'Synbiofilm'- A Friendly Microbial Association in Aquatic Ecosystem

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Authors' contributions

This work was carried out in collaboration between all authors. Author SKS conceived the idea and prepared the draft, author SN reviewed and proof read the draft, organized figures and author SM managed cross-opinion corrections and literature searches. All authors read and approved the final manuscript.

Review Article

ABSTRACT

'Biofilm' is a multi-community microbial association living on submerged substrates. Using the online search engine www.OJOSE.com to identify the 100 most recent journal articles, we found that the term 'biofilm' had been extensively used by journals from medical/pathological disciplines to mean virulent microbial associations in the human body. The question is, whether natural aquatic biofilms different from these virulent ones? Should we hold a separate view regarding natural aquatic biofilms? Ecological journals use 'periphyton' to address similar microbial associations living on submerged substrates. We have discussed the development of biofilm in water and emphasized that natural biofilm is a totally heterogeneous interdependent balanced ecological aggregation of different microbial organisms where 'periphyton' occupies an advanced successional form. Contrary to the pathogenic biofilms, natural biofilms from undisturbed aquatic ecosystems are exclusively 'synbiotic' in their mode of survival. We therefore propose the term 'synbiofilm' to maintain a classical distinction between natural aquatic biofilm and pathogenic biofilms.

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1. INTRODUCTION

Till the mid 19th century, the term 'biofilm' was used to cover all Associated Microbial Populations (AMP) in the aquatic phase. Later on, a large number of publications on AMP emerged, following a 'racial' complexity addressing AMP as 'biofilm', as the majority of journals synonymously employed another term, 'periphyton' to refer to some forms of AMP (or biofilm?). After doing an online survey through (www.OJOSE.com) using the key words 'biofilm' and 'periphyton' for the database Medline/Pubmed, the most recent 100 articles revealed that the term 'biofilm' is the most frequently used one in medical (or clinical) or general microbiology journals. Conversely, 'periphyton' has been widely used by journals dealing with ecology, ecotoxicology and environmental biology. Sometimes both the terminologies are interchangeably used [1]. Except for bacterial colonisation, which in medical and microbiological journals is exclusively termed as 'biofilm', the AMP of benthic cyanobacteria, filamentous algae, protozoan and some other zooplankton are conventionally referred to as 'periphyton' [2,3]. Sometimes, eukaryotic cells (e.g. algae) are also described as an essential component of 'biofilm' [4]. The viewpoint presented in this review is that, when described in medical/clinical terms, 'biofilm' with a host-parasite relationship may differ from similar mutualistic 'biofilm' in an aquatic ecosystem. However, much literature is available that do not distinguish between both these relationships in explaining 'biofilm' [5]. Following the high degree of differences in the abiotic and biotic properties prevailing between hydrated environments (such as the intestinal lumen, dental plaque, aerial-moist surfaces etc.) and the natural aquatic environment, the composition and pattern of development of the biofilm may greatly vary in nature. Thus, a meaningful definition and distinctive classification are necessary for 'biofilm' in these two environments.

Biofilm is often defined as an assemblage of microbial cells irreversibly associated with a surface and enclosed within an Extra Cellular Matrix (ECM) [5]. It is also characterised as a complex containing noncellular materials (e.g. mineral crystals, corrosion particles, clay or silt particles, blood components) depending on the environment along with biotic aggregations of bacteria, algae, fungi and protozoa enclosed within the ECM [5,6]. This is an essential organisation in the survival of the bacterial populations [7] and a prerequisite for the existence of different successional microbial aggregates [8,9]. Further, it as an aggregation that 'may' exclude the eukaryotic primary producers, and mostly include the decomposers and pioneer colonising bacterial groups with the ability to colonize on a number of different substrate surfaces [10]. Wetzel [11] cautiously used the term 'periphyton' instead of 'biofilm' highlighting the micro 'floral' communities, especially the photosynthetic micro-organisms living attached to the underwater substrates. However, the contributions from the early bacterial colonies were ignored in his definition.

It is evident from these definitions that there is a very distinct knowledge gap from 'biofilm' to 'periphyton', bacteria to algae, and Wetzel [11] to Donlan [5]. This gap rests on at least three principal aspects –

(i) The use of multiple terms and definitions to describe analogous ecological communities (i.e. biofilm) is quite an arduous task. A definition or 'term' must strictly adhere to its concept with high uniformity and undebatable scope. There are several grounds on which the term 'biofilm' demands a strict adherence to its properties, such as, to understand the ecological processes and evolution, to analyse decisive ecological behaviour including resilience and climax or basic stable community organisation with multifaceted interactions etc. A justified endorsement for its realistic natural behaviour should take top priority in any study on natural aquatic biofilm.

(ii) The basic question that arises in a natural aquatic ecosystem is whether the aquatic biofilm and periphyton are homologous or two saliently deviating/co-existing ecological communities. After almost three decades since Wetzel [11], the term periphyton continues to receive the 'all or none' kind of treatment from the ecological community. Similarly, the term biofilm has been randomly applied to mean either every kind of surface-based microbial community or restricted strictly to the surface-colonised bacterial populations. We hypothesise that periphyton is a successional product of 'biofilm' and must, therefore, be defined as a function of nutrient sharing and microbial interactions on the substrate. However, such a 'hypothetical fusion' of biofilm and

(iii) Lastly, the main puzzle that confronts us is whether the dynamics of 'biofilm' will lead to 'parasitism' or 'syntrophism? These two ecological relations are completely opposite to each other in relation to their functional organisation of biological communities. Aquatic biologists must, therefore, make a concrete and meaningful selection of the definition for a surface-associated microbial population (irrespective of bacteria and algae) to fit its actual functional role in the system.

periphyton warrants a thorough and in-depth analysis of the characteristics that correlate them.

Understanding the processes and mechanisms of the natural aquatic biofilm will clarify these debates. Hence, a discussion on the ecological background of 'biofilm' through 'periphyton' has been forwarded in regard to prokaryotic and eukaryotic organisms as a friendly and co-existing surface-based organisation.

2. DEVELOPMENT OF BIOFILM

2.1 Pre-Organic Colonisation

Loeb and Neihof [12] were the first to report the formation of a conditioning film on the surfaces exposed in water (seawater). They recorded that such films were formed within minutes of exposure of the surface to seawater, and continued to grow for several hours. Recent studies reported that a conditioning film was initiated by inorganic substances of an acid mucopolysaccharide called Transparent Exopolymer Particle (TEP). These are highly abundant transparent micro-particles visualised by staining with Alcian Blue [13]. These are also defined as 'protobiofilm' as they play a significant role in preconditioning the substrate surface for biofilm formation [14]. Its formation can be prevented by prefiltering the aquatic column which removes the surface conditioning TEPs [14]. TEP primarily originates either from bacterioplankton [15] or from phytoplankton [13,16]. Most natural water sources contain abundant levels of TEP precursor bacteria [17]. A primer compound, glucopolysaccarides, in capsular form and largely resistant to bacterial degradation are continuously released into the ambient environment from metabolically active bacteria [15]. Phytoplanktons belonging to the genera *Skeletonema*, *Chaetoceros*, *Nitzschia*, *Navicula* and *Coscinodiscus* were found to contribute to TEP through the release of

extracellular carbohydrates during their life-cycle [16,18]. In the coastal waters, structures like the mucous nets of the filter feeders [19], the allochthonous sources, exudates of the benthic microbial mats [20] and macroalgal beds are also potential TEP precursors [21]. These precursors could form TEP by the cationic bonds between polysaccharide fibrils [13,22]. In myxobacteria, the fibrilar portion was found to be composed of different monosaccharides i.e. galactose, glucosamine, glucose [23]. Through the divalent cation-bridging and/or ester–sulphate bonding between the anionic ends of the acidic sugars, the surface active TEP polysaccharide fibrils exhibit a strong tendency to form ion bridges and hydrogen bonds. Consequently, TEP becomes extremely sticky, offering a high probability of attachment for aquatic microbiota upon collision with hard substrates [24,25]. Also, the differential settling, surface properties and diffusive boundary layer of the substances may further enhance the sticking rate. Thus, an organic TEP layer gets deposited on the water-solid interface of the substrate [26]. It is likely that this "conditioning layer" neutralises the excessive surface charges and the surface free energy that normally prevent bacterial cells from approaching near enough to the substrate surface under normal conditions for attachment.

2.2 Bacterial Immigration

Once the conditioning layer is formed, a nutritious ion-neutral zone on the substrate attracts and initiates bacterial attachment. We prefer to use the term 'pre-biofilm' to refer to the early colonised bacterial component of the biofilm. Bacteria from the pre-biofilm stage are larger than the free-living bacteria in the surrounding water and their attachment varies from <10% to 90% of the total planktonic bacteria depending on the environment [27]. Consequently, the substrate surface serves as "hot spots" or sites of intense microbial and chemical activity. A few bacteria on TEP layer further produce TEP, thus enriching the nutrient-zone. As the pioneering bacteria and TEP aggregate dissolve the organic materials, they provide favourable and specialised sites (e.g. low oxygen or anaerobic environments) to promote further microbial development on the substrate.

In the lentic system, bacterial colonisation largely depends upon the differential settling of TEP followed by random microbial encounters. However, in the lotic system it also depends upon the fine balance between advective

transport, shear-induced resuspension and bacterial cell motility. In streams, for example, the waterflow largely influences the dispersal and transportation of the microbial cells to the benthic zone where microbial colonisation finally occurs [28,29]. On a small scale, the laminar boundary layer may also influence the bacterial cell motility [30], where the flow velocity approaches zero and the hydrodynamic attraction by the surfaces becomes apparent [31]. Mechanisms like quorum sensing (QS) regulate the colonisation process in multispecies bacterial biofilms in both the lentic and lotic systems [32]. Pioneer bacteria use QS to sense population size, followed by the growth and maintenance of a sizable population, proportionate to nutrient availability. As cell densities increase, cell-to-cell communication becomes enhanced [33]. In many Gram-negative bacteria, this communication occurs via the N-acyl homoserine lactones (AHLs), whereas the autoinducing peptides represent a common signal in the Gram-positive bacteria. This mechanism is very prominent in a diffusion-limiting environment. In the lentic system possessing a high flow velocity, such signalling is disrupted, interrupting the biofilm formation. This could be one of the primary reasons why biofilm formation shifts to the laminar flow boundary layer.

2.3 Heterogeneous Colony Formation

The initial biofilm releases Extracellular Polymeric Substances (EPS) of varied compositions forming Extra Cellular Matrix (ECM). The ECM provides a more suitable protective environment for survival of the biofilm. However, the composition and quantity of the EPS may vary according to the type of microorganisms, substrate, age and different environmental conditions under which the biofilm grows [34]. The ability of the EPS to adsorb and retain the nutrients is especially significant for biofilm development [35]. The EPS from the microbial mats produces more polysaccharides, lipids, proteins and DNA as heteropolymers such as lipopolysaccharides or glycoproteins to the biofilm nutrient pool [36]. Monomers commonly found in the ECM are hexoses, pentoses, uronic acids and deoxy-sugars. The rate of the EPS production reaches its maximum after the initial growth phase of the colonised organisms [37]. Thus, colonisation is encouraged at the ECM-substrate reactive interface.

Subsequently, a succession of other organisms follows, lasting until the reserves or sources of nutrients exhausts. The early colonized bacteria facilitate attachment by providing more diverse adhesion sites besides food. At least two mechanisms accelerate the formation of the heterogeneous colonies. First, the QS factors release the AHL and AHL-like molecules. QS is a common virulent adjustment in pathogenic bacteria, it was reported from the seawater biofilm [38] and also in the cyanobacterium *Gloeothece* sp [39]. This is an adaptive mechanism in the biofilm through which the bacterial population regulates its propagation proportional to the ambient nutrient concentration [40]. Second, the intra- or interspecies competition could exist among the colonising living forms for metabolic requirements. Booth [41] advocated two types of colonisers whose metabolism and growth are dependent on the Carbon (C) sources in the matrix. As and when an organism occupies a certain place, the resources become limited in such a manner that a second organism cannot occupy the same place. If the first type of organism has a short life-span it rapidly colonises the entire surface. In an aquatic ecosystem, this colony, at a specific density, may draw other organisms towards it through QS. Consequently, more attachment structures are formed that keep the biofilm active and stable. The second type of organism multiplies at a slower rate and begins to colonise the surface with less number of individuals. This organism overgrows the first one due to its longer life-span and more effectively exploit the nutrient resources. In the long run, this type of coloniser will replace the first one. However, under unfavourable conditions, the slower colonisers stand little chance to settle on the surface against the faster colonisers, as the latter can quickly compensate their losses.

2.4 Algal Immigration

Phytoplankton absorbs nutrients from the water and captures solar energy. The excess organic C is released during photosynthesis in the form of dissolved organic C (DOC), rich in several compounds such as organic acids, amino acids, peptides and many carbohydrates. Bacteria depend upon phytoplankton-derived DOC as a source of energy and organic C. Thus, a signalling communication gets established between the biofilm bacteria and phytoplankton [38]. Tait et al. [38] observed that the zoospores of *Ulva* sp. respond to the AHL signal molecules and attracted to the substrate. The spores of *U. intestinalis* respond to different long-chain AHL signals and settle down at the 'macromolar AHL

hotspots' through direct attachment with the AHL-producing bacteria [42]. Following a similar strategy, the zoospores of the marine alga *Enteromorpha* were found to respond in different ways to AHL signalling from the monospecific early colonising bacteria [43,44]. It was observed that the settlement of the zoospores of *Enteromorpha* was dependent on the different stages of the biofilm growth [44]. Hodoki [45] added that the algal immigration to the biofilm complex is proportional to the density of the bacteria attached on the substrate. Thus, the heterogeneous biofilm does not favour all the algal immigrants to colonise homogenously on the substrate. There are definite 'hotspots' marked with AHL-production, bacterial density and in some cases even ECM, possessing fibrilar bridges. Patel et al. [44] classified four types of 'association-effects' from the biofilm-algal interactions – (i) no significant effect, resulting in no attachment, (ii) inhibitory, resulting in a nonresponsive and repelling effect, (iii) stimulatory, resulting in attachment and (iv) highly stimulatory, leading to dense attachment. These variable 'association-effects' establish an initial patch formation, a type of adaptive and evolutionary adjustment under a specialised 'phycosphere' like microhabitat in the biofilm to prevent intra- and interspecific competitions. At these hotspots, the bacteria benefit from the EPS, particularly the polysaccharide released from the algae; in return, the algae receive remineralised nutrients, chiefly phosphorus (P) from the bacteria [46].

Initially, the bacteria do not compete with the algae for nutrients, because the high quality C-rich components and other metabolites already sustain the association enriched with nutrients; rather, they maintain a mutualistic relationship. Following the colonisation of the unicellular or colonial algae on the substrate, the bacteria become more senescent and start solubilising the phytodetrital aggregates [47] ultimately transforming the particulate organic matter in the algae to Dissolved Organic Matter (DOM). Algal communities also develop a similar relationship with bacteria for different prokaryotic products. Green algae *Chlamydomonas nivalis* and *Lobomonas rostrata* were found dependent on the vitamin B$_{12}$ or vitamin C produced by *Mesorhizobium* sp [48]. Similarly, the nutrient-stressed algae maintain homeostasis by secreting more polysaccharides into the external medium and such an exudation is at its highest during the stationary growth phase [49,50]. Even at a very low level of their own carbohydrate by-

product (e.g. <1mgL^{-1}), the bacterial population shows better growth [51] by utilising the excess algal nutrient exudates. Guerrini et al. [52] observed an enhanced production of ECM in the associated bacteria-algae complex with major contributions from the algal counterparts.

Although phytoplankton represents a mixture of algal forms, either the unicellular or colonial forms (e.g. blue green algae, diatom) are observed to attach more successfully to the substrate [53]. It is possible that these algae grow faster and reach the stationary growth phase earlier thus creating a nutrient-deficient situation resulting in greater release of polysaccharide. Modelling on this relationship, it is clear that bacterial or algal candidates which cannot sufficiently contribute to ECM will grow faster, increasing its biomass in the periphytic community as it can largely utilise all the nutrients in the periphytic layer [54]. However, at a later stage, under conditions of nutrient stress, the periphytic component with its ability to contribute to ECM only, will thrive and grow. Such an ability of an enhanced contribution to EPS was also observed when the unicellular algal components became senescent [55].

2.5 Algae-Bacteria Interaction

Many algal and invertebrate species are able to regulate the rate of bacterial colonisation on their surfaces [56-58]. The microalgal surfaces are typically covered by bacteria at densities of approximately 10^7 bacteria cm^{-2} [59] and additionally, diatoms have the ability to release TEP to attract the bacteria to their surfaces [13]. Whether QS occurs between the algae and bacterioplankton for such an interaction to occur is not well understood. However, a kind of encounter probability between the bacterioplankton and algal surface is sure [60]. This could be followed by QS through the chance arrival of the bacteria near to an organic source and when the distance range of the bacteria is between 10-20µm from the algal surface [61,62]. These bacteria may be different from the initial colonising bacteria, especially in terms of chemical co-ordination and production of extracellular exudates. The stabilisation of such colonisation is regulated by an enhanced supply of organic C from the algae [63,64]. Bacteria on the algal surface of *Skeletonema costatum* assimilate the extracellular C from the algae [65]. Guerrini et al. [52] observed that the colonised algae *Cylindrotheca fusiformis* exhausts the available P into the phycosphere and their

metabolism shifts to polysaccharide production. Some of this polysaccharide becomes extracellular, creating an algal-bound organic-rich environment. At this primary interactive zone some bacterioplankton get trapped, grow and remineralise the P. Bacterial cells are found to have a higher degree of P assimilation efficiency than do the algal cells and their metabolism triggers the algal P deficiency-stimulating algal cells to release more extracellular products to the ECM.

3. CROSS-KINGDOM INTERACTION

On a broader scale of interactions of the biofilm with the living-world, the earlier sections mainly dealt with two kinds of organisms, viz., bacteria and algae, and demonstrated a kind of two-dimensional ecological organisation. At a later stage, the biofilm progression establishes a three-dimensional ecological organisation, mostly with the micro-zooplankton and occasionally with the macro-zooplankton. These animals or animal-like communities in the biofilm canopy are often termed 'meiofauna', a term originating from meiobenthos [66]. This group of animals, although variously defined by several authors [67,68], comprises a major group of non-attached organisms found in the biofilm complex. The permanent meiofauna frequently observed in the vicinity of biofilm are nematodes, copepods and foraminifera [69]. Earlier, identified cladocera, copepods, hydrachnids, oligochetes, nematodes and rotifers were reported from hard substrate-bound biofilms [70]. Such a diversified group of organisms of animal origin in the biofilm canopy indicates their level of dependency on the natural biofilm complex. The late succesional stage of biofilm, which Wetzel [11] identified as periphyton, meets two needs of the meiofauna – first, as a source of food and the second as a habitat. One of the nutrient components on which these organisms thrives is phytodetritus matter resulting from a senescent algal source. Ostracods like *Candona neglecta* can assimilate phytodetritus material from the truly colonised algae *Skeletonema costatum* [71]. Through a complex pathway the nematodes also colonise in the periphytic habitat, specifically when detritus materials are in abundance [72]. Further, the biotic components of aquatic biofilm-like bacteria, diatoms or protozoa are a good food source for the meiofaunal organisms [73,74] and constitute a large part of their dietary component. The primary driving force of the meiofaunal intrusion is, therefore, nutrient availability in the biofilm habitat. Being good swimmers, the meiofaunal organisms such as copepods can actively colonise and disperse within the periphytic microhabitat [75], while the nematodes disperse passively via water currents [76].

4. BIOFILM IS A FUNCTIONAL ECOLOGICAL UNIT

In light of the present discussion, it is evident that the development of a biofilm describes a nutrient-centred aggregation of AMP, starting from TEP to algae (Fig. 1).

The adaptive behaviours of bacteria, algae and meiofaunal organisms to choose and feed selectively in nutrient-rich biofilm zones regulate the spatial distribution and development of the biofilm components creating spatial heterogeneity with high and rare microbial richness (e.g. algae) on the substrate [77] maintaining a self-regulated balanced biofilm community during the 'Colonisation and settlement period' (Fig. 2). Probably, because of such a heterogeneic effect, the extinction or niche collapse is a rare occurrence for biofilm communities in undisturbed aquatic environments. As and when this community is disturbed, due to different ecological forces (i.e. turbulence period), such as the addition of excess nutrient or macrofaunal/meiofaunal grazing, periphytic heterogeneity is lost, resulting in the breakdown of the biofilm structure.

It is at such a stage that a disturbance to the biofilm stability could occur, disrupting its mutualistic nature. However, as is normal to all natural environments, a resilience phase may result in a recovery period for the disturbed biofilm to once again regain its balanced periphytic interaction stage. Any failure of such a recovery effort, i.e. the post chaotic biofilm, could result in the production of a pathogenic form of the biofilm. Fig. 2 explains this situation at 'recovery phase' where difference exists between natural heterogeneous biofilm to pathogenic biofilm. The fall of the pathogenic line (dashed) after a sharp rise indicates exhaustive nutrient deficiency, diversity loss and decline of synbiotic nature. Once such a situation arises, the biofilm structure which was earlier non-virulent may now be occupied by pathogenic bacteria like *Vibrio cholerae* or *Streptococcus downei*. The form of biofilm addressed by most medical-related journals discusses such a chaotic and turbulent biofilm. The pathogenic property of biofilm may be obvious at this point, but this is also obvious that, prior to the formation of such pathogenic

phase in aquatic ecosystem, a biofilm (of heterogeneous nature) may pass through distinct features that mutually benefit all biotic components in the complex on a time scale. The primary component highlighted here to set such *synbiotic* norms is nutrients. Sharing of nutrients in a non-pathogenic natural biofilm may be multidirectional, leading to advanced successional stage called periphyton. Warnick and Kolter [78] termed this form of multispecies biofilm as city of microbes where one species selects others to be their neighbour for survivability of the self as well as stability of the complex. However, once it turns to pathogenic, heterogeneity is lost, only the pathogenic forms of bacteria (e.g. cholera) will flourish and most interestingly, only unidirectional nutrient flow would occur. This is why, Espinosa-Urgel [79] used the phrase 'residents parking only' to explain a situation where opportunistic pathogen like *Pseudomonas aeruginosa* prevents other non-resident bacteria to colonize in open space in the resident biofilm. The behaviour of such turbulent biofilm is completely different from that of the balanced natural aquatic biofilm. During

this situations inaquatic ecosystem, as the pathogenic bacteria are subject to predation by protozoa, bacterivorous microorganisms, bacteriophages and micro- and macro zooplankton, it is probable that biofilms with pathogenic nature may provide a refuge for the persistence and distribution of pathogenic bacteria from the environment to human beings. We also believe that, in technical terms, biofilm with pathogenic properties at this stage is a subject of microbiology dealing with medical implications. However, natural sciences, like ecology has never addressed heterogeneous synbiotic biofilm to describe ecosystem stability within the preview of its focussed subject areas. Contrasting to the understanding of a microbiologists dealing with medical biofilm (or bacterial biofilm), natural aquatic ecosystem, here, has more and more organised form of biofilm (bacteria to protozoa to meiofauna) contributing unique and substantial characteristics to the stability of an ecosystem. This could be a broad and diverse subject area to understand biological processes of aquatic ecosystem and aquatic biology.
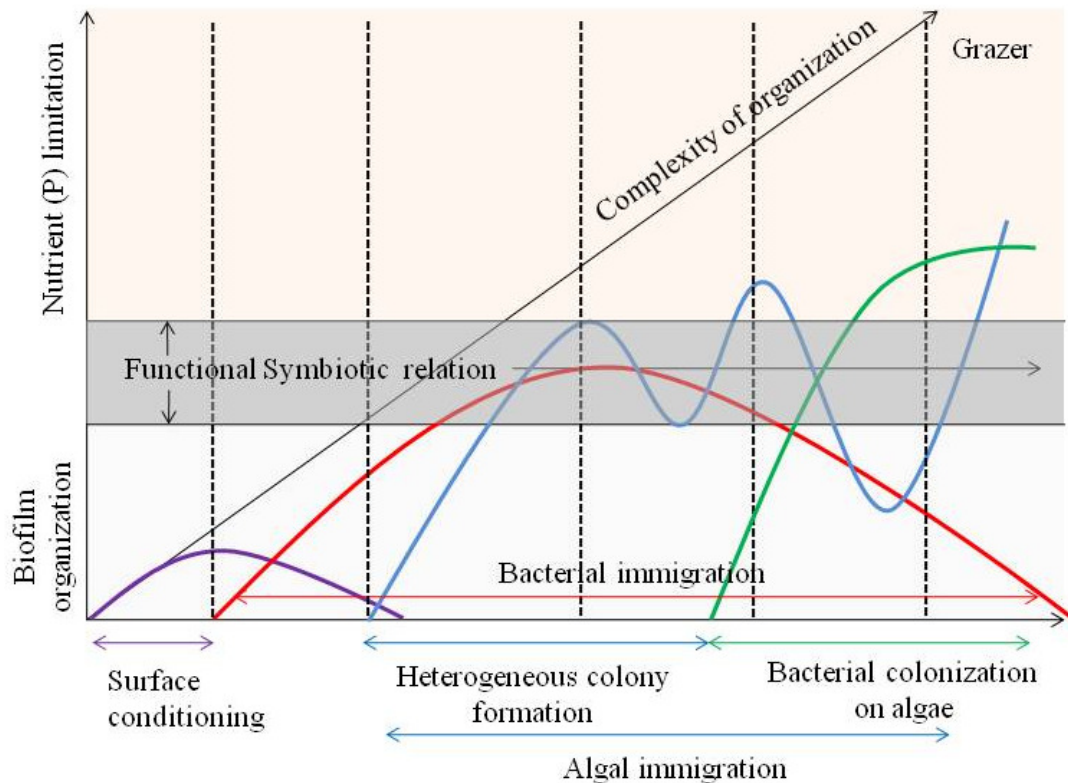

Fig. 1. Formation of biofilm through surface conditioning followed by bacterial immigration, heterogeneous colony (microbial) formation, algal immigration and bacterial colonization on previously colonized algae
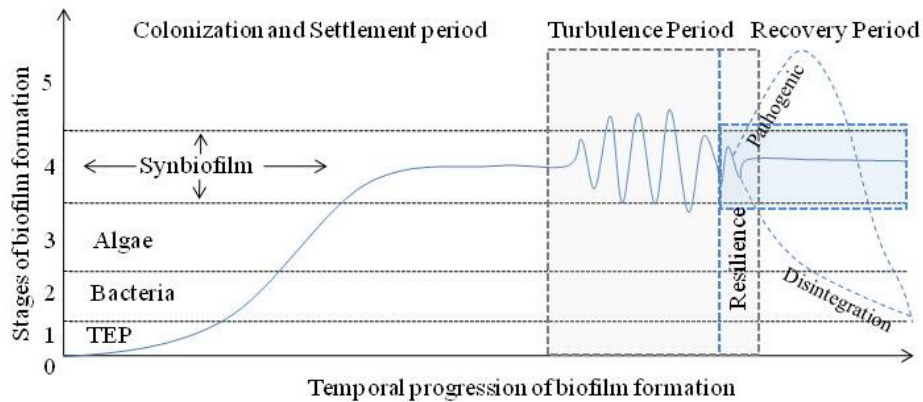
Fig. 2. Different ecological stages of biofilm formation. When encounters ecological disturbances (Turbulence period) the synbiofilm stage may enter into three distinctive forms- (i) disintegrating form (ii) pathogenic biofilm, or (iii) a synbiotic stage after a short turbulence stage (recovery period)

5. CONCLUSION

(i) In the natural aquatic biofilm, both the coloniser and colonist strictly adhere to a mutual sharing of nutrients. The TEP, ECM, patch formation, phycospheric organisation - all favour this relationship. Hence, the biofilm in the aquatic ecosystem is a kind of complex nutrient-bound symbiotic association.

(ii) We propose the use of the term 'synbiofilm' (Greek meaning syn, mutual) for this aggregation because the components mainly include the synbionts in mutual agreement for exploring the nutrients available (a kind of syntrophism) and to act as a nutrient rich hotspot for the upper trophic levels [80,10].

(iii) Contrary to virulent biofilm, synbiofilm here maintains an autoregulatory nutrient management budget with an 'as and when need' pattern. Hence, synbionts are much more complex and of a higher order of aggregation than any other AMP.

(iv) A definition for 'synbiofilm' could be the '*synbiotic and heterogeneous aggregation of associated autotrophic and heterotrophic microbiota (called synbiota) regulated through the manipulations of nutrient sharing and transfer with the ambient as well as within the associated micro-environment.* In future, this definition can be used by ecological journals to describe as an extension of the 'periphyton' (that limits associated microbial research to only the phototrophic algal communities in aquatic ecosystems).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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