing, paper and milk industries biofouling have been found to be very serious problem regarding maintenance of different types of membranes (Anand *et al.*, 2014). It have been still a major challenge in terms of quality of water, plant performance and operating cost in different industries (Fig 2). Four major types of fouling occurs in membranes/filters viz;



Fig. 2. Biofouling (a) management in ship yard (b) membrane sheets in industry (Photocourtesy Yebra et al 2004, Chiellini et al 2012)

- inorganic salt precipitation (contributed by sparingly soluble salts),
- organic (mostly natural organic matter or effluent organic matter),
- colloidal (caused by accumulation of a colloidal cake layer on the membrane surface), and
- microbiological (usually governed by bacterial biofilms and subsequent microfouling formation).

If fouling could not be controlled, it could results in permeate flux decline of the membrane because of the accumulation of retained biofilms on it, which leads to increased differential pressure and feed pressure, increased salt passage, increased energy consumption. Other vigorous problem include membrane biodegradation caused by acidic by-products.

Antifouling strategies and necessity of biotools for antifouling

To control biofouling the proposed methods that concerned about physical, mechanical or chemical means were being a matter of question day by day. Physical or mechanical cleaning of ship hulls or submerged structure basically exposes the substrata for next event of biofouling in successive days. The chemical means of control actually employed different types of biocide or implication of antibiofouling paints (Yebra *et al.*, 2004). Most antifouling paints composed of organotin (tributyltin) or heavy metals (copper plus organic booster biocides, zinc) that, even in very low concentration, served as broad spectrum toxins to target as well as non-target organisms. Use of toxic tributyltin (TBT) coatings has been increasingly banned at global scale (Magin *et al.*, 2010). It has been shown that membrane biofouling chemical clean up stress-up the residual biota and triggers for readily biofilm formation for next session. The fouling organisms generally showed robust nature, that even if 99.9% cells were removed then even the chances exist that films could be easily formed from remaining biostratum (Nguyen *et al.*, 2012). Even frequent chemical cleaning of membrane actually shortens the life time of membrane and it also includes extra maintenance cost as well as extra man power (Chiellinia *et al.*, 2012). So to have alternative, safe, eco-friendly control use of biological tools are gaining importance day by day in biofouling treatment.

Antifouling biotools

Bioinspired biomaterial

Learning from nature’s own defense and transferring the knowledge into application to combat biofouling biomaterials were gaining importance day by day. It has been seen that most of study regarding bioinspired biomaterials were used in the aspect of marine biofouling or ship hull management field. For example, many reports suggested sharkskin mimicking, resulted reduction in drag force and Reynolds number and deterred biofouling. To make nature’s perfect replica there was urged to incorporate biosciences into physical models. Instead of only mimicking the surface topology and texture, many studies were carried on to add bioinspired biomaterials in the coating so that both the physical topography and biochemical phenomenon could be exploited (Salta *et al.*, 2010). For example, besides having groovy scale topography (Baum *et al.*, 2002) whale was also reported to resist micro-organisms as it contains micropores and nano-ridges surrounded by enzymatic gel coating that disintegrates proteins and carbohydrates. Sessile marine organisms do not possess mechanical or dynamic facilities to combat fouling, but they were notably free from macrofouling. Studies revealed that they produce some antimicrobial and antibiofilm exudates and these secondary metabolites keep them free from any fouling condition.

One of the very good examples has been sea weeds. Their unusual chemical structured secondary metabolites were very exclusive and do not share common features with terrestrial tissues. 40% less biofouling was observed when sugar kelp (*Saccharina latissima*) and Guiry's wrack (*Fucus guiryi*) were used as bioinspiration and matrix was replicated by polymorphic reproduction with doping of bromofuranone (Chapman *et al.*, 2014).

Quorum Sensing in biofilms are regulated by releasing and detecting small signaling molecules known as Auto-Inducers (AIs). Three types of AIs have been reported including oligopeptides, N-Acyl Homoserine Lactones (AHL), and autoinducer-2 (AI-2). Cellular communications in Gram-positive and Gram-negative bacteria are achieved by oligopeptides and AHL, respectively. In the case of inter-species communication for both Gram-positive and negative bacteria AI-2 molecules are implied.

Table 1. List of some natural resources producing antifouling biomolecules

Source	Bio molecules	Anti biofouling property
Macroalga (<i>Delisea pulchra</i>)	Furanone/ 2(5H)-Furanone, (5Z)-4-bromo -5-(bromomethylene)-3-butyl-2(5 H)-furanone.	Mimic AHLs and disrupt signaling, disrupt motility and biofilm formation
Green macroalgae <i>Ulva rigida</i> Honaunau Bay coral reef bacterial community, specially marine cyanobacterium <i>Leptolyngbya</i> sp. Seed exudates (<i>Medicago sativa</i>)	Brominated furanone Honaucins A to C L-canavanine(L- α -Amino- γ -(guanidinoxy)-n-butyric acid)	Inhibit quorum sensing V. harvery biofilm formation and E. coli AHL inhibition Inhibit the expression of QS-regulated phenotype exopolysaccharide production.
Streptomyces soil isolate Sweet basil <i>Ocimum basilicum</i>	Tricyclic polypeptide siamycin Rosmarinic acid(R-O-(3,4-Dihydroxycinnamoyl)-3-(3,4-dihydroxyphenyl) lactic acid)	blocked QS regulated feature i.e gelatinase production Inhibit protease, elastase, hemolysin production, biofilm formation and virulence factor
Vanilla beans extract (<i>Vanilla planifolia</i>)	Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	Interfere with AHL receptors. Inhibit C4-HSL, C6-HSL, C8-HSL, 3-oxo-C8-HSL. Inhibit biofilm formation in <i>Aer. hydrophila</i>
<i>Dichotella gemmacea</i>	Juncin	potent nontoxic antilarval settlement
See grass <i>Halodule pinifolia</i> , <i>Cymodocea serullata</i> <i>Avicennia marina</i> , <i>Rhizophora mucronata</i>	carrageenan type amino, carbonyl and phosphoryl functionalities, aliphatic (fatty acids), NH ₂	Antibiofilm
<i>Pseudomonas</i> sp. strain PAI-A	AHL-acylase (PvdQ)	Degrade C10-HSL, 3-oxo-C10-HSL, C12-HSL, 3-oxo-C12-HSL, C14-HSL, C16-HSL
<i>Aspergillus niger</i> IAM 2094 <i>Bacillus megaterium</i>	AHL-lactonase (Gluconolactonase) Oxidoreductase	Lactone ring hydrolysis Oxidizes; C12-HSL, 3-oxo-C12-HSL, C14-HSL, 3-oxo-C14-HSL, C16-HSL,

More than 20,000 metabolites have been reported from marine weeds from 1970s and a considerable percentage showed antibacterial, antifungal, antiprotozoan activities. Though major part of their exact mode of action still remained a secret, they were well reported as antiadhesive, low pH content, toxic, anesthetics and sometime they act as biological signal breaking agents. Maximum secondary metabolites from sea weeds were of terpenoid group (the largest group of natural products) or they were brominated in their biochemical structure.

Quorum quenching molecules

Biofouling may be controlled by using Quorum Quenching (QQ) strategies now adays (Kalia *et al.*, 2015, Feng *et al.*, 2013). Inhibition of biofilm formation in liquid–solid interfaces through quorum quenching is promoted. It has been reported that 60% of bacterial species sampled from biofouled Reverse Osmosis membranes, collected from a water treatment plant produced Quorum Sensing (QS) molecules. Such microorganisms actively participate in biofilm formation on membranes, suggesting that biochemical control of biofilm formation by inhibiting Quorum Sensing signals could be an effective way to reduce membrane biofouling (Diggle *et al.*, 2007, Feng *et al.*, 2013).

Quorum Sensing inhibition can provide considerable and effective means to control biofilm growth without the application of growth-inhibiting agents (Lade *et al.*, 2014). Quorum Quenching (QQ) molecules which have been characterized and reported up to date, generally use three strategies to combat autoinducer systems. The molecules interfere with Quorum Sensing signal production and disturb the synthesis, disrupt accumulation or degrade the Quorum Sensing molecules.

Many reports showed that natural compounds such as vanillin, ajoene, furanones, flavonoids, curcumin, Iberin, patulin etc. (Table 1) and few enzymes notably group of acylase, lactonase, oxidoreductase showed potential quorum quenching activity (Lade *et al.*, 2014, LaSarre and Federle 2013) against biofouling bacteria without interfering with their growth. Extracts of sea grass, mangroves were also studied to reduce quorum sensing controlling phenomenon such as biofilm formation and reports stood at a considerable appreciation (Prabhakaran *et al* 2012). Furthermore, immobilization of quorum quenching bacteria as well as enzymes, by bead-entrapment has been implied to MBR as a new biofouling control technology (Suk OH *et al.*, 2012).

Conclusions

Biofilms develops into biofouling only when “threshold of interference” oversteps and microbiota becomes “nuisance”. It is one of the major vulnerable problems currently disturbing many ecological niches as well as in shipping and other industrial aquatic processes like membrane technology and maintenance. System performance only gets hampered when bacterial count exceeds 10^4 cfu/cm². Ban on oraganotin compounds as antifouling coating agents on ship and ship hull raised the urge of introduction of safe biomaterials into the antifouling research platform. Gradually for controlling membrane biofouling in water systems, biotools has been explored for safe and sustainable management.

Authors' Contribution

AQ initiated and prepared the review text, SG and SP have contributed equally in compiling the technical literature of review, AK and HJP supported overall work.

Competing Interest

None of the authors have any financial and technical competing interest.

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